SURVIVALITY OF *LACTOBACILLUS ACIDOPHILUS* & *BIFIDOBACTERIUM BIFIDUM* AND PHYSICO CHEMICAL PROPERTIES OF FERMENTED ICE CREAM MADE WITH COW MILK, SOYBEAN EXTRACT AND COCONUT MILK INDIVIDUALLY AND IN COMBINATION

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THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE OF BIOLOGICAL SCIENCES
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UNIVERSITY OF MALAYA
KUALA LUMPUR

2015

UNIVERSITY OF MALAYA

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Survivality of *Lactobacillus acidophilus* & *Bifidobacterium bifidum* and physico chemical properties of fermented ice cream made with cow milk, soybean extract and coconut milk individually and in combination

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ABSTRACT

The present study investigated the effects of cow milk (W; control), soybean extract (S), coconut (C) and composite milks (combinations of coconut or cow milks with soybean extract) on the survival of *Bifidobacterium bifidum* (Bb-12; B) and *Lactobacillus acidophilus* (La-05; L) in ice cream and also on the physicochemical and organoleptic properties of bio-ice cream, both with and without fermentation step prior to the freezing of ice cream. The total free amino acids increased considerably in the presence of soybean extract or coconut milk compared to ice cream made with 100% cow milk (control). In comparison to cow milk ice cream, the survival of both probiotics in non fermented ice cream increased slightly in the presence of soybean or coconut extracts. The presence of vegetable extracts in ice creams enhanced the microbial metabolic activity (decreased time required for the pH to reduce 5.50 and colony forming unit). The effect of coconut milk on the microbial metabolic activity and colony forming unit was more pronounced than that by soybean extract. The survival of probiotic bacteria in frozen fermented ice creams after 90 days was higher for Bb-12 than for La-05. Ice creams containing coconut milk had a higher Bb-12 and La-05 survival than ice creams containing cow milk whereas the survival of both probiotics increased with increasing soybean extract content in composite milk ice creams. Simulated gastrointestinal studies demonstrated Bb-12 showing greater tolerance than La-05 to acidic (gastric juice; pH = 2.0) and alkaline conditions (small intestinal juice; 0.3% bile). For composite milk ice cream, the survival of Bb-12 and La-05 in both digestive juices was higher in ice creams containing cow milk than in ice creams containing coconut milk. Increasing soybean extract content in ice creams also increased both probiotics survival. All vegetables and composite milk non fermented ice creams showed a slower melting rate than control ice cream. Amongst ice creams with composite milk, those

containing coconut milk had higher apparent viscosity and fat globule sizes than others. The presence of soybean extract in ice cream made with composite milk increased hysteresis, apparent viscosity and consistency index and decreased the amount of freezable water and the total consumer panelist acceptability. Fermented ice cream made with soybean extract or coconut milk and composite milks showed a slower melting rate than control ice cream. Ice creams containing cow milk had a higher melting rate and lower apparent viscosity than ice creams containing coconut milk, and also those containing La-05 had lower melting rate and higher apparent viscosity than ice creams containing Bb-12. Ice creams without soybean extract had lower apparent viscosity than ice creams containing soybean extract. In conclusion, the replacement of cow milk with vegetable extract markedly improved the physicochemical properties and survival of probiotics. Soybean extract had the strongest influence on increasing the values of the consistency index, apparent viscosity, hysteresis and survival of probiotics under gastric condition whereas coconut milk markedly enhanced the growth of probiotics and their survival during frozen storage.

ABSTRAK

Kajian ini menyiasat kesan susu lembu (W; kawalan), kacang soya (S), santan kelapa (C) dan susu komposit (kombinasi santan kelapa atau susu lembu dengan susu soya) terhadap survival *Bifidobacterium bifidum* (Bb-12; B) dan *Lactobacillus acidophilus* (La-05; L) dalam ais krim dan juga pada fizikokimia nilai sifat organoleptik bio-ais krim, kedua-duanya dengan dan tanpa penapaian sebelum pembekuan ais krim. Jumlah asid amino bebas meningkat dengan ketara dengan kehadiran soya atau santan kelapa susu masing-masing berbanding dengan ais krim dibuat dengan 100% susu lembu (kawalan). Survival kedua-dua probiotik dalam ais krim takditapai meningkat sedikit dengan kehadiran susu soya atau santan kelapa. Kehadiran ais krim susu soya atau ais krim santan kelapa meningkatkan aktiviti metabolisme mikrob dan colony forming unit (masa yang diperlukan untuk pH turun ke 5.50 berkurangan). Kesan santan kelapa adalah lebih ketara terhadap aktiviti metabolik mikrob dan colony forming unit berbanding dengan susu soya. Survival bakteria probiotik dalam ais krim ditapai selepas 90 hari sejuk beku adalah lebih tinggi untuk Bb-12 daripada untuk La-05. Ais krim yang mengandungi santan kelapa mempunyai kelangsungan hidup Bb-12 dan La-05 yang lebih tinggi daripada ais krim yang mengandungi susu lembu manakala survival Bb-12 dan La-05 meningkat dengan peningkatan kandungan susu soya dalam ais krim komposit susu. Kajian simulasi gastriointestinal menunjukkan Bb-12 mempunyai toleransi yang lebih besar daripada La-05 untuk keadaan berasid (jus gastrik; pH = 2.0) dan syarat alkali (jus usus kecil; 0.3% hempedu). Untuk ais krim komposit, survival Bb-12 dan La-05 dalam kedua-dua jus penghadaman adalah lebih tinggi di dalam ais krim yang mengandungi susu lembu daripada ais krim yang mengandungi santan kelapa. peningkatan kandungan susu soya dalam ais krim juga meningkat kan survival Bb-12 dan La-05. Semua ais krim komposit susu tak

