EFFECT OF INCORPORATING ENCAPSULATED AND NON-ENCAPSULATED PROBIOTIC CULTURES ON CULTURE SURVIVAL AND CHEESE QUALITY OF GOUDA CHEESE

By

Yi-Chun Liu

A Thesis

Submitted to the Faculty of Graduate Studies of

The University of Manitoba

In Partial Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Food Science

University of Manitoba

Winnipeg, MB, R3T 2N2

Copyright © 2012 by Yi-Chun Liu

I hereby declare that I am the sole author of this thesis.

I authorize the University of Manitoba to lend this thesis to other institutions or individuals for the purpose of scholarly research.

Yi-Chun Liu

I further authorize the University of Manitoba to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

Yi-Chun Liu

The University of Manitoba requires the signatures of all persons using or photocopying this thesis. Please sign below and give address and date.

ABSTRACT

Gouda is a semi-hard, high fat cheese and was investigated here as a food vehicle for probiotic bacteria.

The purpose of this study was to incorporate non-microencapsulated and microencapsulated probiotic cultures (*L. helveticus* and *B. longum*) into Gouda cheese. *Lactococcus lactis*, *Lactococcus cremoris*, *Lactococcus lactis* subsp. *diacetylactis* and *Streptococcus thermophilus* were used as starter cultures. Each batch was evaluated for its chemical, microbial, textural, and also sensorial properties after 3 and 4 months of aging.

The experimental Gouda cheeses with the addition of probiotic cultures did not alter the chemical properties of the aged cheese. Furthermore, the final levels of both probiotic strains incorporated were meeting the recommendation level suggested by health organizations which is higher than 10^7 cfu/g. Moreover, the addition of probiotic strains and maturation time did not alter the texture of the cheese. Overall, the result from the sensory test also did not show any differences.

ACKNOWLEDGMENTS

I would like to express my gratitude to my advisor, Dr. Susan D. Arntfield, for the continuous support, guidance, and encouragement throughout the program. She has been supportive and patient in answering my every question. Without her, I would not be able to complete this degree and be confident in the things I am doing. From the first day of me being a food science student to this day, she has been a great advisor in everything.

Also, I would like to thank the committee members, Dr. Rick A. Holley, Dr. Arnie Hydamaka, and Dr. Michel Aliani for sharing their knowledge and experience to help me through research and writing this thesis. In addition, I would like to thank Dr. Loreen Onischuk for her help with statistical analyses programs.

To all those who have helped me through my research, Michael Stringer, Denise Aminot-Gilchrist, Patricia Kenyon, Georgina Mejia of the Food Science department for your technical expertise and help in cheese production. Especially to Patricia Kenyon, who has assisted in the transportation of milk cans out to Glenlea Research Facility once a week to pick up milk for cheese productions. Also, I need to thank Richard Simmock and Rosewood Milk Trucking Company for delivering milk and helping me receive the appropriate amount of milk for each batch of cheese production. Thank-you to Larissa from the Milk Marketing Board and Glenlea Research Facility for providing me milk for cheese production.

My appreciation is extended to all my sensory panellists (Roniele, Meghan, Dylan, Senthilkumar, VJ, Pat, Carol, and Allison) for their dedication and invaluable effort in my research.

The financial support of Canadian Dairy Commission and Agri-Food Research and Development Initiative are gratefully acknowledged. I am also thankful for the University of Manitoba supporting me with the student travel award allowing me to present my results at the International Conference of Food Factors held in Taipei, Taiwan in November, 2011.

Thanks to my fellow office mates and other Food Science students who have encouraged and supported me. A special thank you to Sally Diep, Seraphine Liu and Shirley Wu for helping me with my research.

Lastly, I would like to express my sincere appreciation to my family and friends who have motivated and encouraged me during the completion of my research.

TABLE OF CONTENTS

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

LIST OF ABBREVIATIONS

ACE	ANGIOTENSION CONVERTING ENZYME
AOAC	ASSOCIATION OF ANALYTICAL CHEMIST
CFU	COLONY FORMING UNIT
DCU	DANISCO CULTURE UNIT
F6PPK	FRUCTOSE-6-PHOSPHATE PHOSPHOKETOLASE
GI	GASTROINTESTINAL
ISO	INTERNATIONAL ORGANIZATION FOR STANDARDIZATION
ME	MICROENCAPSULATED
MRS	DE MAN, ROGOSA, SHARPE AGAR
NME	NON-MICROENCAPSULATED
RCA	REINFORCED CLOSTRIDIAL AGAR
S.D.	STANDARD DEVIATION
SAS	STATISTICAL ANALYSIS SOFTWARE
ST	*STREPTOCOCCUS THERMOPHILUS*
TRT	TREATMENT

LIST OF APPENDICES

APPENDIX **PAGE**

1. INTRODUCTION

Cheese is widely consumed and one of the most ancient foods around the world. The main purpose of processing milk into cheese is to preserve a perishable food and to extend the variety of products (Walther et al., 2008). Cheese is made based on the coagulation of casein and the separation of whey from milk (Agriculture and Agri-Food Canada, 2006). Cheese is a suitable protein source since it includes most amino acids by having casein as the main protein (Scott, 1986). However, cheese also consists of fat, water, minerals and vitamins. Lactose, the main sugar in milk is rarely present in cheese due to its removal in whey and lactic acid formation (Walther et al., 2008). Cheese can be categorized by the milk used, the manufacturing method, texture, fat content, fermentation type, surface, and interior (Walther et al., 2008). Cheeses also differ in their flavours due to the ripening time when lactose, protein and fat are broken down by fermentation, proteolysis and lipolysis (Walther et al., 2008). The main reasons for consumption of cheese are to supply essential nutrients and enjoyment (Walther et al., 2008).

Cheese consumption has increased over the years in North American and European countries. United States is the largest producer of cheese with 24% of total production in 2005 (Agriculture and Agri-Food Canada, 2006). However, Canada ranked ninth in 2005 for cheese production (Agriculture and Agri-Food Canada, 2006). One of the main companies which dominates cheese production in Canada is Kraft (Agriculture and Agri-Food Canada, 2006). Canadian cheese consumption has increased to 12kg per capita in 2005 from 10kg per capita in

1980s (Agriculture and Agri-Food Canada, 2006). Moreover, there was a 6.8% increase for retail sales of cheese between 2001 and 2005 (Agriculture and Agri-Food Canada, 2006). On the other hand, there was a decline in sales of processed cheese, which dropped by 2.5%, and the reason for this was due to the nutritional concerns of the consumers (Agriculture and Agri-Food Canada, 2006).

The word “probiotic” has been introduced into the food industry and the sales of probiotic foods have been increasing over the years. Most consumers are not fully educated on this term. Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Food and Agriculture Organization of United Nations/World Health Organization – FAO/WHO, 2001). This means that their viability must be maintained throughout all processing steps up to ingestion by the consumers and also must survive in the gastrointestinal tract in the hosts (Gomes da Cruz et al., 2009). Research shows that different strains of probiotic bacteria confer different health effects on hosts (Santosa et al., 2006).

It is estimated that there are 80 probiotic-containing products in the world (Champagne et al., 2005). Most probiotic products on the shelf in the dairy section are yogurt. However, cheese may be a better delivery vehicle for probiotics than yogurt. As cheese has a higher pH, it may provide a more stable environment to support the survival of probiotic strains, and its higher fat content and higher buffering capacity may protect probiotic strains during passage through the GI tract (Champagne et al., 2005). However, the low pH in the

stomach and some processing steps will decrease the level of probiotic in the food matrix. Recently, research showed that encapsulating probiotic cultures can minimize processing effects by maintaining high viability during storage, good growth during the manufacturing process and also preventing the acid in the stomach from destroying the probiotic before arrival in the gut where the most benefits occur. Until recently, the technique of encapsulating probiotic strains has been applied to dairy products such as yogurt, frozen dairy desserts and cheese. Although much research has been conducted on the probiotic cheese, more is needed to understand the whole concept of incorporation of probiotic into cheese. This study is focused on the enumeration of probiotic strains as well as the texture and sensorial characteristics in the presence of encapsulated and non-encapsulated probiotic strains in Gouda cheese during the aging process.

The specific objectives include:

- Enumeration of micro & non-microencapsulated probiotic strains in Gouda cheese.
- Evaluation of the chemical composition of cheese samples throughout the aging process.
- Analysis of texture of cheese samples throughout the aging process.
- Analysis of sensorial attributes (flavour and texture) of cheese samples throughout the aging process.

2. LITERATURE REVIEW

2.1 Cheese

2.1.1 Introduction

Cheeses on the market are mainly produced with cow's milk. Cheese is one type of food which can be consumed by lactose-intolerant individuals; approximately 70% of adults of the global population exhibit some level of lactose-intolerance (Walther et al., 2008). Consumption of milk can result in abdominal pain, diarrhea, nausea, flatulence, and other symptoms for lactose-intolerant individuals (Walther et al., 2008). However, all types of cheese except fresh ones are free of lactose (Walther et al., 2008). Therefore, if one were lactose-intolerant it would be unnecessary to avoid cheese products. Moreover, cheese can provide all the essential amino acids except methionine and cysteine and one portion of full-fat cheese can provide about two-thirds of the recommended daily intake of fat (Walther et al., 2008). For full-fat cheese, the amount of free fatty acid is usually 1 – 5 g per kg of cheese (Renner, 1987). Cheese is also a good source of protein. The protein content of cheese usually ranges between 20 – 35% and the protein content varies with the fat content (Renner, 1987). The most important mineral in dairy products is calcium. Generally, hard and semi-hard cheeses contain 6 to 11 g of calcium per kg of cheese (Walther et al., 2008). However, cheeses produced by rennet coagulation usually contain higher amounts of calcium than those made from acid-coagulated milk (Renner, 1987). Moreover, phosphorus, zinc, magnesium, vitamins A, B2, B6, and B12 can also be found in cheese, although

the amount of vitamins present in cheese depends on the fat content (Walther et al., 200; Renner, 1987). The medicinal benefits of consuming cheese include improved bone and dental health, weight management, anti-hypertension, and anti-carcinogenic compounds (Walther et al., 2008).

2.1.2 Types of cheese

There are at least 500 varieties of cheese throughout the world. The types of cheese are classified based on the manufacturing methods. They can either be grouped by texture (very hard, hard, semi-hard/semi-soft and soft) or method of milk coagulation (rennet-coagulation, acid-coagulation, heat/acid coagulation or concentration/crystallization) (Fox et al., 2000). Most of the cheeses produced in the world are coagulated by rennet; these include Parmesan, Cheddar, Gouda, and Mozzarella. Parmesan is a very hard cheese which is characterized by high cooking temperature. Cheddar, a hard cheese, is ripened by internal bacterial action without the production of eyes. However, there are types of cheese that are ripened by internal bacteria that do produce eyes. Examples are Emmental (big eyes) and Gouda (small eyes) where numerous eyes are created by CO_2 caused by the fermentation of lactate by *Propionibacterium freudenreichii* subsp. *shermanii* or fermentation of citrate by the starter culture (Fox et al., 2000). From the Canadian Dairy Products Regulations (Department of Justice, 2011), there are 46 types of cheese being regulated in Canada each of which has a standardized maximum percentage of moisture and minimum percentage of fat.

2.1.3 Gouda cheese

Gouda is a type of semi-hard, high fat cheese. The cheese is made from cow's milk and it originated in Holland (Rukure & Bester, 2001). Gouda is exported as red or orange waxed wheels that generally weigh 2.5kg (Kosikowski & Mistry, 1999). The cheese is usually yellow from color added in the manufacturing process, and has a mild nutty flavour (Kosikowski & Mistry, 1999). Gouda is renneted cheese with a sweet curd, prepared from partially skimmed or whole milk (Kosikowski & Mistry, 1999). The quality of milk used must be top-grade since the cheese is made from high pH curds (pH 5.4) without the growth restriction of lactic acid against spoilage organisms (Kosikowski & Mistry, 1999). The ripening period for Gouda is at least 40 days (Bertola et al., 2000). Gouda cheese is traditionally ripened within its own packaging at 10°C for 5 to 7 days, turned daily, until the rind changes color. The cheese is then moved to another chamber at 14 – 18°C for 40 days to complete its aging process (Bertola et al., 2000). The maximum percentage of moisture and minimum percentage of fat for Gouda are 43% and 28% respectively, which is regulated in the Dairy Products Regulations from the Canada Agricultural Products Act (Department of Justice, 2011).

2.2 Probiotic

2.2.1 Introduction

According to the Canadian Food Inspection Agency, “Probiotics are microorganisms that are beneficial to human health” (Canadian Food Inspection Agency, 2009). Probiotic strains have been reported to treat or prevent diarrhea, gastroenteritis, irritable bowel syndrome, inflammatory bowel disease, cancer, depressed immune function, inadequate lactose digestion, infant allergies, hyperlipidaemia, hepatic disease, *Helicobacter pylori* infections, and constipation (Parvez et al., 2006). However, the certainty of the health benefits from the probiotic cultures still needs more research to support them. The desired characteristics of probiotic organisms are that they are of human origin (if intended for human use), have acid and bile stability, can adhere to human intestinal cells, are competitive and colonize in the human gut, produce antimicrobial substances, are competitive against carcinogenic compounds and pathogenic bacteria, are safe in food and clinical use, have clinically proven health effects and can be produced on a large scale (Salminen et al., 1998; Ouwehand et al., 2010). The two genera most commonly used as probiotics are *Lactobacillus* and *Bifidobacterium* (Bruhn et al., 2002). Table 1 shows the common species used as probiotics. The Fermented Milks and Lactic Acid Bacteria Beverages Association in Japan stated that the level for probiotic strains necessary to confer health effects on humans should be at least 10^7 per gram or millilitre (Gardiner et al., 1999). These organisms are incorporated into the

fermented food products, especially dairy products. Probiotic products on the market include yogurt, ice-cream, cheese, and pharmaceuticals.

Table 1. Commonly used probiotics

Lactobacilli	Bifidobacteria
Lactobacillus acidophilus[1]	*Bifidobacterium bifidum*[1]
L. delbrueckii (subsp. *bulgaricus*)[1]	*B. adolescentis*[1]
L. brevis[1]	*B. animalis*[1]
L. cellobiosus[1]	*B. infantis*[1]
L. curvatus[1]	*B. longum*[1]
L. fermentum[1]	*B. thermophilus*[1]
L. plantarum[1]	*B. breve*[1]
L. casei[1]	*B. essensis*[2]
L. rhamnosus[1]	*B. lactis*[2]
L. reuteri[1]	
L. gasseri[1]	
L. crispatus[2]	
L. johnsonii[2]	
L. lactis[2]	
L. paracasei[2]	
L. salivarius[2]	

1: Vijaya Kumar et al. (2005)
2: Champagne et al. (2005)

One probiotic formulation which is a combination of *Lactobacillus helveticus* and *Bifidobacterium longum* showed beneficial effects on gastrointestinal (GI) tract symptoms in patients dealing with chronic disease (Diop et al., 2008). Moreover, Messaoudi et al. (2011) reported that the consumption of probiotic formula containing both *L. helveticus* and *B. longum* relieved psychological distress without showing any negative reactions. Therefore, the probiotic combination used in this study is *L. helveticus* with *B. longum* to see the quality and sensory effects on cheese from these organisms.

2.2.2 Lactobacilli

Lactobacilli are generally characterized as Gram-positive, non-spore forming, tolerant to salt and acid, catalase-negative, non-motile anaerobic rods or coccobacilli, and chain formation is common (Gomes & Malcata, 1999; Kandler & Weiss, 1986). It is a diverse genus that is comprised of at least 87 species (Slattery et al., 2010). They are microaerophilic, so surface growth on solid media is generally enhanced by anaerobiosis or reduced oxygen pressure and 5 – 10% CO_2 (Gomes & Malcata, 1999; Kandler & Weiss, 1986). *Lactobacilli* belong to a group of organisms known as lactic acid bacteria (LAB), which produce lactic acid from their metabolism of carbohydrates (Slattery et al., 2010). *Lactobacillus helveticus* is one of the probiotic cultures used in this project. *L. helveticus* is one of the most used starter cultures in the industry, mostly for cheese manufacture (Frece et al., 2009). However, *L. helveticus* was selected as a probiotic strain based on *in vitro* selection criteria (Frece et al., 2009). *L. helveticus* has good growth at 45°C and maximum growth temperature at 50 – 52°C and may be proteolytic (Kandler & Weiss, 1986; Slattery et al., 2010). The optimum growth pH is between 5.5 and 5.8, and it has complex nutritional requirements for growth (Slattery et al., 2010). The requirements of growth factors include calcium pantothenate, niacin, riboflavin, pyridoxal or pyridoxamine (Kandler & Weiss, 1986). This species is characterized by its ability to produce significant levels of bioactive tripeptides that can inhibit the angiotension converting enzyme (ACE) and this action is associated with reducing blood pressure (Slattery et al., 2010). *L. helveticus* can be isolated from sour milk, cheese starter cultures and

Emmental and Gruyere cheeses (Kandler & Weiss, 1986). Moreover, they can also be isolated from intestinal microflora, one of the criteria for being a probiotic strain.

Studies have shown that *L. helveticus* has the ability to survive GI tract conditions, is resistant to bile, has antimicrobial activity against some enteropathogenic and spore-forming bacteria, adheres to epithelial cells *in vitro*, and has been proposed to be a potential probiotic candidate (Beganovic et al., 2011). *L. helveticus* displays a pattern of antimicrobial activity which is similar to other probiotic *Lactobacillus* species such as *L. johnsonii*, *L. acidophilus*, *L. casei* and *L. rhamnosus* (Atassi et al., 2006). Studies have shown that *L. helveticus* was more effective than *L. rhamnosus* in interfering with *Campylobactor jejuni* invasion into intestinal epithelial cells and also was more effective in inhibiting *Escherichia coli* O157:H7 (Wine et al., 2009). Moreover, *in vitro* studies completed on *L. helveticus* have shown that it has the ability to lower serum cholesterol in the presence of bile (Frece et al., 2009). The reason *L. helveticus* has such great potential as a probiotic strain is due to the protective role of its S-layer protein during passage through GI tract (Frece et al., 2009). Furthermore, the S-layer proteins from *L. helveticus* have proven to be resistant to pepsin and pancreatic juice in humans (Frece et al., 2009).

2.2.3 Bifidobacteria

The family Bifidobacteriaceae consists of 7 genera, and there are 36 species of bifidobacteria that are included within the genus *Bifidobacterium* (Nakajo et al., 2010; Champagne et al., 2005). Nine species have been found in the intestine of man and 20 can be isolated from fermented milk and in the intestinal tract of various animals (Champagne et al., 2005). Bifidobacteria compose 5 – 10% of the total colonies of microflora in humans (Champagne et al., 2005). Bifidobacteria are generally characterized as Gram-positive, non-sporeforming, catalase-negative, non-motile anaerobic rods with various shapes (Gomes & Malcata, 1999). The key enzyme used to identify this genus is fructose-6-phosphate phosphoketolase (F6PPK) (Gomes & Malcata, 1999). It utilizes galactose, lactose and fructose as carbon sources (Gomes & Malcata, 1999). The optimum pH for growth is 4.5 – 8.5 and optimum growth temperature is 37 – 41°C (Gomes & Malcata, 1999 & Rokka & Rantamaki, 2010). In general, bifidobacteria grow better in rich synthetic media such as de Man, Rogosa, Sharpe (MRS) broths (Gomes & Malcata, 1999). They can utilize ammonium salts to produce nitrogen (Scardovi, 1986). *Bifidobacterium longum* is the probiotic strain also used in this project. It is usually found in human feces and can also be found in the digestive tract of infants, adults and elderly subjects. It is very closely related to *Bifidobacterium infantis* (Scardovi, 1986; Silva et al., 2004). It can grow well over a wide pH range (Nakajo et al., 2010). It ferments D-ribose, L-arabinose, lactose, melezitose, fructose, galactose, sucrose, maltose, melibiose and raffinose (Scardovi, 1986).

The oxygen tolerance of *B. longum* is a technological advantage for biomass production when compared to more strict anaerobes such as *B. bifidum* and *B. adolescents* (Silva et al., 2004). *B. longum* has been considered as a probiotic culture as it produces organic acids which reduce the colon pH to a level that inhibits pathogenic bacteria (Kiviharju et al., 2005). They also adhere to the colon mucosa to prevent pathogen adherence as well as colon cancer induction (Kiviharju et al., 2005). Research has also shown that bifidobacteria are considered more adequate probiotics for prevention and/or treatment of human intestinal disorders than lactobacilli (Silva et al., 2004). Therefore, it is important to have both genera incorporated into the experiment.

2.2.4 Probiotic cheese

Foods that contain probiotics are categorized as “functional foods” which are defined as “foods claimed to have a positive effect on health” (Stanton et al., 1998). The incorporation of probiotics into cheese would only result in a functional food if the cultures remained viable during processing and storage and there are no alterations of the texture, sensory, shelf-life and composition of the product (Stanton et al., 1998). As more probiotic foods are marketed, a more competitive environment is created and consumers have more choices when selecting the most suitable product for their daily lives. The development of probiotic cheese would fit well in the marketplace.

Research has shown that probiotic bacteria are unable to survive salt concentrations greater than 3.5% (Montoya et al., 2009). Moreover, studies suggest that a high level of inoculation (up to 10 – 20%, based on the volume of yogurt) of probiotic culture in cheese is recommended (Kailasapathy & Chin, 2000). A ratio of 1:1 of bifidobacteria and lactobacilli has been considered adequate for optimum growth to result in symbiosis between these strains (Gomes & Malcata, 1999). However, when adding probiotic strains into cheese, the concern of co-survival with conventional lactic acid bacteria (e.g. starter cultures) is a challenge (Tamime, 2005). Strain survival also depends on pH, the presence of other competing microorganisms, storage temperature, and the presence of inhibitors in the food (Gomes & Malcata, 1999). Cases of lost probiotic viability in dairy products during refrigerated storage at low pH have been reported (Gomes & Malcata, 1999). However, Ross et al. (2002) suggested that to keep the probiotic culture alive in cheese, the cheese needs to be stored in a cool place to ensure high survival rate and stability of the product. In addition, bifidobacteria survive well in low-acid products such as cheese (Gomes & Malcata, 1999). Therefore, proper strain selection for probiotic application into cheese is important. In general, it is necessary to consume probiotic products on regular basis in order to maintain the effect of these microorganisms on the intestinal microflora (Gomes & Malcata, 1999).

2.2.5 Effects of processing on probiotics

The addition of starter culture, salting, and other ingredients may influence the level of probiotics in food. Starter cultures are added for acidification, texture, and flavour development in fermented dairy products. However, studies showed that these starter cultures may slow the growth of probiotics (Champagne et al., 2005). In addition, Vinderola et al. (2002) showed that antagonistic interaction may exist between probiotic and starter cultures. Also, when fast starter culture growth occurs, acidification develops more quickly with shortened fermentation time, resulting in limited growth of probiotics during processing (Champagne et al., 2005).

Heat is commonly used during the manufacture of fermented dairy products and it serves two purposes. High temperatures (over 65°C) can destroy unwanted microorganisms and low temperatures (36 – 39°C) contribute to texture and flavour development (Champagne et al., 2005). However, temperatures below 45°C do not have an effect on probiotics (Champagne et al., 2005).

The maturation time of cheese also affects the viability of probiotic cultures where most ripened cheeses may have maturation times up to 24 months (Ouwehand et al., 2010). Survival of probiotics during storage must also be taken into consideration.

Since many food components and processing steps may interfere with the bioactivity of probiotics, it may be beneficial to encapsulate the probiotic culture to protect it throughout processing, transport and storage (de Vos et al., 2010).

However, the survivability of the probiotic cultures still depends on the species used.

2.3 Microencapsulation

2.3.1 Introduction

Microencapsulation is a chemical or mechanical process that can protect and control release of the active ingredients by covering them with a layer of another material (Chen et al., 2005). Other benefits of microencapsulation include preventing bacteriophage invasion, increasing survival rate during freeze drying and freezing, providing greater stability during storage, and protecting the active content from environmental stresses such as acidity and gastric conditions (e.g. stomach) (Krasaekoopt et al., 2003; Rokka & Rantamaki, 2010). Researchers usually refer to microencapsulation when discussing encapsulation of a probiotic as the size of the encapsulated probiotic is around 1 – 5μm (de Vos et al., 2010). A microcapsule consists of a semi-permeable, spherical, thin, and strong membrane surrounding a solid/liquid core (Anal & Singh, 2007). Microcapsules can be designed to release active ingredients by heat, salvation diffusion and pressure and the coating may also be designed to release active components in specific areas of the body (Anal & Singh, 2007). However, for probiotic encapsulation, the membrane of the microencapsulated system must provide permeability for nutrients to pass through while preventing entry of molecules that may destroy the live bacterial cells (Islam et al., 2010). There are various

methods of microencapsulating probiotic bacteria to increase the survival rate up to 80 – 95%; these include extrusion, formation of oil emulsions and spray-dying (Krasaekoopt et al., 2003; Champagne et al., 2010). Research showed an improved viability of > 10^5 cfu/g for encapsulated probiotic organism when incorporated into frozen dairy desserts when compared to counts < 10^3 cfu/g with non-encapsulated organisms (Tamime, 2005). However, to act as capsules it is essential to select a suitable material that can be incorporated into the foods without interfering with the texture and taste of the food (de Vos et al., 2010).

2.3.2 Extrusion

Extrusion is used to create capsules with hydrocolloids by preparing a hydrocolloid solution, adding microorganisms and then extruding the cells through a syringe needle to form droplets (Krasaekoopt et al., 2003). The size and the shape of the droplets are dependent on the needle (Krasaekoopt et al., 2003). This method is simple, low cost, and uses gentle formulation to ensure high cell viability (Krasaekoopt et al., 2003). The most common material used for extrusion encapsulation is alginate (Krasaekoopt et al., 2003). Talwalkar & Kailasapathy (2003) have shown that encapsulating bacteria in alginate improved survival rates by one log when compared to free cell counts when stored in skim milk for 24 h.

2.3.3 Emulsion formation

In the second method, an emulsion is formed by adding a small volume of the cell-polymer suspension to a large volume of a vegetable oil and then homogenizing to form a water-in-oil emulsion (Krasaekoopt et al., 2003). The bead of suspended cells can then be harvested by filtration (Krasaekoopt et al., 2003). Bead size is controlled by the speed of agitation and the presence and type of emulsifier (Krasaekoopt et al., 2003). For this type of encapsulation, a mixture of κ-carageenan and locust bean gum is most often included (Krasaekoopt et al., 2003). The benefit of the emulsion method is that it is easy for large scale production and can produce smaller size beads than extrusion (Krasaekoopt et al., 2003). However, this technique is fairly new and can result in higher costs than extrusion due to the need for vegetable oil (Krasaekoopt et al., 2003).

2.3.4 Spray drying

The third common method for microencapsulation is spray drying. It is economical and flexible, and produces good quality products (Kailasapathy, 2002). The process is done by dissolving the encapsulated substances in a dispersion of a polymer solution to form an emulsion or dispersion (de Vos et al., 2010). This is followed by atomizing in heated air for fast removal of the solution that contains active ingredients (Kailasapathy, 2002). The advantages of this process are that it can be operated on a continuous basis, has a low cost, and is

easy to operate (de Vos et al., 2010; Kailasapathy, 2002). However, the disadvantage is that the high temperature used during processing may not be compatible with the survival of all types of probiotics. The limited applications for these techniques imply that some bioactive components maybe exposed (de Vos et al., 2010; Kailasapathy, 2002). Nevertheless, it is still a valuable process as it is cost-effective, efficient and uses equipment that is available in the food industry (Kailasapathy, 2002). Furthermore, Gardiner et al. (2002) showed that when *L. paracasei* culture was spray-dried with skim milk, it remained stable in Cheddar cheese during storage for at least 7 weeks.

2.3.5 Microencapsulated probiotics

Microencapsulation can protect the viability of probiotics during processing and storage, but the release of probiotics into the GI tract must also be considered if they are to provide benefits. In the upper intestinal tract the pressure is fairly low due to the large amount of fluid in the stomach and small intestine (de Vos et al., 2010). Microencapsulates used for probiotics can withstand this level of pressure, although they will break when the pressure in the lumen increases thereby releasing the bioactive compounds in the lower gastrointestinal tract (de Vos et al., 2010). Another approach for releasing bioactive compounds is based on pH (de Vos et al., 2010). By using pH sensitive polymers when encapsulating the probiotic they can remain intact in the stomach. They are, however, not resistant to digestive enzymes, so the probiotic can be released in either the small or large intestine (de Vos et al., 2010).

There are two ways of incorporating microencapsulated probiotic into cheese. They can be added with the starter culture or sprinkled on the milled curds to maximize the viability in an end product (Champagne et al., 2005). The reason for adding microencapsulated probiotics with the starter culture is to ensure the incorporation of beads into the curds. The disadvantage of this is that some of the encapsulated probiotics may be drained with the whey since the beads do not readily dissolve in the milk. This leads to the other approach, which is to sprinkle the probiotic on the milled curds so that it minimizes losses of bacterial cells to whey. However, studies have shown that the probiotic cells were not as well incorporated into the curd mass when added at this step. The recovery of probiotics in the cheese curds was almost double when the probiotics were added with the starter culture in the milk despite losses in the whey (Fortin et al., 2011). The value of microencapsulation has been established as studies have shown differences for the level of viability between non-microencapsulated probiotics (decreased to $<10^3$ cfu/g for *L.acidophilus* and bifidobacteria) and encapsulated probiotics ($>10^5$ cfu/g) (Shah, 2000).

However, microencapsulation may have adverse effect on the sensory quality of a food. Particle size usually alters the texture of foods, but when the diameters of the capsules are under 10 μm this should not affect the mouthfeel of most foods and the sizes of encapsulated probiotic are usually 1 – 4 μm (Rokka & Rantamaki, 2010). Also, the color of the capsules may affect the appearance of a food where spray-dried capsules may have color defects due to the Maillard reaction (Rokka & Rantamaki, 2010).

2.4 Texture of cheese

2.4.1 Introduction

The texture of foods, also known as the “eating quality of foods”, includes sight, touch, and sound (Gunasekaran & Ak, 2003). The International Organization for Standardization (ISO) defined texture of a food as “all the rheological and structural (geometric and surface) attributes of the product perceptible by means of mechanical, tactile, and, where appropriate, visual and auditory receptors” (Gunasekaran & Ak, 2003). Consumers expect each food to have its unique texture, so the manufacturer needs to know the texture that is being expected by the public to carefully formulate their products and also to develop quality control criteria (Gunasekaran & Ak, 2003).

Cheese is analyzed as a soft solid material composed mainly of protein, water, and lipid (Foegeding & Drake, 2007). Texture is a primary attribute for cheese (Gunasekaran & Ak, 2003). Many terms have been used to describe the texture of cheese, such as adhesiveness, brittleness, creaminess, crumbliness, chewiness, cohesiveness, crustiness, curdiness, firmness, graininess, hardness, stretchability, lumpiness, mouthfeel, rubberiness, shortness, slipperiness, smoothness, spreadability, springiness, stickiness, stiffness, and thickness (Gunasekaran & Ak, 2003). However, the attributes that are commonly measured for cheese products are firmness, rubberiness, crumbliness, graininess, and mouth-coating (Muir et al., 1997). Cheese sensory texture is done by descriptive analysis and mechanical texture based on rheological and fracture testing (Foegeding & Drake, 2007).

2.4.2 Factors affecting cheese texture

Textural properties of cheese are affected by numerous factors such as properties of milk, cheese making procedures, cheese composition and post manufacturing processes (Gunasekaran & AK, 2003). The breed of cattle, stage of lactation, milking season, and feeding all affect the composition of milk which directly alters the properties of cheese (Gunasekaran & AK, 2003). During the manufacturing of cheese, the addition of a starter culture and a coagulant, cooking temperature, and amount of whey removed affect the final texture of cheese curds (Gunasekaran & AK, 2003). Sodini et al. (2002) reported that by adding different strains of starter culture, the acidification process and the texture are different. They showed that a mixture of starter cultures, composed of *S. thermophilus* and *L. bulgaricus* permitted shorter fermentation time when compared to when single culture of *S. thermophilus*. Longer fermentation times can lead to a grainy texture. A longer fermentation time also leads to a longer milk heating time, and as a result, the pH of milk would become lower due to more acid production from the starter cultures. Again this results in undesirable texture in cheese.

Casein is the most important factor in affecting the texture of cheese where water or serum fills the matrix formed by fat globules that are trapped in the casein protein matrix (Gunasekaran & Ak, 2003). The casein network structure is affected by the moisture of the curd, the fat content, acidity, pH and the scalding temperature of the curd (Gunasekaran & Ak, 2003). For example, during the production of Emmental cheese, the curd scalding temperature needs to be high

in order to create a springy and rubbery curd (Gunasekaran & Ak, 2003). For harder cheese, the pH of milk during the addition of enzyme or the pH of curd during milling needs to be low to result in a harder cheese curd (Gunasekaran & Ak, 2003). Yates & Drake (2007) documented that fat reduced Gouda cheeses were generally characterized by decreased adhesiveness, cohesiveness and degree of breakdown compared to full-fat Goudas. Other researchers reported that an increase in fat content resulted in smoother and softer cheese, and an increase in casein content resulted in firmer cheese (Chen et al., 1979; Gunasekaran & Ak, 2003).

The texture of cheese continues to change during storage due to aging (proteolytic breakdown) and ripening (pH and temperature changes, casein hydrolyzation) (Gunasekaran & Ak, 2003). Yates & Drake (2007) reported that Goudas that had been aged more displayed higher fracturability and were firmer. The texture differences between young and aged Cheddar cheese was documented by Brown et al. (2003) and Watkinson et al. (2001) where longer aging time led to enzymatic breakdown of the casein matrix, which resulted in a crumbly, less cohesive and more fracturable texture.

2.4.3 Analytical methods for cheese texture

Texture of cheese can be measured either by subjective or instrumental methods. The subjective method requires training panellists to perform sensory evaluations. For instrumental texture analysis, there are three approaches: empirical, imitative,

and fundamental (Gunasekaran & AK, 2003). Examples of empirical methods are penetration, puncture test, and ball-compressor test (Gunasekaran & AK, 2003). The imitative method measures texture by attempting to mechanically mimic the sensory evaluation of human evaluators. Texture profile analysis (TPA) is the test most widely used in this approach (Gunasekaran & AK, 2003). For fundamental methods, data are independent of the test instrument used. Tests used are uniaxial compression, bending, and torsion tests (Gunasekaran & AK, 2003). However, fundamental instrumental techniques do not do as good a job of predicting consumer responses to food texture as imitative methods do.

2.5 Flavour analysis of cheese

2.5.1 Flavour compounds in cheese

The flavour of cheese is a key parameter for consumer acceptance and marketing, and is affected by many factors including the quality of the milk, processing parameters, lactic acid formation, proteolysis of casein, breakdown of amino acids, and lipolysis of triacylglycerols (McGorrin, 2007; Tunick, 2007). Volatile components are the major contributors to the flavour of cheese and they are also formed by lipolysis, proteolysis and metabolism of lactose, lactate and citrate (McGorrin, 2007; Van Leuven et al., 2008). There are many volatile compounds that contribute to the flavour of cheese; different varieties of cheese have different signature compounds. Studies showed that bifidobacteria produce acetic and lactic acids during fermentation that may result in a vinegar-like taste

and aroma in products (Gomes & Malcata, 1999). The important flavour components in Gouda cheese are expressed in Table 2.

Table 2. Key and other important flavour components in Gouda cheese with the flavour description (Smit et al., 2005 & Singh et al., 2003)

Probable origin	Flavour compound	Description
Amino acid	3-Methylbutanal	Dark chocolate, malt,
	3-Methylbutanol	Fresh cheese, breathtaking, alcoholic
	Methanethiol	Rotting cabbage, cheese, vegetative, sulphur
	Dimethylsulphide	
	2-Methylpropanol	Banana, malty, chocolate like
	Dimethyltrisulphide	
Sugar	Diacetyl	Buttery, strong
Fat	Butyric acid	Sweaty, butter, cheese, strong, acid
	Butanon	
	Hexanal	
	Pentanal	
Others	Ethyl butyrate	Fruity, buttery, ripe fruit
	Limonene	

2.5.2 Sensory evaluation methods

2.5.2.1Introduction

Sensory evaluation is a science that combines psychophysics, statistics, and other factors that relate to the product of interest (Young et al., 2004). It comprises several methodologies that stimulate subjects to measure their responses, analyze the data, and interpret the results with minimum bias from other factors except product variability. The definition of sensory evaluation is defined by the Sensory Evaluation Division of the Institute of Food Technologists as “scientific discipline used to evoke, measure, analyze and interpret reactions

to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing" (Stone & Sidel, 1993). Sensory evaluations are used for product development, marketing research, and also quality control.

Table 3 demonstrates a list of lexicons that have been used for cheese. Sensory languages or lexicons are sets of words used to describe the flavour (taste and aroma) of a product (Drake, 2004).

Table 3. Basic cheese flavour language (Drake, 2004)

Descriptor	Definition
Cooked/milky	Aromatics associated with cooked milk
Whey	Aromatics associated with Cheddar cheese whey
Diacetyl	Aromatic associated with diacetyl
Milkfat/lactone	Aromatics associated with milkfat
Fruity	Aromatics associated with different fruits
Sulfur	Aromatics associated with sulphurous compounds
Free fatty acid	Aromatics associated with short-chain fatty acids
Brothy	Aromatics associated with boiled meat or vegetable soup stock
Nutty	The sweet roasted aromatic associated with various nuts, wheat germ, unsalted wheat thins
Mothball/feed	Aroma associated with mothballs or protein catabolism, sometimes reminiscent of silage or grass compost
Sour	Fundamental taste sensation elicited by acids
Salty	Fundamental taste sensation elicited by salts
Sweet	Fundamental taste sensation elicited by sugars
Bitter	Fundamental taste sensation elicited by caffeine or quinine
Umami	Chemical feeling factor elicited by certain peptides and nucleotides
Vinegar	Aromatics associated with vinegar
Cheesy/butyric acid	Aromatics associated with butyric acid
Metallic	Chemical feeling factor elicited by metallic objects in the mouth

2.5.2.2Descriptive sensory analysis

Descriptive tests are categorized as analytical-laboratory tests where the results provide complete information about the differences of products. The steps required to complete these tests are recruiting panellists, developing descriptive terms, training the panellists, evaluating their performance, conducting the test, analyzing the data, interpreting results, and presenting the results (Young et al., 2004). A descriptive test involves only 10 to 20 panellists as they are all highly trained. Once a panel is formed then the sensory language used needs to be developed by consensus of the panel. Since descriptive analysis requires training and effort, it gives the most precise analytical results by human subjects. There are various descriptive methods that have different advantages and disadvantages. The different methods include: Flavour Profile, Quantitative Descriptive Analysis, Texture Profile, Spectrum Method, Free Choice Profiling, and Flash Profiling. All these tests give sophisticated and scientific perceptions of the product that were tested. Van Leuven et al. (2008) and Yates & Drake (2007) performed descriptive sensory analysis on Gouda cheese for flavour and texture attributes.

2.5.2.3Affective test

Affective tests focus on the response of the consumer and the results represent a market response used in product development and quality control of foods. This method refers to acceptance, preference or consumer testing and it is usually

measured by a paired-comparison or nine-point hedonic scale. However, the number of panellists should be greater than 50 in order to represent the population. This type of test is very cost-effective and it gives results that show the potential success of a product. The main objective of this test is to determine consumer likes and dislikes. Yates & Drake (2007) performed consumer tests on Gouda cheese for texture attributes.

3. PRELIMINARY EXPERIMENT

3.1 Introduction

The preliminary experiment was designed to have an adequate amount of data for two years' experiments. However, the results of the preliminary experiment were questionable due to some of the choices made in developing the experiment. Therefore, a main experiment was designed to support the preliminary experiment and also to prove the validity of the result. In addition to the uncertainty of the preliminary experiment result, the result from the main experiment could not be combined with the result from the preliminary experiment due to differences during processing and data collection.

3.2 Materials

Most starter cultures that are in use today originate from lactic acid bacteria and generally belong to the genera *Lactococcus*, *Streptococcus*, *Leuconostoc* and *Lactobacillus* (Ouwehand et al., 2010). The starter culture added for Gouda cheese was CHOOZIT MA 4001 LYO 5 DCU (Danisco Culture Unit) obtained from Danisco (Scarborough, ON). This culture is composed of *Lactococcus lactis*, *Lactococcus cremoris*, *Lactococcus lactis* subsp. *diacetylactis*, and *Streptococcus thermophilus*. The probiotic cheeses were made by incorporating non-microencapsulated and microencapsulated *Lactobacillus helveticus* and

Bifidobacterium longum provided by Institut Rosell-Lallemand (Montreal, QC). The milk used to produce cheese was purchased from Dairy Farmers of Manitoba and was produced locally (near Winnipeg, Manitoba). A liquid rennet (microbial vegetarian rennet – Marzyme D.S., Glengarry Cheesemaking and Dairy Supply Ltd., Lancaster, ON., Canada) of microbial origin was used to coagulate. All chemicals were obtained from Sigma-Aldrich Chemical Co. (Milwaukee, Wis., U.S.A.) and Fisher Scientific Co. (Fair Lawn, N.J., U.S.A.) and were of analytical grade.

Three treatments were included in this experiment. They were control (no addition of probiotics), addition of non-microencapsulated probiotic cultures and addition of microencapsulated probiotic cultures. For each treatment, two batches of Gouda cheese were produced resulting in 6 batches in total. All Gouda cheeses were made at the Dairy Facility in the Department of Food Science.

3.3 Methods

3.3.1 Production of Gouda cheese

Cheese making was carried out on a pilot plant scale, adapting a method from the University of Guelph (2011) with modifications for Gouda cheese. Each batch of cheese was made a week apart. Raw milk was received and pasteurized at 76°C for 20 sec. The temperature and time of the pasteurizer was set to meet the standards for Canadian Food Inspection Agency (CFIA). Raw milk composition

can be found in Appendix A. Ten DCU of starter culture (*Lactococcus lactis*, *Lactococcus cremoris*, *Lactococcus lactis* subsp. *diacetylactis*, *S. thermophilus*) were added to a small amount of milk to dissolve and then added to the vat (200L cheese vat) when milk reached 31 – 32°C. Rennet was added at a concentration of 11 mL per 100 L of milk (rennet was diluted in 1 L of cold water prior to adding) while stirring the milk. The milk was then allowed to ripen for 35 – 40 min. When curds cut cleanly, the curds were cut into cubes with vertical and horizontal knives (stainless-steel box framed with stainless-steel wires horizontally and vertically). The curds healed for 5 – 10 min without agitation and were then stirred for 20 – 30 min with slow agitation. One-third of the whey was drained off and water (at 60 – 71°C) representing 25% of the whey removed was added to the vat. The final temperature was between 37 and 39°C. Curds were stirred for 30 – 45 min until the curds were firm and the whey was drained. Curds were filled into 2.5 – 3 kg plastic hoops and pressed for 2 h with occasional turning. The pressure was gradually increased over time until 30 psi was reached. Cheese blocks were removed from the hoops and stored in 30% (w/v) brine solution for 2 days at 12°C. Cheese blocks were removed from brine and air dried for 2 days at 12°C. Cheese blocks were washed with sanitizer (XY-12 liquid sodium hypochlorite, EcoLab, Mississauga, ON) before cutting and packaging. Blocks were vacuum packed and incubated at 12°C for 3 and 4 months. The packaging material was moderate barrier polyamide/polyethylene pouches (stock vacuum pouch, WinPak, Senoia, GA., U.S.A.). Oxygen and moisture permeabilities of the bags were not measured.

For probiotic cheese batches, 5 g of non-microencapsulated probiotic powder or 30 g of microencapsulated probiotic powder were added with the starter cultures. These levels were designed to achieve 2×10^9 cfu/g in cheese.

3.3.2 Chemical analysis

All chemical analyses (moisture, salt, and fat content) for Gouda cheese were done at 3 and 4 months of maturation.

3.3.2.1Moisture content

Cheese moisture content was assessed using the AOAC Moisture Method (926.08 and 948.12) with modifications (AOAC, 2002). This method involved drying 2 g of grated cheese (10 g of cheese from the core of the cheese were transferred into a grater with a knife) in pre-dried aluminum weighing dishes (Fisher Scientific Co., Fair Lawn, N.J., U.S.A.) at 100°C for 16 to 18 h in a preheated air oven (Blue M Electric Co., Blue Island, IL., U.S.A.). Samples were weighed and weights were recorded. Triplicate samples were analyzed for each batch of cheese. Moisture content was calculated using the following formula.

$$\%\ \text{Moisture} = \left(1 - \frac{\text{dry weight}}{\text{wet weight}}\right) \times 100$$

3.3.2.2Salt content

Cheese salt content was assessed using the method adapted from Marshall (1992). Two grams of grated cheese samples were weighed into a 250 mL flask then 100 mL of distilled water was added and brought to a boil. Samples were cooled to room temperature and 6 drops of 25% potassium chromate (Sigma-Aldrich Chemical Co.) were added as indicator. Samples were titrated with 0.171 N silver nitrate (Sigma-Aldrich Chemical Co.) to a very faint orange colour. Duplicate samples were done on each batch of cheese. Salt concentration was calculated using the following formula.

$$\text{Salt (sodium chloride)}\% = \frac{\text{mL } AgNO_3 \times \text{N } AgNO_3 \times 0.0585 \times 100}{\text{weight of sample}}$$

3.3.2.3Fat content

The method used for fat analysis was the Babcock test adapted from Marshall (1992). Nine grams of each grated cheese sample were weighed into 50% Paley cheese bottles (Kimble Kimax brand Babcock bottle for cheese testing, Fisher Scientific Co.). Ten mL of water at 55 – 60°C were added and then the stopper was inserted. Sulphuric acid (sulphuric acid for Babcock tests, Fisher Scientific Co.) (17.5mL of 0.01 N) was added 3 consecutive times. Samples were mixed on a shaker (New Brunswick Scientific Co., New Brunswick, N.J., U.S.A.) with a speed set at 6 for 5 min until a chocolate brown color was reached. Samples were then centrifuged (The Jalco Motor Co., Union City, Ind., U.S.A.) for 5 min at

759 rpm, and water at 55 – 60°C was added until the water reached the base of the reading tube. The sample was centrifuged for 2 min. Additional hot water was then added until the fat column was within the graduated portion of the reading tube and centrifuged for 1 additional min. The bottles were placed in a water bath at 55 – 60°C (Blue M Electric Co., Blue Island, IL., U.S.A.) for 5 min. Two to 3 drops of glymol were added and the height of the fat column was read using callipers. Duplicate samples were done on each batch of samples.

3.3.3 Microbial analysis

3.3.3.1Enumeration of starter cultures

Diluents of peptone were prepared by dissolving 0.1% of trypticase peptone (Becton, Dickinson and Company, Sparks, MD, U.S.A.) in distilled water and autoclaving 90 mL and 9 mL portions at 121°C for 15 min. For each cheese batch, 10 g of cheese from the core of the cheese were transferred into a sterile stomacher bag (sterile filtra bag, Fisher Scientific Co.) using sterilized utensils under aseptic conditions and homogenized in 90 mL of sterile peptone solution for 1 – 2 min using a stomacher (Lab-Blender 400, Intersciences Inc., Markham, ON. Canada). Serial dilutions from 10^{-2} to 10^{-7} were prepared by successively adding 1 mL of the diluted stomached samples into glass tubes containing 9 mL 0.1% sterile peptone water. Enumeration was carried out using an Autoplate 4000 Spiral Plater (Spiral Biotech, Bethesda, MD. U.S.A.). Enumerations of starter cultures were done at 1 week, and after 3 and 4 months of maturation. *L.*

lactis subsp*. lactis*, *L. lactis* subsp. *cremoris*, and *L. lactis* subsp. *lactis* biovar. *diacetylactis* were counted in M17 (Oxoid) and the agar used to enumerate *S. thermophilus* was *Streptococcus thermophilus* agar (Dave & Shah, 1996). Duplicate plates were incubated aerobically at 37°C for 48 h using a Mechanical Convection Incubator (GCA Co., Chicago, IL., U.S.A.). Plates containing 25 to 250 colonies were enumerated and recorded as colony-forming units per gram of culture.

3.3.3.2Enumeration of non-encapsulated and encapsulated probiotic cultures

Enumeration of probiotic cultures were done after 3 and 4 months of maturation. The method by Ahmarani (2010) was used. Diluents of phosphate buffer were prepared by dissolving 0.1% soy peptone (Oxoid), 0.121% potassium phosphate dibasic (Fisher Scientific Co.), and 0.034% potassium phosphate monobasic (Fisher Scientific Co.) in distilled water. Portions of 99mL and 9mL were sterilized for 20 min at 121°C. One gram of cheese from the core of the cheese block was transferred to a sterile mason jar (using sterile utensils) containing 99 mL of phosphate buffer under aseptic conditions and homogenized for 1 min with a 15 sec break between each 30 sec homogenization step using a blender (Osterizer Galaxie, Sunbeam Co., Boca Raton, FL., U.S.A.). The homogenized samples were incubated at 37°C for 15 min in a Mechanical Convection Incubator (GCA Co.) prior to serial dilution. Serial dilutions were prepared by adding 1 mL of

homogenized sample into glass tubes containing 9 mL sterile phosphate buffer until a 10^{-9} dilution was obtained. Enumeration was carried out by pour plating 1 mL of each dilution into petri-dishes (Fisher Scientific Co.) then liquid agar was added and allowed to solidify. The agar used for enumeration of *L. helveticus* was MRS agar (de Man, Rogosa, Sharpe Agar, Oxoid) and the agar used for enumerating *B. longum* was RCA agar (Reinforced Clostridial Agar, Oxoid). Triplicate plates were incubated anaerobically in Oxoid jars (Oxoid) with gas generating packages (Pack-CO_2, Mitsubishi Gas Chemical Company Inc., N.Y., N.Y., U.S.A.) and dry anaerobic indicator strips (Becton, Dickinson and Company, Sparks, MD., U.S.A.) at 37°C for 48 h. Only plates containing between 25 and 250 colonies were counted and recorded as colony-forming units per gram of culture.

3.3.4 Texture analysis

Texture analysis was done with a Zwick/Roell texture analyzer (Zwick/Roell, Kennesaw, GA., U.S.A.) using a Warner-Bratzler blade (BBL-TOFOWBT 002, Zwick/Roell, Kennesaw, GA., U.S.A.) in compression mode. Cheese slabs were cut into 15 cm width and laid across the platform underneath the centre of the blade. Test conditions for compression included a preload of 1kN, and a pre-load speed of 1 mm/min. Force was zeroed after pre-load, cycle speed was controlled at 14 mm/min, with standard travel set to 19 mm. The upper force limit was set at 1000 N and maximum test duration was 2 min. The resulting curve was

evaluated using testXpert II v1.41 software to measure the maximum force (N). Triplicate slabs were evaluated from one block of cheese from each batch.

3.3.5 Sensory analysis

For the sensory analysis, a discriminatory test with a semi-trained panel was used. Further training was not possible due to the short time frame between receiving ethics approval and the 3-month storage time. As a result, attributes were assigned and panellists trained to identify these attributes. The attributes tested for the sensory evaluations were saltiness, bitterness, high acid, flat/lack of flavour, and texture (rubbery/crumbliness). A consent form was made for the signature of the panellists prior to the sensory panels to ensure they understood the purpose of the study and the confidentiality of their information. Examples of the sensory ballots as well as ethics approval forms may be found in Appendices B, C, & D. Thirty panellists were recruited; the results of the 26 which were completed were analyzed. There were 2 training session prior to the sensory panels. They were trained by using store-bought samples and after the training sessions it was assumed that they understood each attribute that was being tested during the sensory panel. Each panellist completed a questionnaire (Appendix C) prior to the training session to provide information on their cheese consumption frequency and cheese type preference. For each sensory panel, instructions were provided with the ballots for the panellists to complete the sensory panels properly. There were 3 samples (one without probiotic, one with

non-microencapsulated probiotic, and one with microencapsulated probiotic) and 1 standard (store bought Gouda cheese) presented at each session. The assessors were presented cubes of coded (3 digit random number) cheese samples, equilibrated to room temperature. They assessed the intensity of the 5 attributes using a 9-point hedonic scale (1 – dislike extremely to 9 – like extremely). When all sessions were completed, each panellist got a $20 giftcard from the University of Manitoba bookstore. For data analysis, mean scores for each attribute were calculated. Individual responses were destroyed upon completion of the analyses due to the rules of Research Ethics Boards of the University of Manitoba.

3.3.6 Statistical analysis

Cheeses were evaluated for significant difference at $p<0.05$ and $p<0.1$ (only for microbial analysis) using ANOVA with Statistical Analysis Software (SAS, version 9.1) and differences located using a Tukey test. The results were recorded as an average with standard deviation (S.D).

3.4 Results and discussion

3.4.1 Chemical analyses

All significant ($p<0.05$) main effects and interactions between effects on the characteristics of treatment and time are summarized in Table 4. Statistical analyses confirmed that the interaction between month and treatment was significant for salt content (Table 4). However, only cheese treatments had significant differences for moisture and fat content.

Table 4. The p-value[1] of the main factors and their interactions on treatments and time

Factor	Moisture	Salt	Fat
Month	0.8935	0.0096	0.1773
Treatment	<0.0001	0.0275	0.0277
Month*Treatment	0.7728	0.0453	0.3086

[1] p-value < 0.05 is significantly different

Table 5 shows that the control cheeses at 3 months of aging were less salty than rest of the batches. In addition, moisture content for the control treatment was lower than the both probiotic treatments (Table 6). Furthermore, the fat content had an inverse relationship when compared to moisture content where lower moisture in the control treatment (26.0%) resulted in a higher fat content (31.1%) (Table 6). Gomes et al (1998) reported similar results in which reduced moisture content was associated with increased fat content. For microencapsulated treatments, the salt, moisture, and fat contents were similar to non-microencapsulated treatments. Differences in chemical components may be due to variation during processing of cheese as much as the effect of adding of probiotic cultures. For example, McBrearty et al. (2001) reported that the addition

of *B. longum* in Cheddar cheese had no adverse effect on the cheese composition. During processing, efforts were made to keep processing variation to a minimal. However, the temperature of the room, the speed of firming of curds, and volume of milk used varied from batch to batch. Wang et al (2011) reported higher moisture (44 – 45%) and similar fat contents (31 – 33%) for their Gouda cheese. However, their method of manufacturing Gouda cheese was different from the one used in this study and these differences could alter the cheese composition.

Table 5. Effect of treatment and time on the salt content (%) of the cheese (n=4)

Month	Treatment	Mean ± S.D.
3	Control	$3.49^{b} \pm 0.39$
3	NME	$4.33^{a} \pm 0.35$
3	ME	$4.21^{a} \pm 0.38$
4	Control	$4.36^{a} \pm 0.25$
4	NME	$4.41^{a} \pm 0.29$
4	ME	$4.38^{a} \pm 0.20$

Different letters within a column indicates significant difference ($p<0.05$)
NME – non-microencapsulated
ME – microencapsulated

Table 6. Chemical analysis of different treatments

	Control	NME	ME
Moisture (%) (n=6)	$26.00^{b} \pm 2.38$	$29.56^{a} \pm 1.17$	$29.67^{a} \pm 1.56$
Fat (%) (n=4)	$31.12^{a} \pm 1.60$	$29.31^{a} \pm 2.30$	$28.75^{b} \pm 1.07$

Different letters within a row indicates significant difference ($p<0.05$)
NME – non-microencapsulated
ME – microencapsulated

The effect of storage time on the chemical constituents of cheese samples as a function of time is shown in Table 7 and, as was indicated by the analysis of variance, fat and moisture contents were unaffected by storage time. The effect of storage time on salt concentration (Table 5) showed an increase in the amount of salt in the control between 3 and 4 months. Gomes et al. (1998) reported that salt from the outer layer diffuses towards the inner layers of the cheese, eventually reaching equilibrium throughout the whole cheese by the 9th week of ripening. The fact that the only difference for salt content was for the control may be an indication that salt migration differs in the presence of probiotics. Overall, when comparing the results of Gomes et al. (1998) to ours, our moisture was lower, salt content was higher and fat contents were similar. According to the Dairy Products Regulations (Department of Justice, 2011), the maximum percentage of moisture and minimum percentage of fat are 43% and 26%, respectively and as a result, all treatments were within the regulation.

Table 7. Chemical changes during aging

	3 month	4 month
Moisture (%) (n=6)	28.4 ^{a} ± 2.7	28.5 ^{a} ± 2.2
Fat (%) (n=4)	30.2 ^{a} ± 2.3	29.3 ^{a} ± 1.5

Different letters within a row indicates significant difference ($p<0.05$)

It is also important to be aware of the salt level as it has been suggested that there is a positive relationship between the death rate of microorganisms and salt concentration (Gomes et al., 1998). Montoya et al. (2009) reported that probiotic bacteria are unable to survive salt concentrations greater than 3.5%. The salt concentrations in our cheese were all above 3.5% (Table 5). Therefore, in future

experiments, the salt concentration needs to be reduced to give probiotic cultures a better chance of survival. Moreover, it is important to analyze the survival rate of starter and probiotic cultures to confirm this hypothesis.

3.4.2 Microbial analyses

All significant ($p<0.05$) main effects and interactions between treatments and time are summarized in Table 8. There was an interaction effect for *L. helveticus*, but not for *Lactococcus* strains, *S. thermophilus* and *B. longum*, where there were no significant differences due to the treatment or storage time. Therefore, the results of *L. helveticus* have been presented as interaction effect while only main effects have been reported for the others. The survival of the *Lactococcus* strains in the cheeses appeared to have an increasing tendency with the addition of probiotic cultures (Figure 1). This may due to the competition for survival between starter cultures and probiotic cultures causing the starter cultures to grow better and compete with the probiotic cultures. The lack of significant difference for these values suggests that this trend needs to be further investigated.

Table 8. The p-value[1] of main factors and their interactions from microbial analysis on treatment and time

Factor	*Lactococcus*	*S. thermophilus*	*L. helveticus*	*B. longum*
Treatment	0.7341	0.5023	< 0.0001	0.1662
Month	0.2516	0.5375	< 0.0001	0.4770
Treatment*Month	0.6364	0.3113	< 0.0001	0.6066

[1] p-value < 0.05 is significantly different

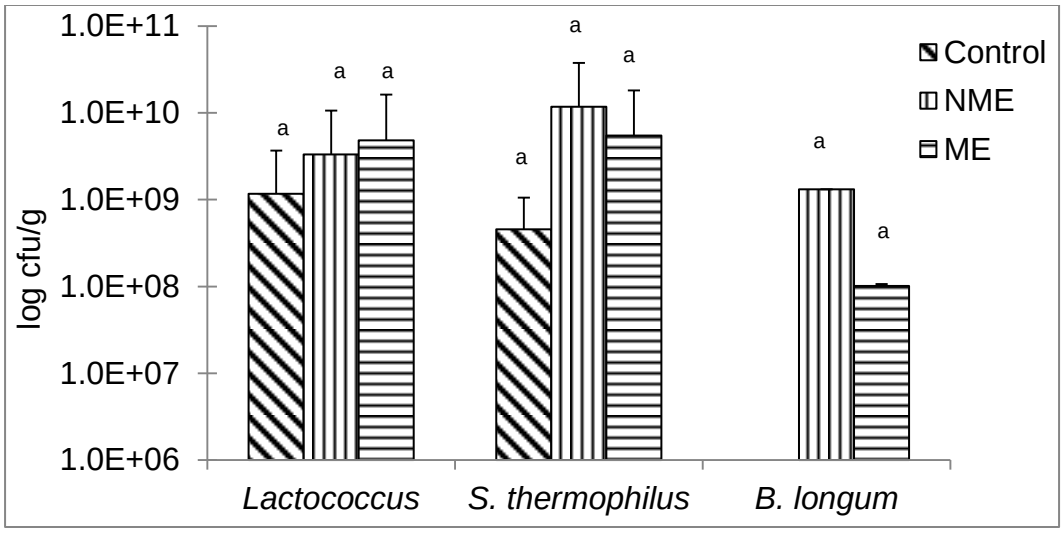


Figure 1. Changes in microbial numbers in treated cheeses (n=4 – *Lactococcus* & *S. thermophilus*; n=6 – *B. longum*). Different letters within each organism indicates significant difference ($p<0.05$)

With respect to non-microencapsulated and microencapsulated treatment in that the number of microencapsulated cultures resulted to be lower than the non-microencapsulated cultures (Figures 1 & 2). This may due to enumeration technique where not all capsules were broken for microbial to be enumerated accurately and it may also be caused by the competition with the starter cultures. Again this requires further investigation.

Levels of *Lactococcus*, *S. thermophilus* and *B. longum* at different storage times are shown in Figure 2. Starter cultures were enumerated at 1 week, 3 months, and 4 months, whereas *B. longum* was only enumerated at 3 and 4 months. While differences were not significant, the survival of *Lactococcus* increased initially, then dropped between 3 and 4 months of maturation (Figure 2). This may indicate that the bacteria did not adapt to the environment, and therefore the level of bacteria dropped after a period of time. In contrast, the number of the

Streptococcus thermophilus starter culture steadily increased during the 4th month of maturation. This suggests this organism is a stronger competitor in the presence of the probiotic cultures; clearly *S. thermophilus* adapted to the growth environment (5.64×10^9 cfu/g and 1.14×10^{10} cfu/g for 3 and 4 month, respectively). The viability of the *B. longum* was similar to the results reported by Phillips et al. (2006) where *Bifidobacterium* sp. was able to survive at 10^8 cfu/g after 12 weeks in Cheddar cheese.

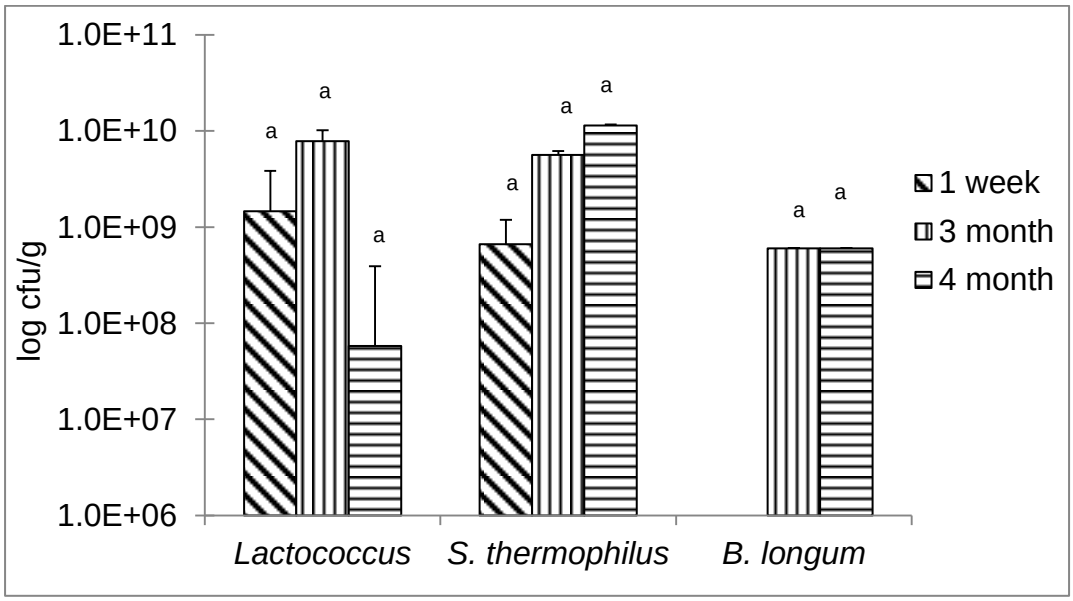


Figure 2. Changes in microbial numbers during aging (n=4 – *Lactococcus* and *S. thermophilus*, n=6 – *B. longum*). Different letters above columns within each organism indicates significant difference ($p<0.1$)

Pavunc et al. (2011) reported that the loss of microencapsulated cells of *L. helveticus* in yogurt was slower than the decline rate of free cells of *L. helveticus* where microencapsulated cells decreased by only 1 log and the free cells decreased by 2 log after 28 days of storage. However, the viability of microencapsulated cells of *L. helveticus* in our experiment tended to be lower

than the free cells of *L. helveticus* (4.34×10^7 cfu/g and 5.84×10^8 cfu/g, respectively) (Figure 3). The lower number of microencapsulated cells detected may due to poor release of the probiotic cells from the capsules. Therefore, additional experimentation to examine the viability of probiotic cultures is required. However, if we assume consumption of a nominal one serving (30g) of cheese per day, the intake of *L. helveticus* and *B. longum* would be between 10^8 and 10^9 per day with the cheese that has been aged for 4 months and that is well above the levels of 10^7/g suggested as providing therapeutic benefits. While there were no significant differences between the two probiotic treatments at 3 and 4 months of aging in this study, increased sample size in future experiments may be necessary to see if any statistical difference exists due to treatments.

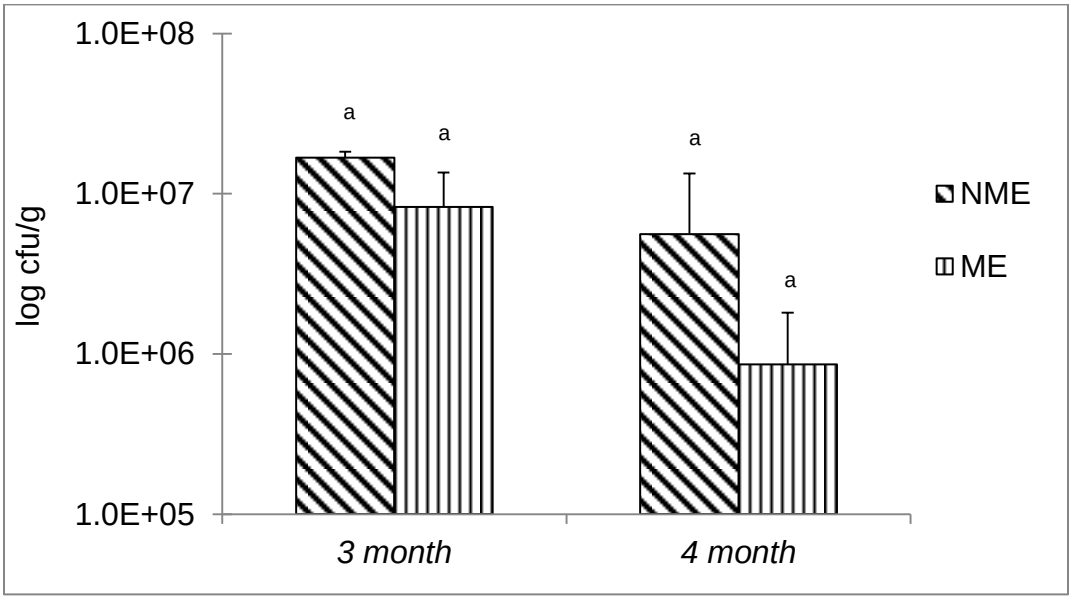


Figure 3. Changes in microbial numbers for *L. helveticus* using MRS agar in treated cheese (n=2). Different letters within each organism indicates significant difference ($p<0.05$).

As was the case with *B. longum*, the levels of *L. helveticus* were not significantly different after 3 and 4 months of aging (Figure 3). Results previously reported on *L. helveticus* strains used in dairy products have varied with the type of dairy products and strains used. Wang et al. (2011) reported that there was a decrease in cell numbers (approximately 2 log reductions) of *L. helveticus* in Gouda cheese over a 6-week maturation period. It is difficult to compare the rate of decline when we do not have week 1 data. The need for these initial values in further work is clear.

Research has shown that probiotic cultures do not survive well with salt content that is greater than 3.5% (Montoya et al., 2009). However, our probiotic cultures survived a salt content of 4% which shows that the strains selected have good level of salt tolerance. No protective effect due to microencapsulation could be seen in this experiment.

3.4.3 Texture analysis

All significant ($p<0.05$) main effects and interactions between treatment and time on cheese for texture analysis are summarized in Table 9. The table showed that there was only a treatment effect on cheese texture.

Table 9. The p-value[1] of main factors and their interactions on texture analysis of cheese.

Factor	Texture
Month	0.1709
Treatment	0.0041
Month*Treatment	0.8059

[1] p-value < 0.05 is significantly different

The maximum forces to cut through the probiotic and control cheeses are shown in Tables 10 and 11. The control treatment (34.50 N) required significantly more force when compared to probiotic treatments (23.26 N and 21.71 N for non-microencapsulated and microencapsulated treatments, respectively). This may be due to the fact that the control sample had lower moisture compared to the probiotic treated cheeses and generally lower moisture content foods tend to be harder than higher moisture foods and require more force to compress. However, large standard deviations were noted for these data. Therefore, the number of samples for evaluating texture needs to be increased for subsequent experiments. When comparing these results to the literature, Bertola et al. (2000) reported Gouda cheese matured at 10°C for 70 days (> 2 month), required breaking force of 37 N which is similar to our control result. Therefore, we could conclude that probiotic cultures have an effect on texture of cheese, possibly

related to moisture level within the cheese. Sensory evaluation is required to see if this difference is detectable by consumers.

As noted in Table 9, storage time had no effect on cheese texture. The actual values are reported in Table 11. Again, the large standard deviations are a concern and further experimentation is needed.

Table 10. Changes in cheese texture with different treatments (n=6)

	Control	NME	ME
Texture (N)	34.50 [a] ± 12.79	23.26 [b] ± 8.46	21.71 [b] ± 3.00

Different letters within a row indicates significant difference ($p<0.05$)

Table 11. Changes in cheese texture during aging (n=6)

	3 month	4 month
Texture (N)	28.69 [a] ± 10.72	24.29 [a] ± 10.07

Different letters within a row indicates significant difference ($p<0.05$)

3.4.4 Sensory analysis

Sensory attributes evaluated were flavour and texture. The results for 3 and 4 month old cheese are given in Tables 12 and 13. Generally, the addition of probiotic bacteria should not have an adverse effect on the taste and aroma of a product (Ross et al., 2000). The results showed there were no significant differences between treatments and aging time in terms of texture and flavour. This was comparable to the results obtained by Zomorodi et al. (2011) who reported Iranian white cheese that contained high levels of probiotic bacteria (either free or microencapsulated) showed no adverse effect on sensory criteria. This indicates that the panellists did not pick up the difference in salt levels that

was observed with the chemical analysis. Moreover, the texture differences between probiotic and control cheese that was seen by the texture analyzer were also not noticed by the panellists. This means that with the addition of probiotic cultures did not alter the taste and texture of the cheese from a consumer's perspective which is the goal of product development. In addition, there were positive comments with respect to flavour and texture for the cheeses which indicated panellists would consider purchasing them if the cheeses were available in the market. However in this study, panellists had only limited training and may need more training to pick up any differences. Also the number of panellists was too low for an untrained evaluation.

Table 12. Sensory evaluation of treatments (n=26 panellists)

	Control	NME	ME
Salty	5.45 [a] ± 0.74	5.68 [a] ± 0.71	5.12 [a] ± 0.99
Bitter	4.58 [a] ± 0.39	4.46 [a] ± 0.38	4.43 [a] ± 0.37
Acid	5.02 [a] ± 0.94	5.28 [a] ± 0.87	5.10 [a] ± 0.86
Flavour	5.97 [a] ± 0.80	5.56 [a] ± 0.95	6.25 [a] ± 0.23
Texture	5.33 [a] ± 1.18	5.28 [a] ± 1.15	6.17 [a] ± 0.32

Different letters within a row indicates significant difference ($p<0.05$)

Table 13. Changes in sensory scores during aging (n=26 panellists)

	3 month	4 month
Salty	5.37 [a] ± 0.93	5.46 [a] ± 0.68
Bitter	4.42 [a] ± 0.32	4.56 [a] ± 0.40
Acid	5.26 [a] ± 0.85	5.00 [a] ± 0.84
Flavour	5.79 [a] ± 0.81	6.07 [a] ± 0.67
Texture	5.68 [a] ± 1.00	5.50 [a] ± 1.03

Different letters within a row indicates significant difference ($p<0.05$)

Overall, this preliminary work indicated that introducing probiotics in Gouda cheese does not alter the flavour and texture of cheese, but advantages associated with using encapsulated probiotic were not evident.

4. MAIN EXPERIMENT

4.1 Introduction

The main experiment was designed to repeat the preliminary experiment. However, some modifications were made in the main experiment to have better control on the process and salt level to prevent death of probiotic cultures. In addition, the level of probiotic cultures added was increased to result in a higher level of probiotics in the end product. All tests done on the probiotic cheese were completed at week 1, and 3 and 4 months of aging. Moreover, techniques for enumerating probiotics were improved by using more agars to confirm the level of viable probiotic cells. For sensory evaluation, a consumer panel was added while a trained panel was also used to obtain more data to understand the change of flavour and texture attributes of probiotic cheese.

4.2 Materials

Raw milk was collected at the Glenlea Research Centre and then transported back to the Fort Gary campus. The compostion of milk can be found in Appendix E. The starter culture (CHOOZIT MA 4001 LYO 25 DCU) was supplied by Danisco (Scarborough, ON). The culture was composed of *Lactococcus lactis*, *Lactococcus cremoris*, *Lactococcus lactis* subsp. *diacetylactis*, and *Streptococcus thermophilus*. The probiotic cheeses were made by incorporating non-microencapsulated and microencapsulated *Lactobacillus helveticus* and

Bifidobacterium longum provided by Institut Rosell-Lallemand (Montreal, QC). Rennet was supplied by Glengary Cheesemaking and Dairy Supply Ltd.

Three treatments were also included in this experiment. They were control (no probiotic cultures added), with the addition of non-microencapsulated probiotic cultures and addition of microencapsulated probiotic cultures. For each treatment two batches were produced, resulting in 6 batches in total.

4.3 Methods

4.3.1 Production of Gouda cheese

The procedure used was the same as in the preliminary experiment. However, there were minor changes such as: the amount of starter culture added was increased to 25 DCU, the amount of rennet added was 10 mL per 100 L of milk, and cheese blocks were stored in 20% (w/v) brine solution. Moreover, cheese blocks were incubated at 10°C.

For cheese batches with non-microencapsulated probiotics, 7.14 g and 5.47 g of *L. helveticus* and *B. longum*, respectively were added. For the microencapsulated probiotic, 14.7 g and 38.6 g of *L. helveticus* and *B. longum*, respectively were added with the starter culture. These levels were designed to achieve 2.0×10^{9} and 2.0×10^{8} cfu/g for non-microencapsulated and microencapsulated probiotic counts in cheese, respectively.

During the preliminary production of microencapsulated probiotic cheese, we noticed that some portion of the probiotic powder was being removed with the

whey since the powder does not dissolve in liquid. Therefore, the level of microencapsulated probiotic may be lower than the non-microencapsulated batches. Despite attempts to standardize the processing of the cheese, there are some processing variations between batches; these included coagulation time and titratable acidity of milk and whey. Therefore, all the processing log sheets are included in the Appendix (Appendix F).

4.3.2 Chemical analyses

4.3.2.1Moisture content

Moisture content was monitored for Gouda cheese at 1 week, and 3 and 4 months of maturation. Composite cheese moisture content was assessed using the AOAC Moisture Method (926.08 and 948.12) with modifications (AOAC, 2002). Samples of cheese were taken by cutting 2.5 cm from the edge of each side and grating the middle portion of cheese. This method involved drying 2 g of grated cheese in pre-dried aluminum weighing dishes (Fisher Scientific Co., Fair Lawn, N.J., U.S.A.) at 100°C for 16 to 18 h in a preheated air oven (Blue M Electric Co., Blue Island, IL., U.S.A.). Samples were weighed and weights were recorded. Triplicate samples were analyzed for each batch of cheese. Moisture content was calculated as follows

$$\%\ \text{moisture} = \left(1 - \frac{\text{dry weight}}{\text{wet weight}}\right) \times 100$$

4.3.2.2Salt content

Salt content was monitored for Gouda cheese at 1 week, and after 3 and 4 months of aging. Cheese salt content was assessed using the method adopted from Marshall (1992) with modifications. Two grams of samples were weighed into a 250 mL flask then 100 mL of distilled water was added and brought to a boil. Samples were cooled to room temperature and 6 drops of 25% potassium chromate (Sigma-Aldrich Chemical Co.) solution were added as indicator. Samples were titrated with 0.171 N silver nitrate (Sigma-Aldrich Chemical Co.) to a very faint orange colour. Triplicate samples were done on each batch of cheese. Salt concentration was calculated as follows

$$\text{Salt (sodium chloride)}\% = \frac{\text{mL } AgNO_3 \times \text{N } AgNO_3 \times 0.0585 \times 100}{\text{weight of samle}}$$

4.3.2.3Fat content

Fat content was monitored for Gouda cheese at 1 week, and after 3 and 4 months of maturation. The Babcock test from Marshall (1992) with modification was used for determination of percentage of fat in cheese. Nine grams of grated sample were weighed into 50% Paley cheese bottles (Kimble Kimax brand Babcock bottle for cheese testing, Fisher Scientific Co.). Ten mL of water at 55 – 60°C were added and then the stopper was inserted. Sulphuric acid (sulphuric acid for Babcock tests, Fisher Scientific Co.) (17.5mL of 0.01 N) was added 3 consecutive times. Samples were mixed on a shaker for 5 min until a chocolate

brown color was reached. Samples were then centrifuged for 5 min at 759 rpm, and water at 55 – 60°C was added until the water reached the base of the reading tube. The sample was centrifuged for 2 additional min, hot water was then added until the fat column was within the graduated portion of the reading tube and centrifuged for 1 additional min. The bottles were put in a water bath (Blue M Electric Co., Blue Island, IL., U.S.A.) at 55 – 60°C for 5 min. Two to 3 drops of glymol were added and the height of the fat column was read using callipers. Triplicate samples were done on each batch of sample.

4.3.3 Microbial analyses

4.3.3.1Enumeration of starter cultures

Peptone for dilution was prepared by dissolving 0.1% of trypticase peptone (Becton, Dickinson and Company, Sparks, MD, U.S.A.) in distilled water and autoclaving 90 mL and 9 mL portions at 121°C for 15 min. Ten grams of culture/sample were transferred into a sterile stomacher bag (sterile filtra bag, Fisher Scientific Co.) under aseptic conditions and homogenized in 90 mL of sterile peptone solution for 1 – 2 min using a stomacher (Lab-Blender 400, Interscience Inc., Markham, ON., Canada) to achieve 10^{-3} to 10^{-7} serial dilution. Enumeration was carried by using an Auto-plate technique (Spiral Biotech, Bethesda, MD., U.S.A.). Enumerations of starter cultures were done at 1 week, and after 3 and 4 months of maturation. The agar used for enumerating *L. lactis* subsp*. lactis*, *L. lactis* subsp. *cremoris*, and *L. lactis* subsp. *lactis* biovar.

diacetylactis was M17 (Oxoid) and the agar used for enumerating *S. thermophilus* was *Streptococcus thermophilus* agar (Dave & Shah, 1996). Triplicate plates were incubated aerobically at 37°C for 48 h. Plates containing 25 to 250 colonies were enumerated and recorded as colony-forming units per gram of culture/sample.

4.3.3.2Enumeration of non- and encapsulated probiotic cultures

Enumerations of probiotic cultures were done at 1 week, and after 3 and 4 months of maturation. The method by Ahmarani (2010) was used. Diluents of phosphate buffer was prepared by dissolving 0.1% soy peptone (Oxoid), 0.121% potassium phosphate dibasic (Fisher Scientific Co.), and 0.034% potassium phosphate monobasic (Fisher Scientific Co.) in distilled water. Portions of 99 mL and 9 mL were sterilized for 20 min at 121°C. One gram of sample was diluted in 99 mL phosphate buffer and incubated at 37°C for 15 min and then serially diluted until a 10^{-9} dilution was obtained. Enumeration was carried by using an Autoplate 4000 (Spiral Biotech, Bethesda, MD., U.S.A.) for non-microencapsulated cultures and pour plate technique for microencapsulated cultures. The agars used to enumerate *L. helveticus* were MRS agar (de Man, Rogosa, Sharpe Agar, Oxoid) and MRS agar containing 0.25 µg/mL of clindamycin hydrochloride (Sigma-Aldrich Chemical Co.)) and the agar used to enumerate *B. longum* was RCA agar (Reinforced Clostridial Agar, Oxoid) and

RAF 5.1 agar (method adapted from Ahmarani, 2010). Triplicate plates were incubated anaerobically in Oxoid jars (Oxoid) with CO_2 gas generating packages (BD GasPak EZ anaerobe container system; Becton, Dicksinson & Co., MD., U.S.A.) and anaerobic indicator (Oxoid) at 37°C for 48 h. Only plates containing between 25 and 250 colonies were counted and recorded as colony-forming units per gram of culture.

For microencapsulated probiotic cultures, one gram of culture was diluted with 99 mL of phosphate buffer in autoclaved mason jar and blended using Osterizer Galaxie 8 blender (Sunbeam Corporation Canada Ltd., Mississauga, ON.) for 1 min with a 15 sec break between each 30 sec prior to incubation at 37°C for 15 min. Otherwise the protocol was the same.

4.3.4 Texture analyses

4.3.4.1Warner-Bratzler blade test

The Warner Bratzler blade test was done with a Zwick/Roell texture analyzer using a Warner-Bratzler blade in compression mode. Cheese slabs were cut into 15 cm widths and laid across the platform underneath the centre of the blade. Test conditions for compression included a preload of 1kN, and a pre-load speed of 1 mm/min. Force was zeroed after pre-load, cycle speed was controlled at 14 mm/min, with standard travel set to 19 mm. The upper force limit was set at 1000 N and maximum test duration was 2 min. The resulting curve was evaluated using testXpert ǁ v1.41 software to measure the maximum force (N). Six slabs

from two different blocks (3 slabs per block) of cheese within one batch were tested.

4.3.4.2Penetration ball test

A penetration ball test was included to mimic the interactions between the molars and a piece of cheese. This test was done with a Zwick/Roell texture analyzer using a penetration ball in compression mode. Cheese cubes were cut into 15 cm width and laid in the middle of the platform underneath the centre of the ball. Test conditions for compression used a preload of 1kN, and a pre-load speed of 1 mm/min. Force was zeroed after pre-load, cycle speed was controlled at 1 mm/min, standard travel set to 19 mm, the upper force limit was set to 1000 N and maximum test duration was 1 min. The resulting curve was evaluated using testXpert ǁ v1.41 software to measure the maximum force (N). Six cubes from two different blocks of cheese (3 cubes from one block) from each batch were tested.

4.3.5 Sensory analyses

4.3.5.1 Discriminative test

For the sensory analysis, a discriminatory test with a trained panel was used. A consent form was made for the signature of the panellists prior to the sensory panels to ensure they understood the purpose of the study and the confidentiality of their information. Copies of the consent form and ethics approval form are included in Appendices G & H. A total of twelve panellists were recruited; but only eight panellists (three male, five female, aged between 20 and 60 years) completed the evaluation and their data were analyzed. There were seven training sessions prior to the sensory panels. They were trained by using store-bought samples, experimental cheeses and samples prepared to demonstrate specific attributes that were identified and agreed upon by the panellists. During training sessions, the panellists developed a vocabulary of four flavour attributes and three texture attributes (Table 14). Using these attributes, panellists were trained until the results were consistent. The results from the training sessions were checked by using PanelCheck V1.4.0 to monitor the progress on variability of each panellist. The results of the PanelCheck can be found in Appendix J. There were variations from panellist, but the panels had to be conducted within the short time of research. All panellists were analyzed as random effects. Random effects were obtained by presenting the samples in random order and different order for each panellist. During the sensory sessions, each panellist sat at different seats. Each panellist completed a questionnaire prior to the training session to provide information on their cheese consumption frequency and

cheese type preference. A copy of the questionnaire can be found in Appendix H. For each sensory panel, instructions were provided with the ballots for the panellists to complete the sensory panels properly (Appendix I). There were six samples (two without probiotic, two with non-microencapsulated probiotic, and two with microencapsulated probiotic) and one standard for each attribute presented at each session. The cheese samples were presented as cubes of coded (3 digit random number) cheese samples, equilibrated to room temperature. The standards were presented with letter coded dairy products (Table 15), also equilibrated to room temperature. They assessed the intensity of the 7 attributes using a 15 mm score line with standards marked on the agreed spot. Sessions were conducted at the end of 3 and 4 month maturation of cheese. After sensory analysis, the scores were converted to numerical values. For data analysis, mean scores for each attribute were calculated. Individual responses were destroyed upon completion of the analyses as of Research Ethics Board. When all sessions were completed, each panellist got $60 ($50 visa gift card and $10 University of Manitoba bookstore gift card) as compensation for volunteering for the sensory evaluation.

Table 14. Descriptive vocabulary of 7 attributes for descriptive sensory analysis of Gouda cheeses

Flavour attributes	Texture attributes
Cheese flavour	Firmness (First bite)
Buttery/Creamy	Crumbliness/Cohesiveness
Acid/Tangy	Creaminess/Smoothness of Mass
Salty	

Table 15. Standards Used for each of the attributes for descriptive sensory analysis of Gouda cheeses

Attributes	Letter code	Type of sample of as standard
Cheese flavour	C_R	Third batch of Gouda cheese
Buttery/Creamy	B_R	33% cream
Acid/Tangy	A_R	0.44% citric acid added to cream cheese
Salty	S_R	0.88% salt added to cream cheese
Firmness (First bite)	R_F	Kraft Gouda cheese
Crumbliness/Cohesiveness	R_C	Kraft Gouda cheese
Creaminess/Smoothness of Mass	R_S	Safeway Gouda cheese

4.3.5.2 Affective test

For the affective test, a preference test with a consumer panel was used. The attributes being tested for the sensory evaluations were flavour liking, texture liking, and overall liking. A consent form was made for the signature of the panellists prior to the sensory panels to ensure they understood the purpose of the study and the confidentiality of their information. The consent form and sensory ballots are included in Appendix I. One hundred and twelve untrained panellists were recruited from the University of Manitoba, primarily from the Faculty of Agricultural and Food Science; the results of one hundred and eleven (55 females and 56 males; 65 panellists in age group 18 – 25, 30 panellists in age group 25 – 40, and 16 panellists in age group 40+) which were complete were analyzed due to incomplete ballot. The panellists were presented with one square of each of the cheese tested in a random order and coded with different colored toothpicks (red for control cheese, green for non-microencalsupated probiotic cheese and blue for microencapsulated probiotic cheese), which they

were asked to assess in a random order from left to right. Panellists did not know the treatment-color combination that was assigned for their sample. Panellists were given water and unsalted soda crackers to rinse their palate between tasting of different samples. Panellists rated the samples using 9-point hedonic scales where 1 equals dislike extremely and 9 equals like extremely. Volunteers were given a small snack as compensation for their time. In addition volunteers were included a draw for one of 3 of $20 gift cards from University of Manitoba bookstore.

Only one batch from each of the treatments was selected for sensory evaluation based on the desire to see differences between addition of 2 different probiotic cultures and control cheese. From the chemical and textural results, there were no batch variations.

4.3.6 Statistical analysis

Cheese samples were evaluated for significant differences at $p<0.05$ using ANOVA with Statistical Analysis Software (SAS, version 9.1). Six samples were used for chemical, microbial, texture and sensorial analyses and the results were recorded as an average with standard deviation (S.D.). The sensorial data was normalized upon statistical analysis.

4.4 Results and discussion

4.4.1 Chemical analyses

All significant ($p<0.05$) main effects and interactions of chemical analyses are summarized in Table 16.

Table 16. Significant effects of the main factors and their interactions on chemical analysis of cheese

Factor	Fat	Moisture	Salt
Treatment	0.6698	0.1578	< 0.0001
Time	0.6491	0.0089	< 0.0001
Treatment*Time	0.3340	0.5257	0.0042

Statistical analyses confirmed that fat was unaffected by the treatment and maturation time, while moisture was affected by maturation time and salt was affected by an interaction between treatments and maturation time (Table 16). Therefore, the results of moisture and fat content were presented as main effects (Table 17 & 18). The lack of change in fat and moisture with the addition of probiotics has been reported previously. An Irish study found that when *L. paracasei* were spray-dried with skim milk, the composition of Cheddar cheese was not altered (Gardiner et al., 2002). However, there were significant effects due to maturation time for moisture content (Table 18). The moisture content of the cheeses aged for 1 week were highest, and dropped at 3 months maturation. However, the moisture content at 4 months maturation was not significantly different from the control or the 3-month samples. The fact that the 3-month sample had a lower moisture content may reflect the fact that not all blocks of cheese were pressed the same.

Table 17. Fat and moisture content of different treatments of cheese (n=18)

	Control	NME	ME
Fat (%)	29.0 [a] ± 1.5	29.0 [a] ± 1.1	29.3 [a] ± 0.6
Moisture (%)	43.8 [a] ± 2.2	43.4 [a] ± 1.3	42.8 [a] ± 1.4

Different letters within a row indicates significant difference ($p<0.05$)

Table 18. Fat and moisture content changes during aging (n=18)

	1 week	3 month	4 month
Fat (%)	29.2 [a] ± 1.2	29.3 [a] ± 1.1	28.9 [a] ± 1.2
Moisture (%)	44.2 [a] ± 1.9	42.5 [b] ± 1.3	43.4 [ab] ± 1.2

Different letters within a row indicates significant difference ($p<0.05$)

For the salt content, the statistical analysis confirmed that the interaction between treatments and maturation time was significant (Table 19). The salt content of non-microencapsulated (NME) and microencapsulated (ME) cheese at 1 week of aging were lower than the salt content of the control cheese (Table 19). However, at 3 and 4 months of aging, the salt content increased for the probiotic cheeses such that the results were the same as the control batch. This may reflect differences in salt migration from the outer layer to the centre portion where the sample for analysis was selected. Since Montoya et al. (2009) reported that probiotic strains do not survive salt content above 3.5%, the salt concentration in brine had been reduced from 30% (preliminary experiment) to 20% for this experiment. This resulted in lower salt concentrations in the cheeses where the salt content of preliminary experiment was on average above 4% and for this experiment the salt content was reduced down to an average of 2.85%. As a result, the survival rate of microbes should be higher for this experiment when compared to the preliminary experiment.

Table 19. Effect of treatment and time on the salt content of the cheese (n=6)

Time	Treatments	Mean ± S.D.
1 week	Control	3.11 [a] ± 0.99
1 week	NME	1.92 [b] ± 0.15
1 week	ME	1.44 [c] ± 0.24
3 month	Control	3.63 [a] ± 0.54
3 month	NME	3.23 [a] ± 0.45
3 month	ME	3.04 [a] ± 0.22
4 month	Control	3.17 [a] ± 0.51
4 month	NME	3.17 [a] ± 0.23
4 month	ME	2.95 [a] ± 0.20

Different letters within a column indicates significant difference ($p<0.05$)

4.4.2 Microbial analyses

All significant ($p<0.05$) main effects and interactions for microbial contents are summarized in Table 20

Table 20. Significant effects of the main factors and their interactions on microbial analysis of treated cheese and maturation

Factor	M17	ST	MRS	MRS w/ C	RCA	RAF
Trt[1]	<0.0001	0.0792	<0.0001	0.0007	0.0001	<0.0001
Time	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001
Trt*Time	0.0001	0.0481	<0.0001	0.0003	<0.0001	<0.0001

[1] Trt – Treatment

Statistical analyses confirmed that the interactions between treatments and maturation time for all the microbial analyses were significant (Table 20). Therefore, all the microbial results were expressed as interaction effects.

For the enumeration of *Lactococcus* strains (starter culture) with M17 agar, there was a significant drop of numbers of Lactococci from 1 week to 3 months of maturation (Figure 4). However, it stabilized from 3 months to 4 months of

maturation. Moreover, the numbers of lactococci were higher for the control batches at 3 and 4 months of maturation when compared to non-microencapsulated and microencapsulated batches. This may due to the competition for survival between *Lactococcus* strains and probiotic cultures to cause a higher level of lactococci in control cheeses than the probiotic cheeses.

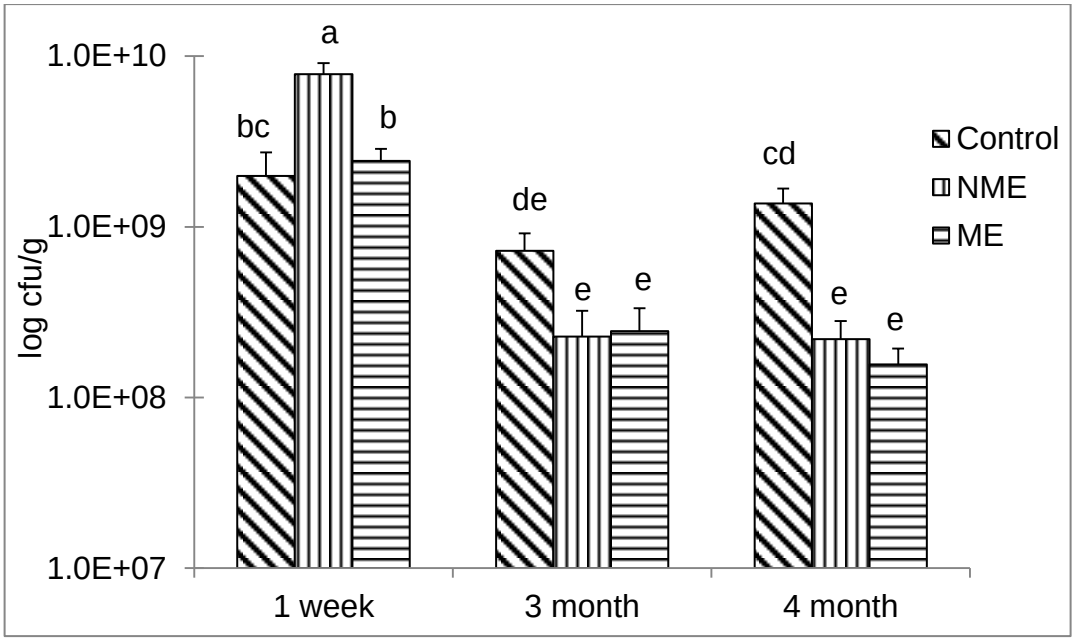


Figure 4. Changes in numbers of Lactococcus strains (starter cultures) using M17 agar in treated cheese (n=6). Different letters within the graph indicates significant difference ($p<0.05$).

When the levels of *S. thermophilus* was studied using ST agar, there was also a significant drop from 1 week to 3 months of maturation (Figure 5). However, the level also stabilized after 3 months of maturation. There were no significant differences in the level of *S. thermophilus* between treatments during 3 and 4 months of maturation. This may mean that *S. thermophilus* was able to compete

better with the probiotic cultures than *Lactococcus* strains used. Therefore, the level of *S. thermophilus* in probiotic cheeses was the same as the control.

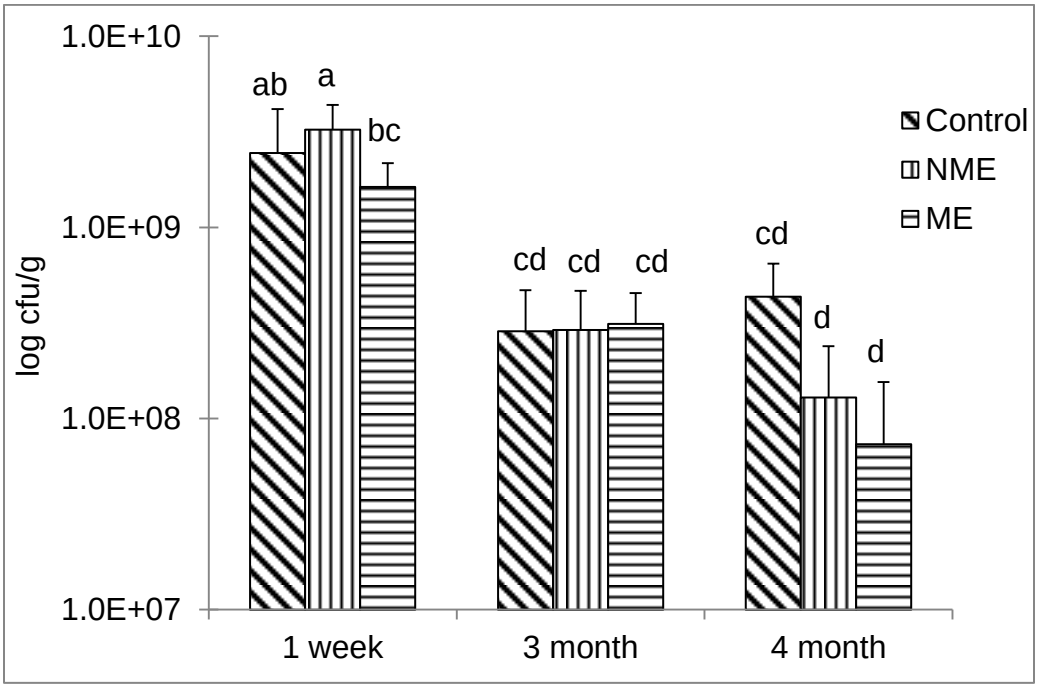


Figure 5. Changes in numbers of *S. thermophilus* (starter cultures) using ST agar in treated cheese (n=6). Different letters within a graph indicates significant difference ($p<0.05$).

For the probiotic cultures, there was a significant difference with respect to the NME and ME treatment in that the numbers of ME organisms were lower than the NME organisms (Figures 6, 7, 8 & 9). For both organisms and methods of evaluation, this difference was seen at week 1 of storage. As a result, this may be due to the removal of ME cultures with the whey during the processing of Gouda cheese. Microencapsulated probiotic powder did not dissolve well in the milk when it was added. Therefore, some of ME cultures could have been removed during whey removal and this appeared to be the case during the production of Gouda cheese. However, the level of NME cultures decreased after 3 months of aging and remained at this level after 4 months (Figures 6, 7, 8 & 9).

In the study of Pavunc et al (2011), the decline rate of microencapsulated cells of *L. helveticus* in yogurt was slower than the decline rate of free cells of *L. helveticus* after 28 days of storage. This is similar to our result except the decline in encapsulated cells was not significant. Moreover, Adhikari et al. (2000) and Sultana et al. (2000) also reported that increased viability of encapsulated bifidobacteria in yogurt was observed. However, it should be noted that there were some issues during enumeration of *B. longum* using RAF 5.1 agar such that they would not grow at 4 months of aging on some plates. Therefore, the level of *B. longum* using RAF 5.1 agar is significantly lower than the level of *B. longum* using RCA agar (Figures 8 & 9). The levels of *L. helveticus* were similar using either MRS agar or MRS with clindamycin agar (Figures 6 & 7). However, regardless of the type of probiotic culture (either free cells or encapsulated), the number of viable cells of *L. helveticus* and *B. longum* decreased by 1 – 2 logs during storage.

In this experiment, the amount of probiotic cultures (either free cells or encapsulated cultures) added was increased due to low level of probiotic bacteria in the preliminary experiment. Therefore, the initial level of both non- or microencapsulated cultures of this experiment was higher than in the preliminary experiment. Fortin et al. (2011) reported that the inoculation rate of probiotic population does not affect the rate of viability loss during storage. However, in this experiment, the final level of *L. helveticus* at 4 months of aging was higher than the preliminary experiment. On the other hand, the final level of *B. longum* was not as high as the preliminary experiment. On average NME had a level of

9.56×10^{8} cfu/g and ME had a level of 3.51×10^{8} cfu/g for the preliminary experiment, but on average NME had a level of 9.80×10^{8} cfu/g and ME had a level of 8.60×10^{7} cfu/g in the main experiment.

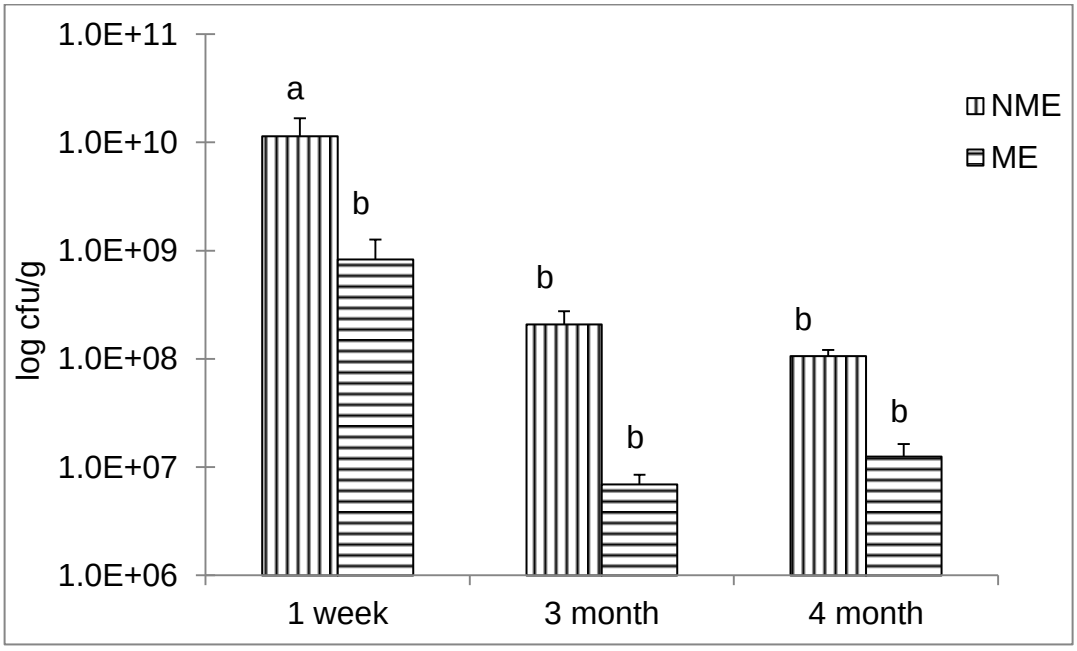


Figure 6. Changes in numbers of *L. helveticus* (probiotic cultures) using MRS agar in treated cheese (n=6). Different letters within a column indicates significant difference ($p<0.05$).

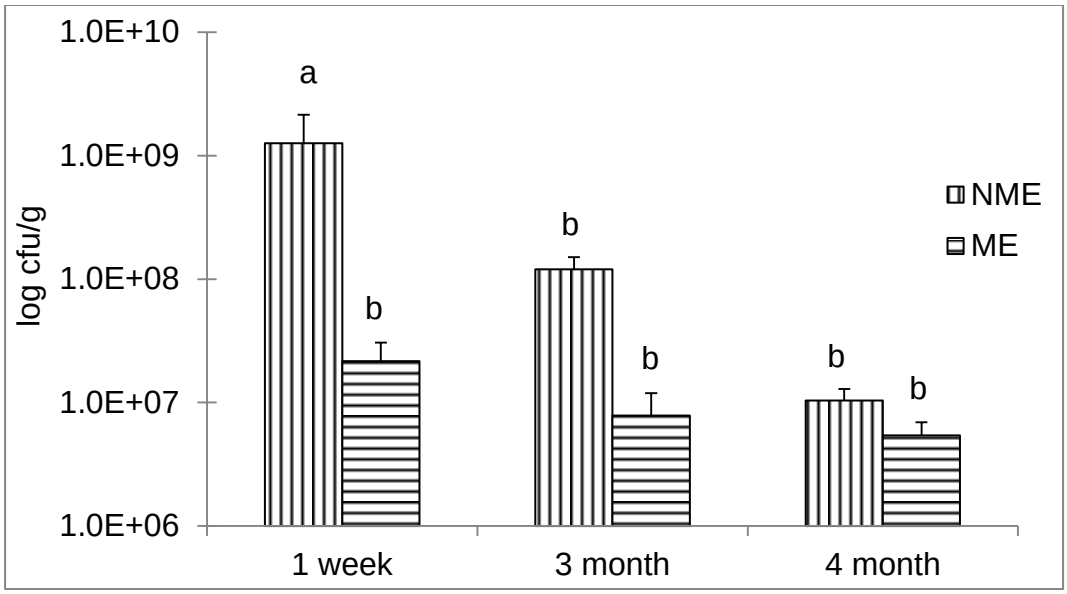


Figure 7. Changes in numbers of *L. helveticus* (probiotic cultures) using MRS with clindamycin agar in treated cheese (n=6). Different letters within a column indicates significant difference ($p<0.05$).

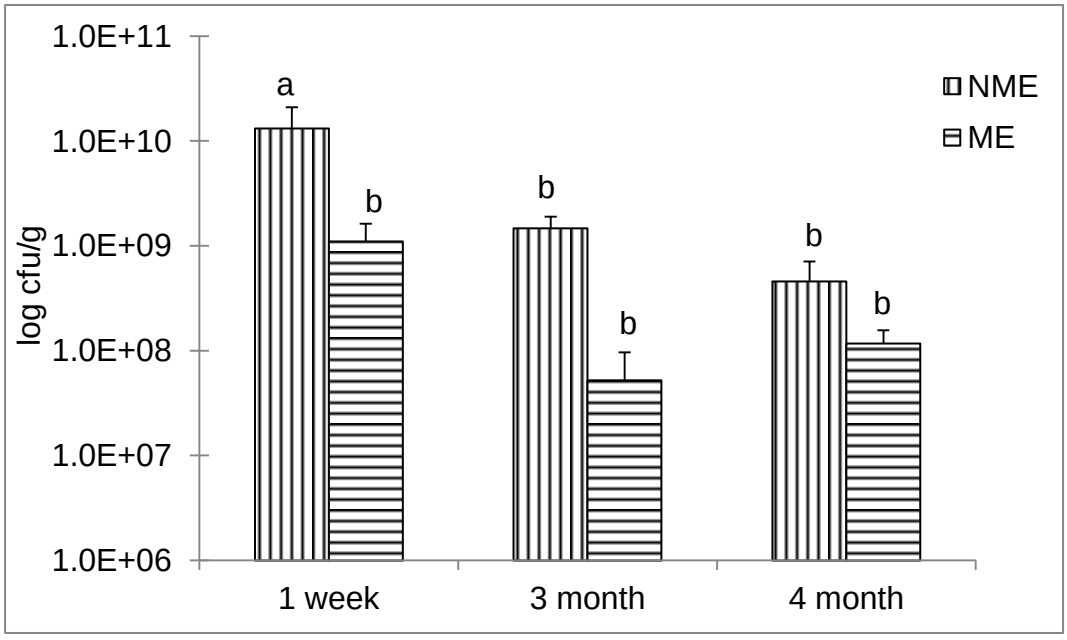


Figure 8. Changes in numbers of *B. longum* (probiotic cultures) using RCA agar in treated cheese (n=6). Different letters within a column indicates significant difference ($p<0.05$).

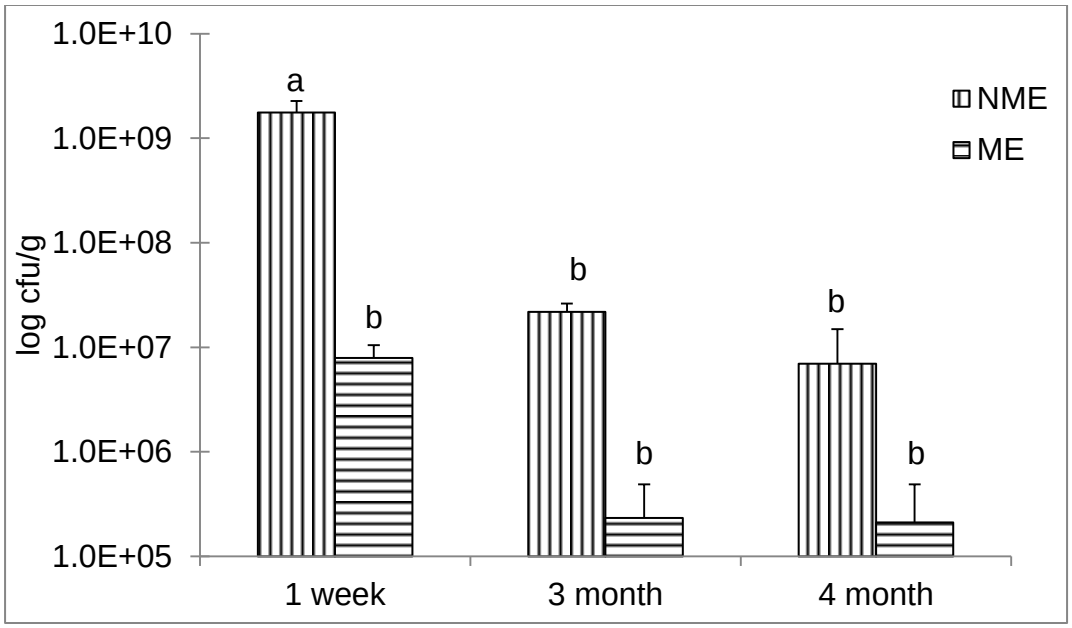


Figure 9. Changes in numbers of *B. longum* (probiotic cultures) using RAF 5.1 agar in treated cheese (n=6). Different letters within a column indicates significant difference ($p<0.05$).

If we assume consumption of a nominal one serving (30g) of cheese per day, the intake of *L. helveticus* and *B. longum* would be between 10^8 and 10^7 per day with the cheese that has been aged for 4 months and that is well above the levels suggested as providing therapeutic benefits which is levels higher than 10^7 per gram. Kurmann & Rasic (1991) recommended 10^5 – 10^7 bifidobacteria per g at the date of consumption, and the level in experimental Gouda was above thus recommendation.

4.4.3 Texture analyses

All significant ($p<0.05$) main effects and interactions for textural analysis are summarized in Table 21. The only significant effect was for the blade test where there was a significant interaction between treatment and time; there were no differences for either main factors or interactions for the ball test.

Table 21. Significant effect of the main factors and their interactions on textural analysis of treated cheese and maturation

Factor	Blade	Ball
Treatment	0.0101	0.8916
Time	0.9225	0.0796
Treatment*Time	0.0490	0.9850

The maximum force to cut through the probiotic and control cheeses using the blade are shown in Table 22. The microencapsulated cheese at 4 months maturation (23.18 N) required more force to cut through when compared to the control cheese at 4 months maturation (17.21 N). As all samples had similar moisture contents, this difference may indicate that microencapsulated probiotics add to the overall structure. Alternately, sampling effects where some blocks of cheese may not have been pressed as well as others may have contributed to texture differences.

However, when comparing our result from this main experiment to the preliminary experiment, the cutting force was lower in the main experiment. This may be due to the higher moisture content for cheese in the main experiment resulting in a softer cheese. Bertola et al. (2000) reported that ripening time and temperature can significantly effect the breaking force of Gouda cheese. It was

determined that as ripening time increased the breaking force decreased. In addition, the decreasing rate of breaking force increased with increasing ripening temperature. While we can conclude that moisture content does have an effect on the texture of the cheese, it is also possible that the lower temperature (10°C) during aging in the main experiment contributed to the change of texture. But during the storage period, the ripening temperature rose to 20°C for couple days due to technical problems with the ripening room. Therefore, that may also have had an effect on the texture of the cheese. In the end, time was not a significant factor in the main experiment; it appeared that conditions during aging could have an impact on the texture of the cheese.

Table 22. Effect of treatment and time on texture from the blade test (N) of the cheese (n=12)

Time	Treatments	Mean ± S.D.
3 month	Control	19.47 ^{ab} ± 7.48
3 month	NME	20.76 ^{ab} ± 2.33
3 month	ME	20.14 ^{ab} ± 2.46
4 month	Control	17.21 ^{b} ± 1.34
4 month	NME	20.24 ^{ab} ± 2.60
4 month	ME	23.18 ^{a} ± 2.64

Different letters within a column indicates a significant difference ($p<0.05$)

The compression forces from the ball texture test on the cheese samples are shown in Table 23 and 24, where there were no significant differences due to treatment and aging time. Lee et al. (1978) correlated instrumental compression force with sensory parameters such as hardness, chewiness, adhesiveness, and springiness. Therefore, sensory evaluation will determine if these similar textural properties are also seen during cheese consumption.

Table 23. Changes in cheese compression test with different treatments (n=24)

	Control	NME	ME
Texture (N)	5.36 [a] ± 1.12	5.50 [a] ± 0.89	5.46 [a] ± 0.07

Different letters within a row indicates significant difference ($p<0.05$)

Table 24. Changes in cheese compression test during aging (n=36)

	3 month	4 month
Texture (N)	5.65 [a] ± 0.86	5.23 [a] ± 1.09

Different letters within a row indicates significant difference ($p<0.05$)

4.4.4 Sensory analyses

All significant ($p<0.05$) main effects and interactions for attributes evaluated by the trained panel are summarized in Tables 25 and 26. There were no significant interactions of treatment and time for any of the flavour attributes. However, the salty and buttery flavours were affected by both aging time and treatment. For textural attributes, there was a significant interaction between treatment and time for creaminess and significant treatment effects for both firmness and cohesiveness. Therefore, the results of flavour analysis, firmness and cohesiveness are presented as main effects while the creaminess data are presented as an interaction effect.

Table 25. Significant effect of the main factors and their interactions on sensory flavour analysis (trained panellists) of treated cheese and maturation

Factors	Salty	Acid	Buttery	Flavour
Treatment	0.0368	0.7699	0.0030	0.2424
Time	0.0004	0.7028	0.0274	0.3490
Treatment*Time	0.6150	0.1934	0.8483	0.6854

Table 26. Significant effect of the main factors and their interactions on sensory texture analysis (trained panellists) of treated cheese and maturation

Factors	Firmness	Creaminess	Cohesiveness
Treatment	0.0003	<0.0001	0.0178
Time	0.1244	0.0476	0.1457
Treatment*Time	0.5057	0.0362	0.7255

The effects of treatment for all flavour and textural parameters except creaminess for trained panellists are given in Table 27. Significant differences were seen for salty, buttery, firmness and cohesiveness but not for acid and cheese flavour. The significant differences for saltiness indicated the NME samples were saltier than the ME samples although salt levels (Table 19) for the two cheeses were not significantly different. This could have been due to processing variation and choice of sample or other factors (lower buttery scores) that influence panellists' perception of salt. The panellists noticed a more buttery flavour for control cheeses than probiotic cheeses. It is possible that the probiotic cultures take away the buttery/creamy flavour in the cheese. In previous studies, probiotic cultures did not alter the cheese flavour in the cheeses even in studies that have shown that *L. helveticus* has the ability to decrease bitterness and accelerate flavor development in cheese (Broadbent et al., 2011). It can be concluded that, despite these differences, probiotic cultures did not give an unpleasant flavour to the Gouda cheese. This result is comparable to those obtained by Zomorodi et al. (2011) who reported Iranian white cheese that contained high levels of probiotic bacteria (either free or microencapsulated) showed no adverse effect on sensory criteria. Kailasapathy (2005) also reported that encapsulated bacteria did not affect the color, flavor, or aftertaste of yogurt.

Further, Gardiner et al. (2002) reported that the addition of spray dried *L. paracasei* to skim milk used in the production of Cheddar cheese gave sensory scores equivalent to those of commercial Cheddar cheese.

Ong & Shah (2009) reported that there were no significant differences in the hardness of cheese following addition of probiotic cultures (*B. longum*, B. *animalis*, *Lactobacillus casei*, and/or *Lactobacillus acidophilus*). However, Table 27 showed that there was a significant difference in firmness of the cheese with the addition of probiotics when compared to the control cheese. This may possibly have been due to the different strain combinations added which resulted in a different textural score. Moreover, Ong & Shah (2009) also reported that with the addition of probiotic cultures, the crumbliness of the cheese was significantly greater and the present results were similar. The addition of microencapsulated probiotic cultures resulted in a significant increase in the cohesiveness of the cheese when compared to the control cheese.

Table 27. Sensory evaluation (trained panellists) of treatments (n=32)

	Control	NME	ME
Salty	$7.63^{ab} \pm 0.87$	$7.84^{a} \pm 1.09$	$7.24^{b} \pm 0.95$
Acid	$7.84^{a} \pm 0.85$	$7.99^{a} \pm 1.18$	$7.84^{a} \pm 0.91$
Buttery	$8.50^{a} \pm 0.78$	$7.92^{b} \pm 0.92$	$7.78^{b} \pm 0.93$
Cheese flavour	$8.91^{a} \pm 0.97$	$8.65^{a} \pm 0.96$	$8.48^{a} \pm 1.09$
Firmness	$6.99^{b} \pm 1.09$	$7.76^{a} \pm 1.00$	$8.04^{a} \pm 1.00$
Cohesiveness	$7.18^{b} \pm 1.45$	$7.76^{ab} \pm 1.11$	$8.05^{a} \pm 1.11$

Different letters within a row indicates significant difference ($p<0.05$)

The interactions between time and treatment of creaminess scores are given in Table 28. The results showed that control cheeses had higher scores than probiotic cheeses, but only after 4 months of maturation.

Table 28. Effect of treatment and time on sensory scores (trained panellists) for the texture parameter of creaminess (n=16)

Time	Treatment	Mean ± S.D.
3 month	Control	10.86 ^{a} ± 1.00
3 month	NME	10.16 ^{ab} ± 0.93
3 month	ME	10.28 ^{ab} ± 1.39
4 month	Control	11.19 ^{a} ± 1.05
4 month	NME	9.57 ^{b} ± 1.04
4 month	ME	9.21 ^{b} ± 1.01

Different letters within a column indicates significant difference ($p<0.05$)

The time effect results of the trained panel are shown in Table 29, where only salty and buttery parameters were significantly affected. Trained panellists detected saltiness differences between the 3 and 4 month matured cheese samples, where the 4 month old cheeses were saltier than the 3 month old cheeses. This may have been due to the salt migration from the outer to the inner layer of the cheese within 30 days of maturation since the cheese samples were taken from the centre. However, the panellists detected a significant decrease in the buttery flavour of cheese samples over the same time period. Therefore, it could be concluded that during the longer aging time the salt does migrate to the centre portion gradually and the buttery flavour in cheese disappears slowly.

Ong & Shah (2009) reported that hardness of cheese decreased as ripening time increased possibly due to increased proteolysis with time. In addition, they also reported that a difference was seen in crumbliness with increasing ripening time. However, the trained panellists in the present experiment did not detect any textural differences between 3 and 4 months of aging. Therefore, it could be

concluded that the trained panellists could not detect the differences in firmness of the cheese that were seen by instrumental analysis.

Table 29. Changes in sensory scores (trained panellists) during aging (n=48)

	3 month	4 month
Salty	7.23 [b] ± 0.98	7.92 [a] ± 0.88
Acid	7.85 [a] ± 0.80	7.93 [a] ± 1.14
Buttery	8.27 [a] ± 0.84	7.87 [b] ± 0.98
Cheese flavour	8.78 [a] ± 1.02	8.58 [a] ± 1.01
Firmness	7.76 [a] ± 1.21	7.43 [a] ± 0.99
Cohesiveness	7.85 [a] ± 1.41	7.48 [a] ± 1.10

Different letters within a row indicates significant difference ($p<0.05$)

All significant ($p<0.05$) main effects and interactions between effects of treatments and time difference for consumer panel are summarized in Table 30. Data in Table 30 showed that there were no interaction effects between gender, age and treatments. Therefore, the results are presented as main effects since there were differences for age groups for all three parameters.

Table 30. Significant effect of the main factors and their interactions on sensory analysis (consumer test) of treated cheese and maturation

Factors	Flavor liking	Texture liking	Overall liking
Gender	0.6600	0.4047	0.6291
Age	0.0043	0.0050	0.0023
Trt	0.1489	0.8705	0.3634
Gender*Age	0.5760	0.4941	0.7907
Gender*Trt	0.9866	0.8825	0.9955
Age*Trt	0.8422	0.8004	0.6528
Gender*Age*Trt	0.3544	0.8999	0.6996

Summaries of the means and standard deviations of the sensory scores for the consumer test with gender, age, and treatment differences are shown in Tables 31, 21, and 33. There were no significant differences between gender and treatments for all three parameters tested. However, there was a significant difference for age groups where older (40+) panellists (7.00 for overall liking) gave higher scores than younger (18-35) panellists (6.09 for overall liking). It is possible that older age groups had more experience in tasting cheeses and they happen to prefer the cheeses that were presented to them. Yates and Drake (2007) reported that consumers usually eat more young Gouda cheese (< 6 month) than aged Gouda. However, the study also reported that most consumers eat cheese as a topping or in sandwiches and less was eaten alone or as a snack. When the main experiment was performed, the samples were presented alone. Therefore, the presentation style of the sample may have affected the scores in this evaluation. Moreover, when the main experiment was conducted, the number of panellists in the age group of 18 – 25 (64 panellists) was greater than the number of panellists aged 40+ (14 panellists). Overall, the probiotic cheeses were liked as much as the control cheeses (Table 33).

Table 31. Sensory evaluation (consumer test) of different gender (n=111)

	Male	Female
Flavour liking	6.44 [a] ± 1.74	6.55 [a] ± 1.88
Texture liking	6.25 [a] ± 1.55	6.44 [a] ± 1.85
Overall liking	6.43 [a] ± 2.46	6.53 [a] ± 1.62

Different letters within a row indicates significant difference ($p<0.05$)

Table 32. Sensory evaluation (consumer test) of different age (n=111)

	18 – 25	25 – 40	40+
Flavour liking	6.06 [b] ± 1.85	6.41 [ab] ± 1.71	7.02 [a] ± 1.62
Texture liking	5.92 [b] ± 1.60	6.34 [ab] ± 1.83	6.78 [a] ± 1.70
Overall liking	6.09 [b] ± 1.59	6.35 [ab] ± 1.48	7.00 [a] ± 1.56

Different letters within a row indicates significant difference ($p<0.05$)

Table 33. Sensory evaluation (consumer test) of treatments (n=111)

	Control	NME	ME
Flavour liking	6.23 [a] ± 1.81	6.46 [a] ± 1.86	6.79 [a] ± 1.75
Texture liking	6.37 [a] ± 1.73	6.40 [a] ± 1.69	6.26 [a] ± 1.72
Overall liking	6.30 [a] ± 1.52	6.47 [a] ± 1.66	6.66 [a] ± 1.55

Different letters within a row indicates significant difference ($p<0.05$)

5. CONCLUSIONS

Probiotic cultures are added to foods to help consumers improve their health by adding value to food and giving them more choices in selecting healthy foods.

The general aims of microencapsulation are to protect probiotic cultures from processing treatments and passage through stomach since free cells are usually unable to survive in the gastric environment, and then to release them in their target area (e.g. gut) in humans. On the other hand, the challenge in using microencapsulated probiotic cultures is that the quality of a food (flavour and texture attributes) to which microencapsulated probiotic cultures are added should not be changed. Many studies have been done on incorporating probiotic adjuncts into cheese and determining the effect of probiotics on sensory attributes and also the survivability of the probiotic cultures. Most of these studies were done on Cheddar and fresh cheese where the Cheddar was usually aged for 6 months and fresh cheeses were usually consumed within a month of their production. Therefore, the survivability of probiotics in cheese that was aged around 3 – 4 months needed to be studied.

The chemical, microbial, textural, and sensory analyses were evaluated on Gouda cheese with and without the addition of non-encapsulated and microencapsulated probiotic cultures. The salt content was principally affected by the percentage of the brine solution and aging also increased the salt content of the cheese due to salt migration from the outer to the inner portion. It was noticed that initially the probiotic cheeses had lower levels of salt. However, with

increased ripening time, the salt level of probiotic cheeses reached the same level as the control cheese. Therefore, it could be concluded that probiotic cultures lowered the speed of salt migration into the centre of the cheese. The fat and moisture content were also affected by processing variables. In general, probiotic cultures (without or with microencapsulation) did not affect the chemical properties of the aged cheese. Moreover, the fat and moisture content of the Gouda cheese made in both experiments all met the limits set by the Dairy Products Regulations (Department of Justice, 2011).

The enumeration of starter and probiotic cultures (with and without microencapsulation) was conducted. The results showed that higher level of *Lactococcus* strains occurred in the control cheese than in probiotic cheese after 4 months of aging. This may have been due to nutrient competition between starter culture and probiotic strains. But the levels of *S. thermophilus* (starter culture) were similar in the control and probiotic cheese at 4 months of aging, suggesting *S. thermophilus* was better able to compete in the presence of probiotic cultures. The enumeration results from probiotic strains showed that the level of microencapsulated cultures did change significantly during aging, although there was a loss during processing. On the other hand, free probiotic cells, which were higher at week 1 decreased significantly after 3 months of aging. Overall, the final level of both probiotic strains incorporated (either free cells or microencapsulated) meet the requirements suggested by researchers and health organizations.

From the texture results, it has been concluded that treatment and aging time did not alter the cutting and compression resistance forces of the cheese. The lower force to break the control cheese with the blade test was not seen with the ball test or in the sensory evaluation. This may have been a sampling effect or an indication that the probiotics had some impact on the casein network.

The findings obtained from the trained panel sensory result showed that probiotic cultures (whether non-encapsulated or microencapsulated) affected the perception of saltiness, where the non-encapsulated cheese appeared saltier. Buttery flavour was lower for the two probiotic cheese. Other flavour scores were similar to the control cheese. Addition of probiotic cultures made the cheese firmer and more cohesive. Additionally, the consumer panel sensory result concluded that there were no treatment and aging time differences between control and probiotic cheeses. The only significant differences found from the sensory result was that older (40+) panellists gave higher flavour, texture and overall liking scores than the younger panellists.

Probiotics can be successfully incorporated into an aged Gouda cheese without adversely affecting quality. These cheeses can be used as a food ingredient in the preparation of ready-to-eat food, such as salad and sandwiches, making probiotic cheese a convenient food. However, the food industry still needs to promote and understand probiotic bacteria.

The survival rate of probiotic bacteria after various processing and storage treatments has generally been studied by the plate count method and these tests

indicated there were sufficient levels of probiotic organisms after 4 months of aging to provide the health benefits associated with probiotics. However, growth on agar plates is not consistently accurate.

This study focused on the viability of probiotic cultures and the changes resulting from incorporating probiotic cultures (chemical, textural, and sensorial) in Gouda cheese. In the future, research is needed to evaluate microencapsulated probiotics in terms of their viability through food processing, storage, and the delivery in the GI tract. Furthermore, methods for incorporation of microencapsulated probiotic into cheese needs more research as difficulty was observed when incorporating microencapsulated probiotic cultures into cheese because a portion of the capsules appeared to be lost during whey removal. Therefore, the technological aspects of the use of microencapsulated probiotics in cheese still needs investigation.

REFERENCES

Adhikari, K., Mustapha, A., Grun, I.U., Fernando, L. 2000. Viability of microencapsulated bifidobacteria in set yogurt during refrigerated storage. Journal of Dairy Science. 83:1946-1951

Agriculture and Agri-Food Canada. 2006. Sector profile: cheese. [Online Database] Government of Canada. URL: http://www4.agr.gc.ca/resources/prod/doc/dairy/pdf/prof_cheese_e.pdf (23 January, 2012)

Ahmarani, J. 2010. Differential quantification of microorganisms in Protecflor. Internal Lallemand R&D report #BCE-003-00

Anal, A.K., & Singh, H. 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends in Food Science & Technology. 18:240-251

AOAC. 2002. AOAC Moisture Method (926.08 & 948.12). Association of Official Analytical Chemists. Gaithersburg, MD.

Atassi, F., Brassart, D., Grob, P., Graf, F., Servin, A.L. 2006. In vitro antibacterial activity of *Lactobacillus helveticus* strain KS300 against diarrhoeagenic, uropathogenic and vaginosis-associated bacteria. Journal of Applied Microbiology. 101:647-654

Beganovic, J., Frece, J., Kos, B., Lebos Pavunc, A., Habjanic, K., Suskovic, J. 2011. Functionality of the S-layer protein from the probiotic strain *Lactobacillus helveticus* M92. Antonie van Leeuwenhoek. 100:43-53

Bertola, N.C., Califano, A.N., Bevilacqua, A.E., Zaritzky, N.E. 2000. Effects of ripening conditions on the texture of Gouda cheese. International Journal of Food Science & Technology. 35:207-214

Broadbent, J.R., Cai, H., Larsen, R.L., Hughes, J.E., Welker, D.L. De Carvalho, V.G., Tompkins, T.A., Ardo, Y., Vogensen, F., De Lorentiis, A., Gatti, M., Neviani, E., Steele, J.L. 2011. Genetic diversity in proteolytic enzymes and amino acid metabolism among *Lactobacillus helveticus* strains. Journal of Dairy Science. 94:4313-4328

Brown, J.A., Foegeding, E.A., Daubert, C.R., Drake, M.A., Gumpertz, M. 2003. Relationships among rheological and sensorial properties of young cheeses. Journal of Dairy Science. 86:3054-3067

Bruhn, C.M., Bruhn, J.C., Cotter, A., Barrett, C., Klenk, M., Powell, C., Stanford, G., Steinbring, Y., West, E. 2002. Consumer attitudes toward use of probiotic cultures. Journal of Food Science. 67:1969-1972

Canadian Food Inspection Agency. 2009. Health Claims. [Online Database] Government of Canada. URL: http://www.inspection.gc.ca/english/fssa/labeti/guide/ch8ae.shtml#a8_7 (23 January, 2012)

Champagne, C., Gardner, N., Roy, D. 2005. Challenges in the addition of probiotic cultures to foods. Critical Reviews in Food Science and Nutrition. 45:61-84

Champagne, C.P., Raymond, Y., Tompkins, T.A. 2010. The determination of viable counts in probiotic cultures microencapsulated by spray-coating. Food Microbiology. 27:1104-1111

Chen, A.H., Larkin, J.W., Clark, C.J., Irwin, W.E. 1979. Textural analysis of cheese. Journal of Dairy Science. 62:901-907

Chen, K-N., Chen, M-J, Liu, J-R., Lin, C-W., Chiu, H-Y. 2005. Optimization of incorporated prebiotics as coating materials for probiotic microencapsulation. Journal of Food Science. 70:M260-M266

Dave, R.I., & Shah, N.P. 1996. Evaluation of media for selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp *bulgaricus*, *Lactobacillus acidophilus*, and bifidobacteria. Journal of Dairy Science. 79:1529-1536

de Vos, P., Faas, M.M., Spasojevic, M., Sikkema, J. 2010. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. International Dairy Journal. 20:292-302

Department of Justice. 2011. Dairy Products Regulations.[Online Database] Government of Canada. URL: http://laws-lois.justice.gc.ca/PDF/SOR-79-840.pdf (23 January, 2012)

Diop, L., Guillou, S., Durand, H. 2008. Probiotic food supplement reduces stree-induced gastrointestinal symptoms in volunteers: a double-blind, placebo-controlled, randomized trial. Nutrition Research. 28:1-5

Drake, M.A. 2004. ADSA Foundation Scholar Award: defining dairy flavors. Journal of Dairy Science. 87:777-784

Foegeding, E.A., & Drake, M.A. 2007. Sensory and mechanical properties of cheese texture. Journal of Dairy Science. 90:1611-1624

Food and Agriculture Organization of United Nations & World Health Organization. 2001. Guidelines for the evaluation of probiotics in food. [Online Databse] URL: http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf (23 January, 2012)

Fortin, M.H., Champagne, C.P., St-Gelais, D., Britten, M., Fustier, P., Lacroix, M. 2011. Viability of *Bifidobacterium longum* in Cheddar cheese curd during manufacture and storage: effect of microencapsulation and point of inoculation. Dairy Science & Technology. 91:599-614

Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H. 2000. *Fundamentals of Cheese Science*. Aspen Pub. Inc., Gaithersburg, MA. 587pp.

Frece, J., Kos, B., Svetec, I.K., Zgaga, Z., Beganovic, J., Lebos Pavunc, A. Suskovic, J. 2009. Synbiotic effect of *Lactobacillus helveticus* M92 and prebiotics on the intestinal microflora and immune system of mice. The Journal of Dairy Research. 76:98-104

Gardiner, G., Stanton, C., Lynch, P.B., Collins, J.K., Fitzgerald, G., Ross, R.P. 1999. Evaluation of Cheddar cheese as a food carrier for delivery of a probiotic strain to the gastrointestinal tract. Journal of Dairy Science. 82:1379-1387

Gardiner, G.E., Bouchier, P., O'Sullivan, E., Kelly, J., Kevin Collins, J., Fitzgerald, G., Ross, P.R., Stanton, C. 2002. A spray-dried culture for probiotic Cheddar cheese manufacture. International Dairy Journal. 12:749-756

Gomes da Cruz, A., Alonso Buriti, F.C., Batista de Souza, C.H., Fonseca Faria, J.A., Isay Saad, S.M. 2009. Probiotic cheese: health benefits, technological and stability aspects. Trends in Food Science & Technology. 20:344-354

Gomes, A.M.P., & Malcata, F.X. 1999. *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trends in Food Science & Technology. 10:139-157

Gomes, A.M.P., Vieira, M.M., Malcata, F.X. 1998. Survival of probiotic microbial strains in a cheese matrix during ripening: simulation of rates of salt diffusion and microorganism survival. Journal of Food Engineering. 36:281-301

Gunasekaran, S., & Ak, M.M. 2002. *Cheese Rheology and Texture*. CRC Press LLC., Boca Raton, FL. 437pp.

Islam, M.A., Yun, C-H., Choi, Y-J., Cho, C-S. 2010. Microencapsulation of live probiotic bacteria. Journal of Microbiology and Biotechnology. 20:1367-1377

Kailasapathy, K. 2002. Microencapsulation of probiotic bacteria: technology and potential applications. Current Issues in Intestinal Microbiology. 3:39-48

Kailasapathy, K. 2005. Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. LWT – Food Science and Technology. 39:1221-1227

Kailasapathy, K., & Chin, J. 2000. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunology and Cell Biology. 78:80

Kandler, O., & Weiss, N. 1986. Genus Lactobacillus. pp. 1063-1065 in: *Bergey's Manual of Systematic Bacteriology, Volume 2*. Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holt, J.G. (9th Ed.) Williams and Wilkins, Baltimore, MD

Kiviharju, K., Leisola, M., Eerikainen, T. 2005. Optimization of a *Bifidobacterium longum* production process. Journal of Biotechnology. 117:299-308

Kosikowski, F.V., & Mistry, V.V. 1999. *Cheese and Fermented Milk Foods, Volume 2. Procedures and Analysis* (3rd Ed.) FV Kowikowski, LLC., Great Falls, VA. 429pp.

Krasaekoopt, W., Bhandari, B., Deeth, H. 2003. Evaluation of encapsulation techniques of probiotics for yoghurt. International Dairy Journal. 13:3-13

Kurmann, J.A., & Rasic, J.L. 1991. The health potential of products containing bifidobacteria. Pages 117-158 in: *Therapeutic Properties of Fermented Milks*. Robinson, R.K. ed. Elsevier Science Publishers Ltd, London.

Lee, C.H., Imoto, E.M., Rha, C. 1978. Evaluation of cheese texture. Journal of Food Science. 43:1600-1605

Marshall, R.T. 1992. *Standard Methods for the Examination of Dairy Products*. American Public Health Association, Washington, DC. 546pp.

McBrearty, S., Ross, R.P., Fitgerald, G., Collins, J.K., Auty, M.A.E., Wallace, J.M. 2001. Influence of two commercially available bifidobacteria cultures on Cheddar cheese quality. International Dairy Journal. 11:599-610

McGorrin, R.J. 2007. Flavor analysis of dairy products. Pages 23-49 in: *Flavor of Dairy Products*. Cadwallader, K.R., Drake, M.A., McGorrin, R.J. ed. American Chemical Society, Washington, DC.

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., Bisson, J-F., Rougeot, C., Pichelin, M., Cazaubiel, M., Cazaubiel, J-M. 2011. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helvetiucs* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. The British Journal of Nutrition. 105:755-764

Montoya, D., Boylston, T.D., Mendonca, A. 2009. Preliminary screening of Bifidobacteria spp. and *Pediococcus acidilactici* in a Swiss cheese curd slurry model system: Impact on microbial viability and flavor characteristics. International Dairy Journal. 19:605-611

Muir, D., Hunter, E.A., Banks, J.M. 1997. Aroma of cheese. 2. contribution of aroma to the flavour of Cheddar cheese. Milchwissenschaft-Milk Science International. 52:85-88

Nakajo, K., Takahashi, N., Beighton, D. 2010. Resistance to acidic environments of caries-associated bacteria: *Bifidobacterium dentium* and *Bifidobacterium longum*. Caries Research. 44:431-437

Ong, L., & Shah, N.P. 2009. Probiotic Cheddar cheese: influence of ripening temperatures on proteolysis and sensory characteristics of Cheddar cheese. Journal of Food Science. 74:91

Ouwehand, A.C., Ibrahim, F., Forssten, S.D. 2010. Cheese as a carrier for probiotics: in vitro and human studies. Australian Journal of Dairy Technology. 65:165-169

Parvez, S., Malik, K.A., Ah Kang, S., Kim, H-Y. 2006. Probiotics and their fermented food products are beneficial for health. Journal of Applied Microbiology. 100:1171-1185

Pavunc, A.L., Beganovic, J., Kos, B., Buneta, A., Beluhan, S., Suskovic, J. 2011. Influence of microencapsulation and tranglutaminase on viability of probiotic strain *Lactobacillus helveticus* M92 and consistency of set yoghurt. International Journal of Dairy Technology. 64:254-261

Phillips, M., Kailasapathy, K., Train, L. 2006. Viability of commercial probiotic cultures (*L. acidophilus*, *Bifidobacterium* sp., *L. casei*, *L. paracasei* and *L. rhamnosus*) in Cheddar cheese. International Journal of Food Microbiology. 108:276-280

Renner, E., 1987. Nutritional aspects of cheese. Ch. 9 in *Cheese: Chemistry, Physics and Microbiology. Volume 1. General Aspects*, P.F. Fox (Ed.), p. 345-363. Elsevier Applied Science, London and New York.

Rokka, S., & Rantamaki, P. 2010. Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. European Food Research and Technology. 231:1-12

Ross, R.P., Fitzgerald, G., Collins, K., Stanton, C. 2002. Cheese delivering biocultures – probiotic cheese. Australian Journal of Dairy Technology. 57:71-78

Ross, R.P., Stanton, C., Hill, C., Fitzgerald, G.F., Coffey, A. 2000. Novel cultures for cheese improvement. Trends in Food Science & Technology. 11:96-104

Rukure, G., & Bester, B.H. 2001. Survival and growth of *Bacillus cereus* during Gouda cheese manufacturing. Food Control. 12:31-36

Salminen, S., Ouwehand, A.C., Isolauri, E. 1998. Clinical applications of probiotic bacteria. International Dairy Journal. 8:563-572

Santosa, S., Farnworth, E., Jones, P.J.H. 2006. Probiotics and their potential health claims. Nutrition Reviews. 64:265-274

Scardovi, V. 1986. Genus Bifidobacterium Orla-Jensen. pp. 1418-1434 in: *Bergey's Manual of Systematic Bacteriology, Volume 2*. Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holt, J.G. (9th Ed.) Williams and Wilkins, Baltimore, MD

Scott, R. 1986. *Cheesemaking Practice*. Elsevier Applied Science Publishers., London. 529pp.

Shah, N.P. 2000. Probiotic bacteria: selective enumeration and survival in dairy foods. Journal of Dairy Science. 83:894-907

Silva, A.M., Barbosa, F.H.F., Duarte, R., Vieira, L.Q., Arantes, R.M.E., Nicoli, J.R. 2004. Effect of *Bifidobacterium longum* ingestion on experimental salmonellosis in mice. Journal of Applied Microbiology. 97:29-37

Singh, T.K., Drake, M.A., Cadwallader, K.R. 2003. Flavor of cheddar cheese: a chemical and sensory perspective. Comprehensive Reviews in Food Science and Food Safety. 2:139-162

Slattery, L., O'Callaghan, J., Fitzgerald, G.F., Beresford, T., Ross, R.P. 2010. Invited review: *Lactobacillus helveticus* – A thermophilic dairy starter related to gut bacteria. Journal of Dairy Science. 93:4435-4454

Smit, G., Smit, B.A., Engels, W.J.M. 2005. Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. FEMS Microbiology Reviews. 29:591-610

Sodini, I., Lucas, A., Oliveira, M.N., Remeuf, F., & Corrieu, G. 2002. Effect of milk base and starter culture on acidification, texture, and protiotic cell counts in fermented milk processing. Journal of Diary Science, 85:2479-2488

Stanton, C., Gardiner, G., Lynch, P.B., Collins, J.K., Fitzgerald, G., Ross, R.P. 1998. Probiotic cheese. International Dairy Journal. 8:491-496

Stone, H., & Sidel, J.L. 1993. *Sensory Evaluation Practices*. Academic Press, Inc., San Diego, CA. 338pp.

Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P., Kailasapathy, K. 2000. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. International Journal of Food Microbiology. 62:47-55

Talwalkar, A., & Kailasapathy, K. 2003. Effect of microencapsulation on oxygen toxicity in probiotic bacteria. Australian Journal of Dairy Technology. 58:36-39

Tamime, A. 2005. *Probiotic Dairy Products*. Blackwell Publishing, Oxford, UK. 216pp.

Tunick, M.H. 2007. Origins of cheese flavor. Pages 155-173 in: *Flavor of Dairy Products*. Cadwallader, K.R., Drake, M.A., McGorrin, R.J. ed. American Chemical Society, Washington, DC.

University of Guelph. 2010. Semi-hard cheese – washed: Gouda. Selected Recipes. [Online Databse] URL: http://www.foodsci.uoguelph.ca/cheese/sectionf.htm (2 June, 2010)

Van Leuven, I., Van Caelenberg, T., Dirinck, P. 2008. Aroma characterisation of Gouda-type cheeses. International Dairy Journal. 18:790-800

Vijaya Kumar, S.G., Singh, S.K., Goyal, P., Dilbaghi, N., Mishra, D.N. 2005. Beneficial effects of probiotics and prebiotics on human health. Die Pharmazie. 60:163-171

Vinderola, C.G., Mocchiutti, P., Reinheimer, J.A. 2002. Interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. Journal of Dairy Science. 85:721-729

Walther, B., Schmid, A., Sieber, R., Wehrmuller, K. 2008. Cheese in nutrition and health. Dairy Science & Technology. 88:389-405

Wang, H., Cui, L., Chen, W., Zhang, H. 2011. An application in Gouda cheese manufacture for a strain of *Lactobacillus helveticus* ND01. International Journal of Dairy Technology. 64:386-393

Watkinson, P., Coker, C., Crawford, R., Dodds, C., Johnston, K., McKenna, A., White, N. 2011. Effect of cheese pH and ripening time on model cheese textural properties and proteolysis. International Dairy Journal. 11:455-464

Wine, E., Gareau, M.G., Johnson-Henry, K., Sherman, P.M. 2009. Strain-specific probiotic (*Lactobacillus helveticus*) inhibition of *Campylobacter jejuni* invasion of human intestinal epithelial cells. FEMS Microbiology Letters. 300:146-152

Yates, M.D., & Drake, M.A. 2007. Texture properties of Gouda cheese. Journal of Sensory Studies. 22:493-506

Young, N.D., Drake, M., Lopetcharat, K., McDaniel, M.R. 2004. Preference mapping of Cheddar cheese with varying maturity levels. Journal of Dairy Science. 87:11-19

Zomorodi, S., ASL, A.K., Rohani, S.M.R., Miraghaei, S. 2011. Survival of *Lactobacillus casei*, *Lactobacillus plantarum* and *Bifidobacterium bifidum* in free and microencapsulated forms on Iranian white cheese produced by ultrafiltration. International Journal of Dairy Technology. 64:84-91

APPENDIX A

Milk Composition – Preliminary Test

Dairy Farmers of Manitoba

COPY

University of Manitoba			Litres Purchased	1,336
Food Sciences Dept. Atten: Karola Lang			kg Butterfat	47.400
252 Ellice Building			kg protein	43.143
R3T 2N2			kg other solids	75.370
		INVOICE NUMBER	INVOICE DATE	MONTH
	Average Tests	B1008-13	September 10, 2010	August 2010
Butterfat	3.54790	Fixed Tests Class 1a	Fixed Tests Class 1B	Fixed Tests Class 1C
Protein	3.22927	3.4092	2.7606	3.4200
O/Solids	5.64147	5.7921	4.6901	5.8104
Protein Skim Test		0.036417	O. Solids Skim Test	0.063619

UTILIZATION	LITRES	COMPONENTS	WEIGHT	PRICE/KG	VALUE
CLASS 2		Butterfat		$7.53450	
		Protein		$5.64530	
		Other Solids		$5.64530	
		Totals			
CLASS 3A	1,336	Butterfat	47.400	$7.53450	$357.14
		Protein	43.143	$13.18700	$568.93
		Other Solids	75.370	$0.89460	$67.43
		Totals			$993.49
CLASS 3B		Butterfat		$7.53450	
		Protein		$12.93430	
		Other Solids		$0.87860	
		Totals			
CLASS 4A		Butterfat		$7.53450	
		Protein		$5.24690	
		Other Solids		$5.24690	
		Totals			
CLASS 4C		Butterfat		$7.53450	
		Protein		$5.24690	
		Other Solids		$5.24690	
		Totals			
CLASS 4D		Butterfat			
		Other Solids			
		Protein			
		Totals			
CLASS 4D(j)		Butterfat		$7.53450	
		Protein		$5.24690	
		Other Solids		$5.24690	
		Totals			
CLASS 5A		Butterfat			
		Protein			
		Other Solids			
		Totals			
LITRES UTILIZED	1,336				

Total Component Values	$993.49
Component Adjustment (+/-)	
Interim Advance Received	
Amount Due - Current Month:	$993.49

Final payment due 8 days subsequent to date of invoice.
1.5% per month service charge on all overdue accounts.

36 Scurfield Boulevard, PO Box 724, Stn Main Winnipeg, Manitoba R3C 2K3
Tel (204) 488-6455 Fax (204) 488-4772

Appendix B

Sensory Ethics Approval Letter – Preliminary Experiment

UNIVERSITY OF MANITOBA | Ethics
Office of the Vice-President (Research)

CTC Building
208 - 194 Dafoe Road
Winnipeg, MB R3T 2N2
Fax (204) 269-7173
www.umanitoba.ca/research

APPROVAL CERTIFICATE

October 29, 2010

TO: **Yi-Chun Liu** (Advisor S. Arntfield)
Principal Investigator

FROM: **Brian Barth, Chair**
Joint-Faculty Research Ethics Board (JFREB)

Re: **Protocol #J2010:123**
"Effect of Incorporating Encapsulated and Non-encapsulated Probiotic Culture on Culture Survival and Cheese Quality of Gouda Cheese"

Please be advised that your above-referenced protocol has received human ethics approval by the **Joint-Faculty Research Ethics Board**, which is organized and operates according to the Tri-Council Policy Statement. This approval is valid for one year only.

Any significant changes of the protocol and/or informed consent form should be reported to the Human Ethics Secretariat in advance of implementation of such changes.

Please note:

- **If you have funds pending human ethics approval, the auditor requires that you submit a copy of this Approval Certificate to the Office of Research Services, fax 261-0325 - please include the name of the funding agency and your UM Project number. This must be faxed before your account can be accessed.**

- **if you have received multi-year funding for this research, responsibility lies with you to apply for and obtain Renewal Approval at the expiry of the initial one-year approval; otherwise the account will be locked.**

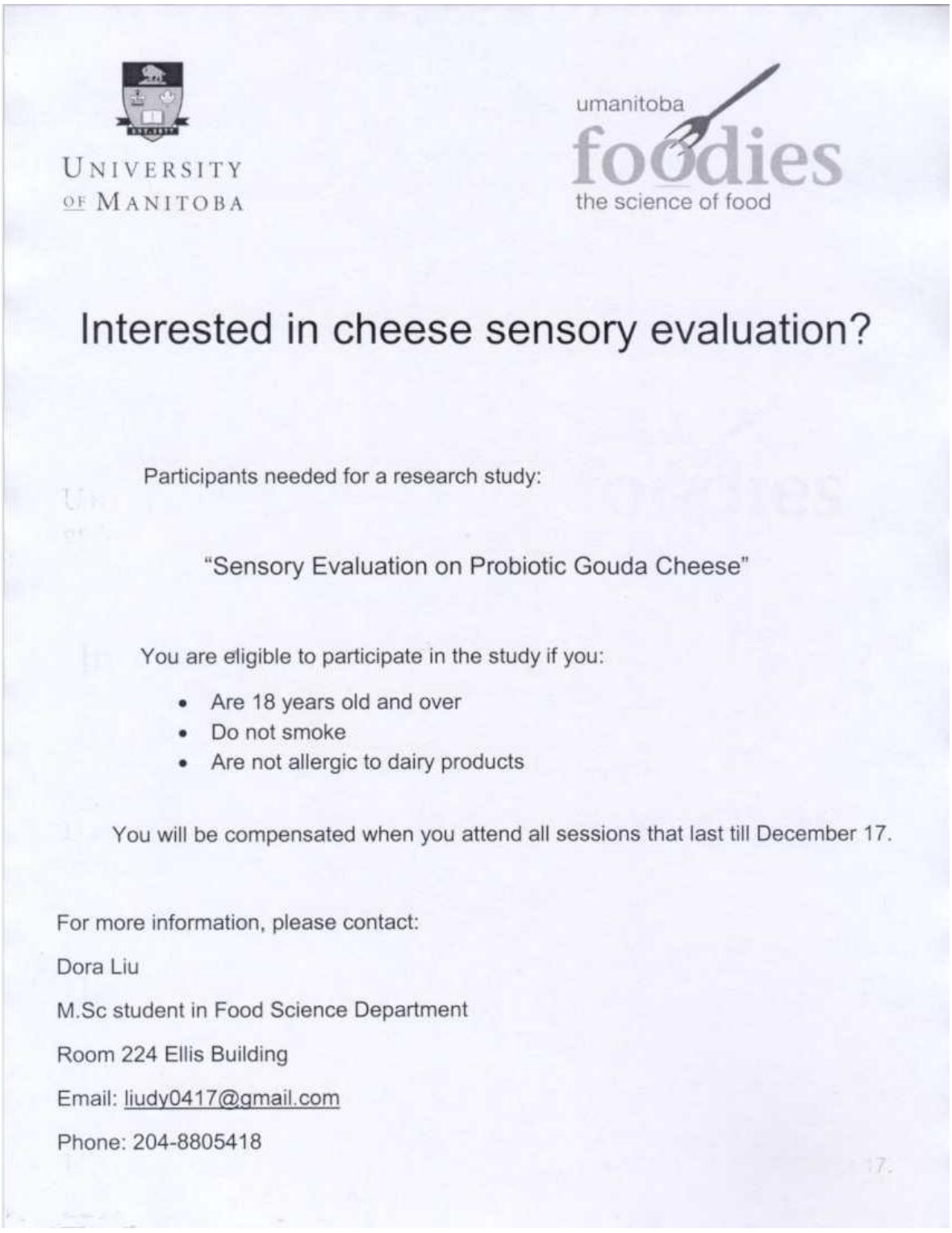
The Research Ethics Board requests a final report for your study (available at: http://umanitoba.ca/research/ors/ethics/ors_ethics_human_REB_forms_guidelines.html) **in order to be in compliance with Tri-Council Guidelines.**

Bringing Research to Life

APPENDIX C

Sensory Evaluation Forms – Preliminary Experiment

Recruitment Poster



UNIVERSITY OF MANITOBA

umanitoba foodies
the science of food

Interested in cheese sensory evaluation?

Participants needed for a research study:

"Sensory Evaluation on Probiotic Gouda Cheese"

You are eligible to participate in the study if you:

- Are 18 years old and over
- Do not smoke
- Are not allergic to dairy products

You will be compensated when you attend all sessions that last till December 17.

For more information, please contact:

Dora Liu

M.Sc student in Food Science Department

Room 224 Ellis Building

Email: liudy0417@gmail.com

Phone: 204-8805418

Consent Form

UNIVERSITY OF MANITOBA | Faculty of Agricultural and Food Sciences

Department of Food Science
Winnipeg, Manitoba
Canada R3T 2N2
Telephone: (204) 474-9621
Head: (204) 474-9065
Fax: (204) 474-7630

Consent form for Gouda cheese sensory evaluation

Department of Food Science

University of Manitoba

This consent form, a copy of which will be left with you for your records and reference, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

The approval of this sensory evaluation has been received from the Joint-Faculty Research Ethics Board (JFREB)

Researcher: Yi-Chun (Dora) Liu
Sponsor: Canadian Dairy Commission

UNIVERSITY OF MANITOBA | Faculty of Agricultural and Food Sciences

Department of Food Science
Winnipeg, Manitoba
Canada R3T 2N2
Telephone: (204) 474-9621
Head: (204) 474-9065
Fax: (204) 474-7630

Dear Fellow Colleague,

You are being asked to evaluate the flavour and texture of Gouda cheese. You will be asked to place samples of the Gouda cheese provided into your mouth to evaluate the flavour and texture and complete a short evaluation form. The time evolved in completing each panel will be about 15 minutes. The frequency of the panel is approximate once or twice a week. You will be trained at least two to three times before panels start. Each training panel will take about 15 minutes. The frequency of the training panel is two or three times per week and it will start one week before the panel starts. This study will start October 25th 2010. This study will possibly end by the week of December 17th. You may choose the time of the sensory panel within the week of evaluation of the sample (please let me know the time you would like to come in for a session at least two days in advance). Your participation is strictly voluntary and will provide research data for a graduate studies thesis.

These tests may pose risks or discomfort to some people; a list of ingredients is included below in the event you know of a substance (cow milk) to which you have allergic reactions or intolerance. Other discomfort feeling you might have is from the which may not be to your liking flavour of cheese, therefore a waste cup is provided so you would not have to ingest it. You may voluntarily withdraw from this sensory evaluation test at any time throughout testing without penalty.

No reference will be made to individual judges in any presentation of discussion of data and no record will remain once the data is analyzed.

The evaluation will be done in Room 221 (Sensory panel) in the Ellis Building (Department of Food Science). Please direct any questions or concerns you may have about the project or the tests to me at (204)880-5418 or you can email me at liudy0417@gmail.com. The sensory evolutions will be supervised under Dr. Susan Arntfield. She can be contacted at (204)474-9866 or susan_arntfield@umanitoba.ca. If you have any regarding research ethics, you can contact the coordinator of Human Ethics – Margaret Bowman at margaret_bowman@umanitoba.ca or (204)474-7122.

Your willingness to participate in this panel is greatly appreciated. There will be compensation of $20 gift card from the University of Manitoba Bookstore when all sessions are completed.

List of ingredients in this test: Gouda cheese (cow's milk and probiotic), water, and unsalted soda cracker.

Name: _____ (Please PRINT)

Signature: _____ Date: _____

Phone Number: _____

Email Address: _____

Researcher: Yi-Chun (Dora) Liu
Sponsor: Canadian Dairy Commission

Questionnaire

Panellists Questionnaire
Cheese Evaluation

THIS INFORMATION WILL REMAIN STRICTLY CONFIDENTIAL

Name: _____

Phone: _____

Email address: _____

1. Have you participated in a sensory evaluation before?

   ☐ Yes ☐ No

   If yes, what product(s) did you evaluate?

   _____

   _____

   Was training part of the evaluation procedure?

   ☐ Yes ☐ No

   If yes, indicate which product(s)

   _____

   _____

2. Are you allergic to any food product(s)?

   ☐ Yes ☐ No

   If yes, please note them

   _____

3. Are there any food specifically, or any food flavour that you prefer not to evaluate?

   _____

4. Do you take any medication that will affect your senses?

   ☐ Yes ☐ No

5. Do you have any dental work that will affect your evaluation of texture?

   ☐ Yes ☐ No

6. Do you expect to be away prior to December 18th/2010?

   ☐ Yes ☐ No

7. Are there any weekdays (M-F) that you will not be available on a regular basis?

   _____

8. How often do you consume cheese?

   ☐ <1 per month ☐ 1 – 3 times per month ☐ >5 times per month

9. Have you tried Gouda cheese?

   ☐ Yes ☐ No

   If yes, how often?

   ☐ <1 per month ☐ 1 – 3 times per month ☐ >5 times per wk

10. What type of cheese do you prefer?

    ☐ Mild ☐ Medium ☐ Mature/Sharp Cheese

APPENDIX D

Sensory Evaluation Instruction and Ballot – Preliminary Experiment

Sensory Evaluation Instruction

Sensory Evaluation Instruction

- Please be seated and knock on the door in front of you to let me know the seat number you are using.
- Please put your panel number and date of evaluation on the questionnaire.
- Please cleanse your mouth with water provided before beginning your first sample.
- Some flavour standards have been provided; please cleanse your mouth with water or unsalted soda cracker provided between each sample and standard.
- Evaluate the samples from left to right. Please do not go back to tasting previous samples.
- Please taste the control before begins tasting the samples.
- Taste the cheese for flavour.
- Touch or use your mouth to evaluate the cheese for texture.
- Cleanse your mouth with water or unsalted soda cracker in between samples to remove the flavour from the previous sample.
- If you do not like the sample, the waste cup is provided for you to spit out the sample. Ingestion of sample is not mandatory.
- When you are done, please knock on the door in front of you to inform me that you are finished with all the samples.
- **Don't forget to take your treat and thank you for your participation!**

Ballot

Sensory Evaluation of Gouda Cheese

Sample number: _____ Panellist Number: _____

Date: _____

You are presented with Gouda cheese that is letter coded and a standard that is coded as S1
To evaluate the texture, put a small sample between your fingers and feel the texture of the sample, although do use your month to evaluate the texture too. To evaluate the flavour, put a small sample into your mouth and taste the sample. Spit out the sample into the waste cup if the flavour discomforts you. Ingestion is not mandatory.
Evaluate all parameters in the samples provided and circle the number of the intensity detected. Go to the next sample. Cleanse your mouth with water or unsalted soda cracker provided.

AROMA & FLAVOUR:

Salty: (1 = not salty, 9 = extremely salty)

1 2 3 4 5 6 7 8 9

Bitterness: (1 = not bitter, 9 = extremely bitter)

1 2 3 4 5 6 7 8 9

High Acid: (1 = no acid taste, 9 = high acid)

1 2 3 4 5 6 7 8 9

Flat/lack of flavour: (1 = flat, 9 = rich)

1 2 3 4 5 6 7 8 9

TEXTURE:

Crumbliness/rubbery: (1 = extremely crumbly, 9 = extremely rubbery)

1 2 3 4 5 6 7 8 9

OVERALL LIKING: (1 = dislike extremely, 9 = like extremely)

1 2 3 4 5 6 7 8 9

APPENDIX E

Milk Composition – Main Experiment

Dairy Farmers of Manitoba

COPY

University of Manitoba		Litres Purchased	856
Food Sciences Dept. Atten: Karola Lang		kg Butterfat	30.110
252 Ellice Building		kg protein	26.493
R3T 2N2		kg other solids	48.749

		INVOICE NUMBER	INVOICE DATE	MONTH
	Average Tests	B1105-13	June 9, 2011	May 2011
Butterfat	3.51752	Fixed Tests Class 1a	Fixed Tests Class 1B	Fixed Tests Class 1C
Protein	3.09498	3.4092	2.7606	3.4200
O/Solids	5.69498	5.7921	4.6901	5.8104
Protein Skim Test		0.034864	O. Solids Skim Test	0.064152

UTILIZATION	LITRES	COMPONENTS	WEIGHT	PRICE/KG	VALUE
CLASS 2		Butterfat		$7.64370	
		Protein		$5.72570	
		Other Solids		$5.72570	
		Totals			
CLASS 3A	856	Butterfat	30.110	$7.64370	$230.15
		Protein	26.493	$13.38660	$354.65
		Other Solids	48.749	$0.90720	$44.23
		Totals			$629.03
CLASS 3B		Butterfat		$7.64370	
		Protein		$13.13390	
		Other Solids		$0.89120	
		Totals			
CLASS 4A		Butterfat		$7.64370	
		Protein		$5.32730	
		Other Solids		$5.32730	
		Totals			
CLASS 4C		Butterfat		$7.64370	
		Protein		$5.32730	
		Other Solids		$5.32730	
		Totals			
CLASS 4D		Butterfat			
		Other Solids			
		Protein			
		Totals			
CLASS 4D(j)		Butterfat		$7.64370	
		Protein		$5.32730	
		Other Solids		$5.32730	
		Totals			
CLASS 5A		Butterfat			
		Protein			
		Other Solids			
		Totals			

LITRES UTILIZED	856

Total Component Values	$629.03
Component Adjustment (+/-)	
Interim Advance Received	-$521.08
Amount Due - Current Month:	$107.95

Final payment due 8 days subsequent to date of invoice.
1.5% per month service charge on all overdue accounts.

36 Scurfield Boulevard, PO Box 724, Stn Main Winnipeg, Manitoba R3C 2K3
Tel (204) 488-6455 Fax (204) 488-4772

APPENDIX F

Processing Log Sheets for Main Experiment

Food Science Department

Gouda Cheese Manufacturing Sheet

DATA	Amount of Rennet (ml) (25ml)/Lot #
Cheesemaker: Dora, Michael, Denise	Time Curd Cut: 12:20
Date: Apr 18/11	Acidity After Cutting (0.100): 0.12
Lot #: 1	Time Healing Started (5 – 10 minutes): 12:25
Milk Supplier: Glenlea	Time Agitation Started (20 – 30 minutes): 12:40
Milk type: Cow	Amount of Whey Removed (L) (1/3 of total whey) (~~58~~L)(66L): 66L
Temp of milk Rec'd (<5°C): 5.6°C	Acidity of Removed Whey (0.115): 0.13
Volume (L): 330	Hot Water Temperature (60 – 71°C): 70.7°C
Odor/Flavor	Amount of Water Added (L) (25% of whey removed) (~~38~~L) (16.5L): 17 L
Antibiotics: - ve.	Final Temperature of Whey (38 – 39°C): 35.1°C → 38.5°C
Acidity (~0.17): 0.16	Time Stirring Started (30 – 45 minutes): 1:25
Milk Fat %	Time Curd Hooped: 2:00
PROCESSING ACITIVITY	Acidity of Whey (0.08): 0.13
Past Temp / Time	Time Cheese Immersed into Brine: 2:30
Temp of Milk when culture added (31 – 32°C): 31.3°C	pH of Curd (5.3 – 5.5)
Time Culture Added: 10:20 am	Amount of Salt Added in Brine (kg) (~~18%~~)~~20%~~: 12kg → 60L
Type of Culture/Lot #: Lot 947151B143 CHOOZIT MA4001 1/0 25DCU	Salt Lot #
Amount of Culture Added: 25 DCU (9.7g)	pH of Brine (5.15 – 5.25): 7.81
Type of Probiotic /Amt of Probiotic Added: N/A	Cheese Yield
Acidity after Ripening: 0.17	Date Packaged
Time Rennet Added: 10:50	Samples Taken for Microbiology

Food Science Department

Gouda Cheese Manufacturing Sheet

DATA	Amount of Rennet (ml) (25ml)/Lot # 28 ml
Cheesemaker Dora Michael Denise	Time Curd Cut 11:15
Date Apr 26, 11	Acidity After Cutting (0.100) 0.12
Lot # 2	Time Healing Started (5 – 10 minutes) 11:20
Milk Supplier Glenlea	Time Agitation Started (20 – 30 minutes) 11:30
Milk type cow	Amount of Whey Removed (L) (1/3 of total whey) (50L) 66 L
Temp of milk Rec'd (<5°C)	Acidity of Removed Whey (0.115) 0.12
Volume (L) 230 L	Hot Water Temperature (60 – 71°C)
Odor/Flavor	Amount of Water Added (L) (25% of whey removed) (~~38L~~) 16.5 L
Antibiotics	Final Temperature of Whey (38 – 39°C) 39.9°C
Acidity (~0.17) 0.17	Time Stirring Started (30 – 45 minutes) 12:03
Milk Fat %	Time Curd Hooped 1:50
PROCESSING ACITIVITY	Acidity of Whey (0.08) 0.16
Past Temp / Time	Time Cheese Immersed into Brine 3:30
Temp of Milk when culture added (31 – 32°C) 32.3	pH of Curd (5.3 – 5.5) 5.27
Time Culture Added 9:25	Amount of Salt Added in Brine (kg) (19%) 22%
Type of Culture/Lot # Lot 44715181 43 Chozit MA 4001 LYO 25DCU	Salt Lot #
Amount of Culture Added 25 DCU	pH of Brine (5.15 – 5.25)
Type of Probiotic /Amt of Probiotic Added N/A	Cheese Yield
Acidity after Ripening ~~0.15~~ 0.16	Date Packaged
Time Rennet Added 10:07	Samples Taken for Microbiology

Food Science Department

Gouda Cheese Manufacturing Sheet

DATA	Amount of Rennet (ml) (25ml)/Lot #
	30ml
Cheesemaker Dora, Michael, Denise, Shirley	Time Curd Cut 11:50
Date May 3	Acidity After Cutting (0.100) 0.11
Lot # 3	Time Healing Started (5 – 10 minutes) 11:52
Milk Supplier Glenloa	Time Agitation Started (20 – 30 minutes) 12:20
Milk type cow	Amount of Whey Removed (L) (1/3 of total whey) ~~(50L)~~ 66L
Temp of milk Rec'd (<5°C)	Acidity of Removed Whey (0.115) 0.12
Volume (L) 250	Hot Water Temperature (60 – 71°C) 70°C
Odor/Flavor	Amount of Water Added (L) (25% of whey removed) ~~(38L)~~ 17.5L
Antibiotics	Final Temperature of Whey (38 – 39°C) 39.5°C
Acidity (~0.17) 0.12	Time Stirring Started (30 – 45 minutes) 12:45
Milk Fat %	Time Curd Hooped 1:35
PROCESSING ACITIVITY	Acidity of Whey (0.08) 0.14
Past Temp / Time	Time Cheese Immersed into Brine 4:20
Temp of Milk when culture added (31 – 32°C) 32.1°C	pH of Curd (5.3 – 5.5) 5.32
Time Culture Added 9:30	Amount of Salt Added in Brine (kg) (19%) 21.43%
Type of Culture/Lot # CHOOZIT MA 4001 LOT 447151843	Salt Lot #
Amount of Culture Added 25DCU	pH of Brine (5.15 – 5.25)
Type of Probiotic /Amt of Probiotic Added L ~~447~~ 7.24 B 5.74 NME	Cheese Yield
Acidity after Ripening 0.14	Date Packaged
Time Rennet Added 10:08	Samples Taken for Microbiology

Food Science Department

Gouda Cheese Manufacturing Sheet

DATA	Amount of Rennet (ml) (25ml)/Lot # 30ml Glengarry Marzyme Supreme
Cheesemaker Dan, Michael, Denise	Time Curd Cut 1:20
Date May 10/11	Acidity After Cutting (0.100) 0.13
Lot # 4	Time Healing Started (5 – 10 minutes) 11:20
Milk Supplier Glenlea	Time Agitation Started (20 – 30 minutes) 11:35
Milk type cow	Amount of Whey Removed (L) (1/3 of total whey) (50L) 64L
Temp of milk Rec'd (<5°C)	Acidity of Removed Whey (0.115) 0.12
Volume (L) 220.	Hot Water Temperature (60 – 71°C) 71
Odor/Flavor	Amount of Water Added (L) (25% of whey removed) (38L) 17.5L
Antibiotics	Final Temperature of Whey (38 – 39°C) 39.7°C
Acidity (~0.17) 0.18	Time Stirring Started (30 – 45 minutes) 12:06
Milk Fat %	Time Curd Hooped 1:20
PROCESSING ACITIVITY	Acidity of Whey (0.08)
Past Temp / Time	Time Cheese Immersed into Brine 3:25
Temp of Milk when culture added (31 – 32°C)	pH of Curd (5.3 – 5.5) 5.69
Time Culture Added 9:10	Amount of Salt Added in Brine (kg) (19%) 20%
Type of Culture/Lot # Lot 44715181/43 Chozit UA4001	Salt Lot #
Amount of Culture Added 25 DCU	pH of Brine (5.15 – 5.25)
Type of Probiotic /Amt of Probiotic Added NME B&L 5.7782853g	Cheese Yield
Acidity after Ripening 0.18	Date Packaged
Time Rennet Added 9:45	Samples Taken for Microbiology

Food Science Department

Gouda Cheese Manufacturing Sheet

DATA	Amount of Rennet (ml) (25ml)/Lot # 31ml
Cheesemaker Dora, Michael, Denise, Elena	Time Curd Cut 11:30
Date May 17	Acidity After Cutting (0.100) 0.1, 0.11
Lot # 5	Time Healing Started (5 – 10 minutes) 11:30
Milk Supplier Glenbea	Time Agitation Started (20 – 30 minutes) 11:45
Milk type cow	Amount of Whey Removed (L) (1/3 of total whey) (50L) 16L
Temp of milk Rec'd (<5°C)	Acidity of Removed Whey (0.115) 0.12
Volume (L)	Hot Water Temperature (60 – 71°C) 70°C
Odor/Flavor	Amount of Water Added (L) (25% of whey removed) (38L) 17.5L
Antibiotics -ve	Final Temperature of Whey (38 – 39°C) 39.7°C
Acidity (~0.17) 0.14	Time Stirring Started (30 – 45 minutes) 12:35
Milk Fat %	Time Curd Hooped 1:35
PROCESSING ACITIVITY	Acidity of Whey (0.08) 0.13
Past Temp / Time	Time Cheese Immersed into Brine 3:25
Temp of Milk when culture added (31 – 32°C) 32.1°C	pH of Curd (5.3 – 5.5) 5.69
Time Culture Added 9:45	Amount of Salt Added in Brine (kg) (19%) 21.07
Type of Culture/Lot # Choozit MA4001 44715181 43	Salt Lot #
Amount of Culture Added 25DCU	pH of Brine (5.15 – 5.25)
Type of Probiotic /Amt of Probiotic Added ME BAL 38.6g & 15g	Cheese Yield
Acidity after Ripening 0.15	Date Packaged
Time Rennet Added 10:18	Samples Taken for Microbiology

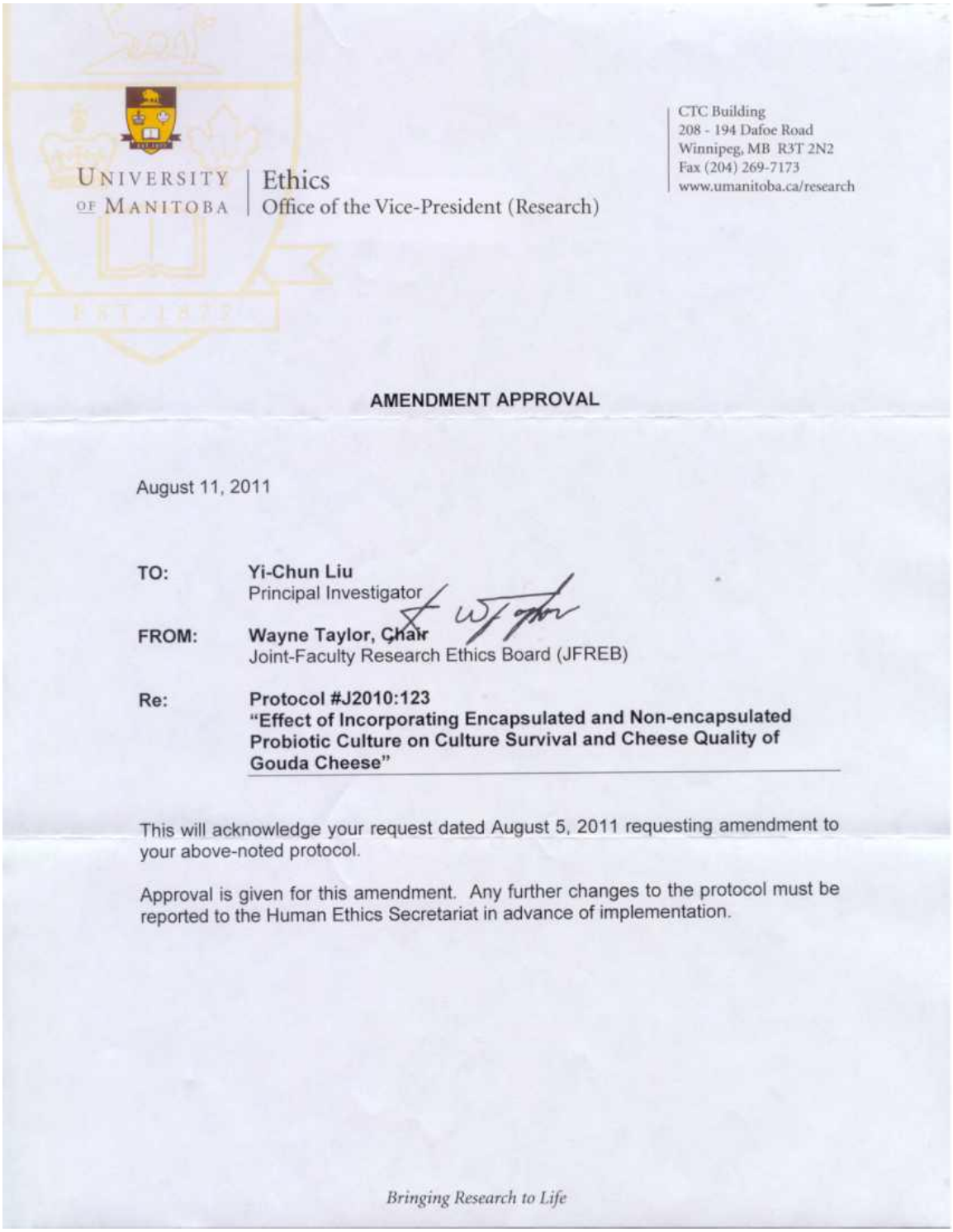
Food Science Department

Gouda Cheese Manufacturing Sheet

DATA	Amount of Rennet (ml) (25ml)/Lot # 32ml
Cheesemaker Denise, Michael, Dan	Time Curd Cut 12:30
Date May 24	Acidity After Cutting (0.100) 0.12, 0.11
Lot # 6	Time Healing Started (5 – 10 minutes) 12:30
Milk Supplier Glenlea	Time Agitation Started (20 – 30 minutes) 12:45
Milk type cow	Amount of Whey Removed (L) (1/3 of total whey) (50L) 66 L
Temp of milk Rec'd (<5°C)	Acidity of Removed Whey (0.115) 0.15
Volume (L) 220 L	Hot Water Temperature (60 – 71°C) 70°C
Odor/Flavor	Amount of Water Added (L) (25% of whey removed) (38L) 17.5 L
Antibiotics -ve	Final Temperature of Whey (38 – 39°C) 39.5
Acidity (~0.17) ~~0.17~~ 0.155	Time Stirring Started (30 – 45 minutes) 1:30
Milk Fat %	Time Curd Hooped 2:10
PROCESSING ACITIVITY	Acidity of Whey (0.08) 0.165
Past Temp / Time	Time Cheese Immersed into Brine
Temp of Milk when culture added (31 – 32°C) 31°C	pH of Curd (5.3 – 5.5)
Time Culture Added 10:00	Amount of Salt Added in Brine (kg) (19%) 20.36 kg
Type of Culture/Lot # CHOOZIT 4001 447 15/08/13	Salt Lot #
Amount of Culture Added 25DCU	pH of Brine (5.15 – 5.25)
Type of Probiotic /Amt of Probiotic Added ME BSL 38.73/14.71	Cheese Yield
Acidity after Ripening 0.15	Date Packaged
Time Rennet Added 10:30	Samples Taken for Microbiology

APPENDIX G

Sensory Ethics Approval Letter – Main Experiment



UNIVERSITY OF MANITOBA | Ethics
Office of the Vice-President (Research)

CTC Building
208 - 194 Dafoe Road
Winnipeg, MB R3T 2N2
Fax (204) 269-7173
www.umanitoba.ca/research

AMENDMENT APPROVAL

August 11, 2011

TO: **Yi-Chun Liu**
Principal Investigator

FROM: **Wayne Taylor, Chair**
Joint-Faculty Research Ethics Board (JFREB)

Re: **Protocol #J2010:123**
"Effect of Incorporating Encapsulated and Non-encapsulated Probiotic Culture on Culture Survival and Cheese Quality of Gouda Cheese"

This will acknowledge your request dated August 5, 2011 requesting amendment to your above-noted protocol.

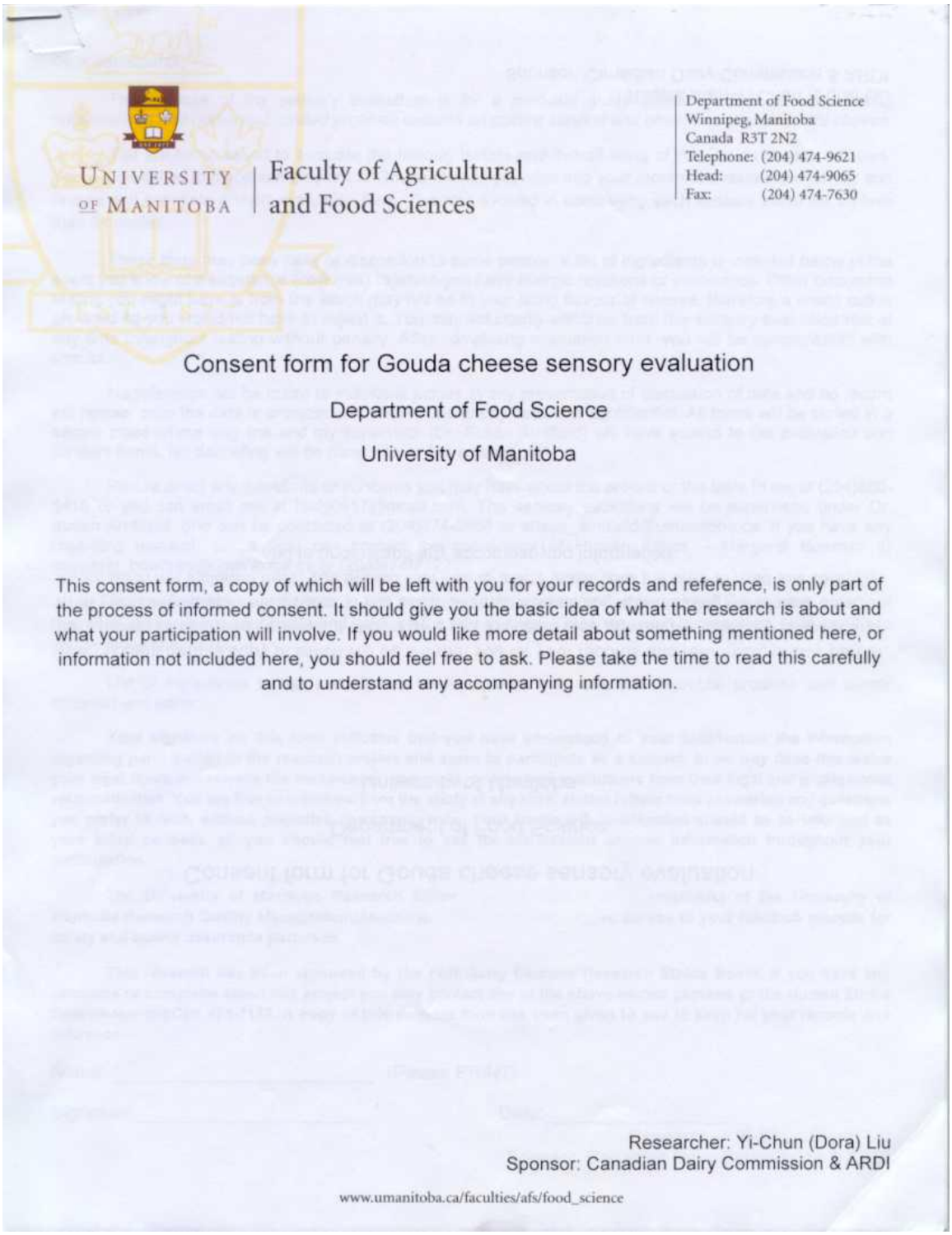
Approval is given for this amendment. Any further changes to the protocol must be reported to the Human Ethics Secretariat in advance of implementation.

Bringing Research to Life

APPENDIX H

Sensory Evaluation Forms – Main Experiment

Consent Form – For Both Descriptive and Affective Tests



UNIVERSITY OF MANITOBA | Faculty of Agricultural and Food Sciences

Department of Food Science
Winnipeg, Manitoba
Canada R3T 2N2
Telephone: (204) 474-9621
Head: (204) 474-9065
Fax: (204) 474-7630

Consent form for Gouda cheese sensory evaluation

Department of Food Science

University of Manitoba

This consent form, a copy of which will be left with you for your records and reference, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

Researcher: Yi-Chun (Dora) Liu
Sponsor: Canadian Dairy Commission & ARDI

www.umanitoba.ca/faculties/afs/food_science

Dear participants:

The purpose of the sensory evaluation is for a graduate study thesis – *Effect of incorporating encapsulated and non-encapsulated probiotic cultures on culture survival and cheese quality of Gouda cheese.*

You are being asked to evaluate the flavour, texture and overall liking of the Gouda cheese samples. You will be asked to place samples of the Gouda cheese provided into your mouth to evaluate the flavour and texture and complete a short evaluation form. The time evolved in completing each sensory panel will be less than 5 minutes.

These tests may pose risks or discomfort to some people; a list of ingredients is included below in the event you know of a substance (cow milk) to which you have allergic reactions or intolerance. Other discomfort feeling you might have is from the which may not be to your liking flavour of cheese, therefore a waste cup is provided so you would not have to ingest it. You may voluntarily withdraw from this sensory evaluation test at any time throughout testing without penalty. After completing evaluation form, you will be compensated with snacks.

No reference will be made to individual judges in any presentation of discussion of data and no record will remain once the data is analyzed. All data will be anonymous and confidential. All forms will be stored in a secure place where only me and my supervisor (Dr. Susan Arntfield) will have access to the evaluation and consent forms. No debriefing will be done prior to the sensory test.

Please direct any questions or concerns you may have about the project or the tests to me at (204)880-5418 or you can email me at liudy0417@gmail.com. The sensory evolutions will be supervised under Dr. Susan Arntfield. She can be contacted at (204)474-9866 or susan_arntfield@umanitoba.ca. If you have any regarding research ethics, you can contact the coordinator of Human Ethics – Margaret Bowman at margaret_bowman@umanitoba.ca or (204)474-7122.

Your willingness to participate in this panel is greatly appreciated. If you would like to have a copy of the result, please leave your name and email on the sign-in sheet. Once the result is organized, I will email it to you by November, 2011.

List of ingredients in this test: Gouda cheese (cow's milk, salt and microbial probiotic and starter cultures) and water.

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release the researchers, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time, and/or refrain from answering any questions you prefer to omit, without prejudice or consequence. Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation.

The University of Manitoba Research Ethics Board(s) and a representative(s) of the University of Manitoba Research Quality Management/Assurance office may also require access to your research records for safety and quality assurance purposes.

This research has been approved by the Fort Garry Campus Research Ethics Board. If you have any concerns or complaint about this project you may contact any of the above-named persons or the Human Ethics Coordinator (HEC)at 474-7122. A copy of this consent form has been given to you to keep for your records and reference.

Name: _____ (Please PRINT)

Signature: _____ Date: _____

Researcher: Yi-Chun (Dora) Liu
Sponsor: Canadian Dairy Commission & ARDI

Questionnaire – Descriptive Test

Panellists Questionnaire
Cheese Evaluation

THIS INFORMATION WILL REMAIN STRICTLY CONFIDENTIAL

Name: _____

Phone: _____

Email address: _____

1. Have you participated in a sensory evaluation before?

   ☐ Yes ☐ No

   If yes, what product(s) did you evaluate?

   _____

   _____

   Was training part of the evaluation procedure?

   ☐ Yes ☐ No

   If yes, indicate which product(s)

   _____

   _____

2. Are you allergic to any food product(s)?

   ☐ Yes ☐ No

   If yes, please note them

   _____

3. Are you taking any medications which affect your senses, especially taste and smell? _____

4. Do you have any dental work that will affect your evaluation of texture?

   ☐ Yes ☐ No

5. How often do you consume cheese?

   ☐ <1 per month ☐ 1 – 3 times per month ☐ >5 times per month

6. Have you tried Gouda cheese?

   ☐ Yes ☐ No

   If yes, how often?

   ☐ <1 per month ☐ 1 – 3 times per month ☐ >5 times per wk

7. What type of cheese do you prefer?

   ☐ Mild ☐ Medium ☐ Mature/Sharp Cheese

Recruitment Poster – Affective Test

APPENDIX I

Sensory Evaluation Instruction and Ballot – Main Experiment

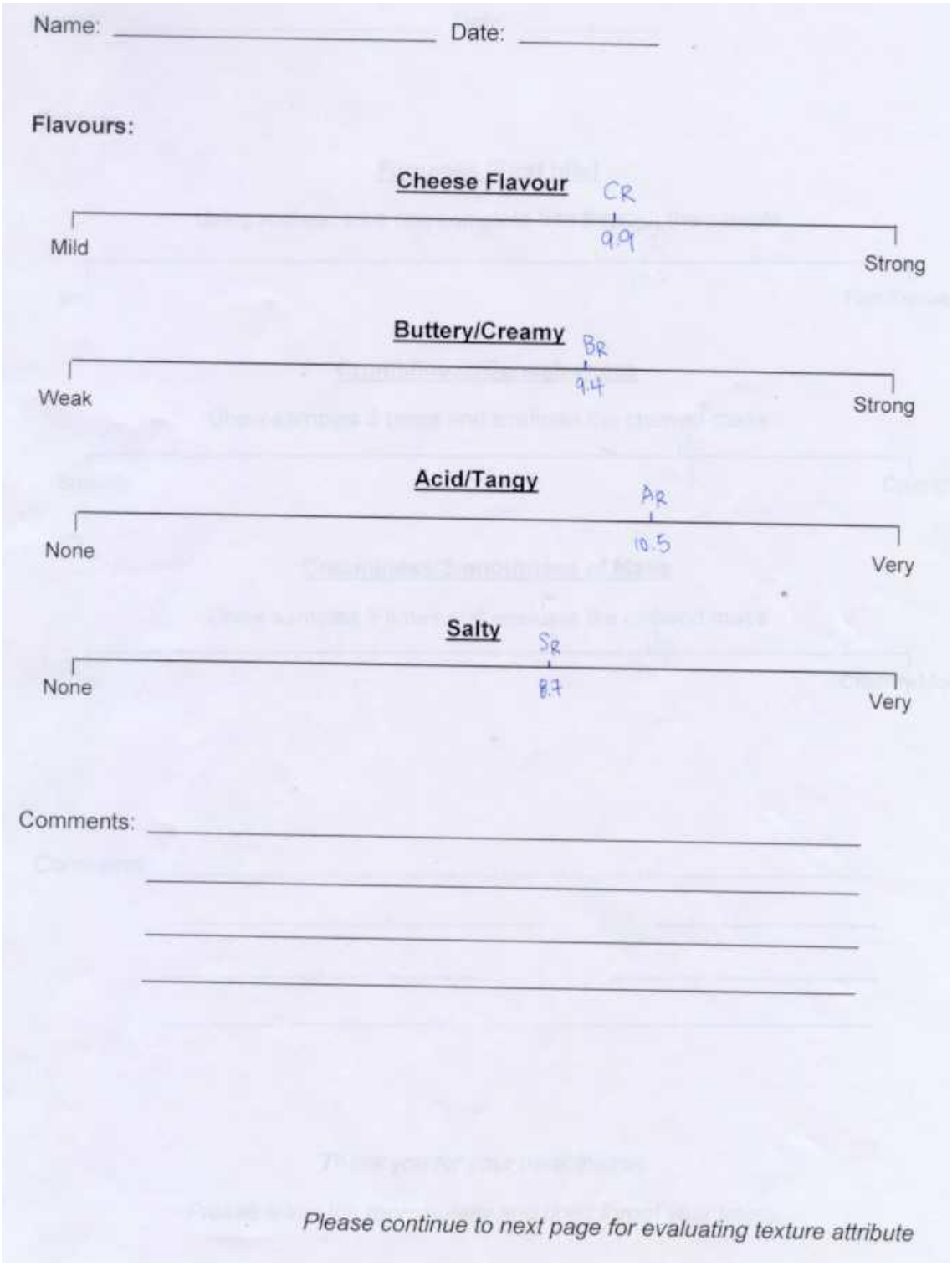
Sensory Instruction – Descriptive Test

PLEASE READ BEFORE BEGINS EVLUATION OF SAMPLES

Sensory Evaluation Instruction

- Please put your name and date of evaluation on the questionnaire.
- Please cleanse your mouth with unsalted soda crackers and water provided before beginning your first sample.
- Standards have been provided as references. Rinse the mouth thoroughly with unsalted soda crackers and water between standards and samples.
- Evaluate the samples from left to right.
- Evaluate the flavour parameters FIRST and then evaluate the texture attributes.
- Taste the cheese cubes for its flavour and texture attributes.
- Please evaluate each parameter.
- Please mark the sample code on each tick you put on the line. There should be 6 marks on each line.
- Please taste the standards before begins tasting the samples. Refer back to standards if needed.
- Cleanse your mouth with unsalted soda cracker and water in between samples to remove the flavour from the previous sample.
- If you do not like the sample, the waste cup is provided for you to expectorate the sample. Ingestion of sample/standard is not mandatory.
- When you are done, please leave quietly.
- **Don't forget to take your treat and thank you for your participation!**

Sensory Ballot – Descriptive Test



Name: _____ Date: _____

Flavours:

Cheese Flavour CR

Mild — 9.9 — Strong

Buttery/Creamy BR

Weak — 9.4 — Strong

Acid/Tangy AR

None — 10.5 — Very

Salty SR

None — 8.7 — Very

Comments: _____

_____

_____

_____

Please continue to next page for evaluating texture attribute

Name: _____ Date: _____

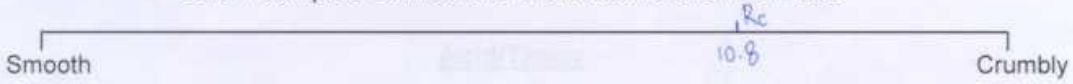
Texture:

Firmness (First bite)

Using molars, take one complete bite through the sample

Crumbliness/Cohesiveness

Chew samples 5 times and evaluate the chewed mass



Creaminess/Smoothness of Mass

Chew samples 5 times and evaluate the chewed mass

Comments: _____

_____

_____

_____

Thank you for your participation.

Please leave the room quietly and don't forget your treats.

Sensory Instruction – Affective Test

PLEASE READ BEFORE BEGINS EVALUTION OF SAMPLES

SENSORY EVALUATION INSTRUCTIONS

- Please put the color of the toothpick as sample name
- Please cleanse your mouth with unsalted soda crackers and water provided before beginning your first sample.
- Evaluate the samples from left to right
- Please evaluate all samples
- Please answer all the questions (there are two pages)
- Cleanse your mouth with unsalted soda crakers and water in between samples to remove the flavour from the previous sample
- If you do not like the sample, the waste cup is provided fro you to expectorate the sample. Ingestion of sample is not mandatory.
- **Don't forget to take your treat and thank you for your participation.**
- **The winners of the bookstore gift cards will be announced in October.**

Sensory Ballot – Affective Test

Questionnaire For Gouda Cheese Consumer Test

Gender: ☐ Male ☐ Female

Age Group: ☐ Under 18 ☐ 18 - 25 ☐ 25 – 40 ☐ 40 - 60 ☐ Over 60

- Please rinse your mouth before starting.
- Evaluate the samples in front of you by tasting it to evaluate flavour, texture, and overall liking of each sample by checking one box [√].

Sample_____

Flavour

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike Extremely — Neither Like or Dislike — Like Extremely

Texture

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike Extremely — Neither Like or Dislike — Like Extremely

Overall Liking

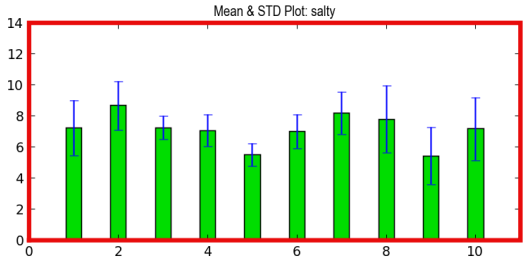
☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

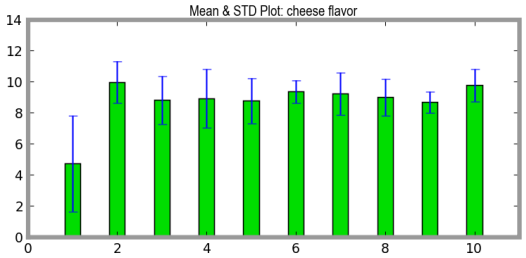
Dislike Extremely — Neither Like or Dislike — Like Extremely

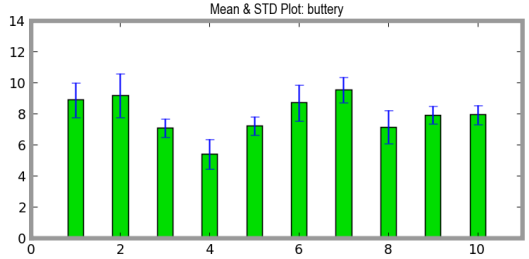
APPENDIX J

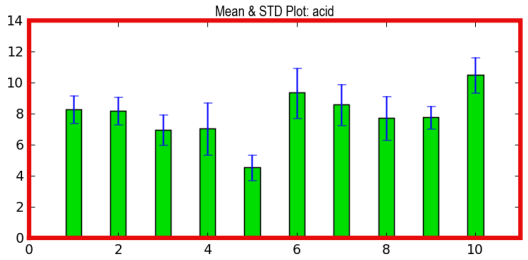
PanelCheck Result – Trained Panel

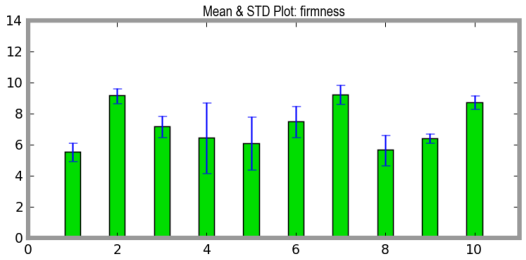
First session

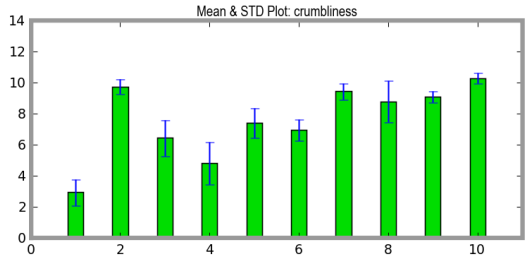


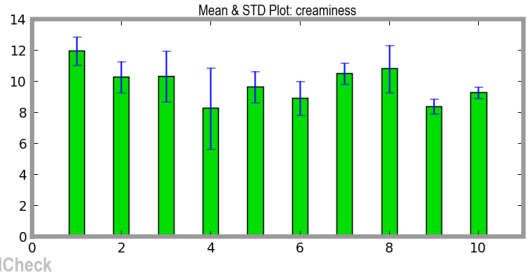






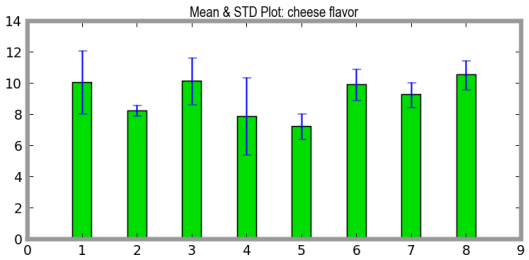


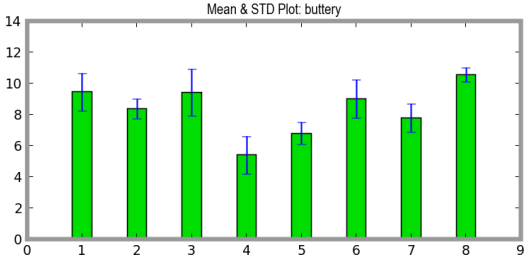


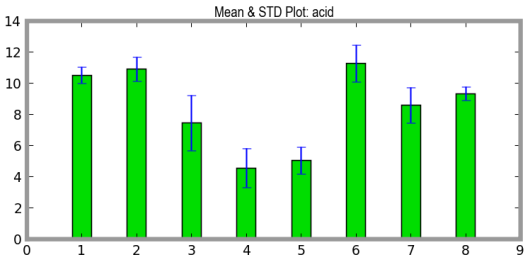


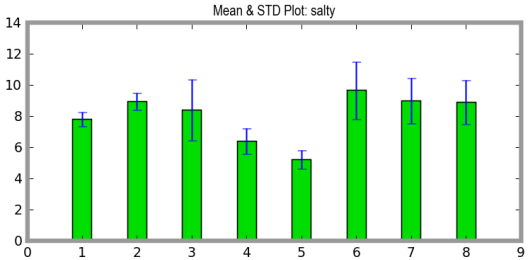
PanelCheck

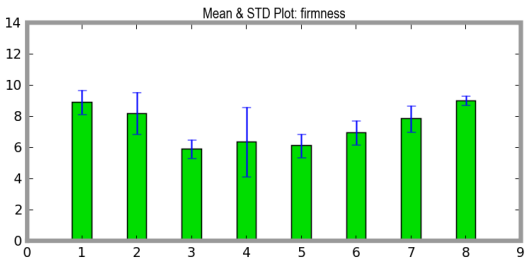
Second session

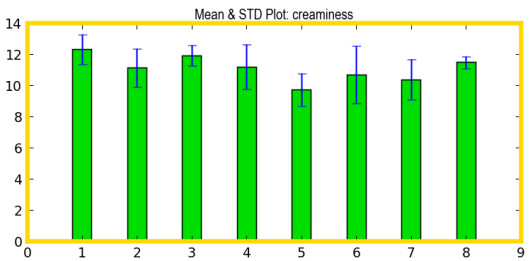












PanelCheck

Third session