ditapai menunjukkan kadar pencairan yang lebih perlahan daripada ais krim susu lembu. Di antara ais krim dengan susu komposit, yang mengandungi santan keapa mempunyai kelikatan ketara yang lebih tinggi dan saiz titisan lemak mPa s daripada yang lain. Kehadiran susu soya dalam ais krim yang dibuat dengan susu komposit mengurangkan jumlah air boleh beku yang menyebabkan peningkatan hysterisis, kelikatan ketara dan indeks ber konsisten dan pengurangan jumlah kebolehterimaan ahli panel konsumer. Ais krim tertapai yang dibuat dengan susu soya atau santan kelapa dan susu komposit menunjukkan kadar cair yang lebih perlahan daripada susu lembu ais krim (kawalan). Ais krim yang mengandungi susu lembu mempunyai kadar yang pancairan lebih cepat dan kelikatan ketara lebih rendah daripada ais krim yang mengandungi santan kelapa, dan ais krim yang mengandungi La-05 mempunyai kadar cair yang lebih rendah dan kelikatan ketara lebih tinggi daripada ais krim yang mengandungi Bb-12. Ais krim tanpa susu soya mempunyai kelikatan ketara lebih daripada ais krim yang mengandungi susu soya. Kesimpulannya, penggantian susu lembu dengan susu soya atau santan kelapa jelas meningkatkan ciri-ciri fizikokimia dan kelangsungan hidup probiotik. Susu soya mempunyai pengaruh yang paling kuat untuk meningkatkan nilai-nilai indeks berkonsisten, kelikatan ketara, hysterisis dan survival probiotik hidup dalam keadaan gastrik manakala santan meningkatkan dangan ketara pertumbuhan probiotik dan kelangsungan hidup mereka semasa penyimpana sejo beku.

DEDICATION

I would like to dedicate this thesis to my parents and sisters. There are no words to describe the love, appreciation and admiration that I have for you guys. You have made me the person who I am today; you have always believed in me and supported every single one of my actions, and for that, I am forever grateful. I am truly blessed to have such loving and supportive family like you. THANK YOU!

ACKNOWLEDGEMENT

First, I want to thank God for giving me life and faith and strength in everything I do and guiding my steps all of the time.

My appreciation goes to my supervisor Associate Professor Dr. Ahmad Salihin Baba and my co supervisor Professor Dr. Misni Misran for their guidance, patience and understanding throughout the years and always encouraging me to do my best academically and personally. For believing in me and affording me the much needed opportunity to work with them.

I would like to thank all lab assistance for their advice to use the equipment in the laboratories especially colloids laboratory team.

I would like to extend my sincerest thank you and dedication of this dissertation to my parents for their continuous me support, love, advice and financial support throughout all my education. To my sisters for always "having my back" and listening to me when I needed someone to talk, for that I love them and thank them.

Last but not least, I would like to express my sincere and heartiest thanks to all good hearted people who helped me during the completion of my research and thesis but are not mentioned here, I grateful thank you all for your help.

Fatemeh Aboulfazli

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

LAB = Lactic acid bacteria

ssp = subspecies

Bb-12 = *Bifidobacterium bifidum*

La-05 =*Lactobacillus acidophilus*

B = *Bifidobacterium bifidum*

L = *Lactobacillus acidophilus*

W = ice cream made with 100% cow milk;

C = ice cream made with 100% coconut milk;

S = ice cream made with 100% soybean extract.

SW1 = ice cream made with 75% soybean extract+25% cow milk

SW2 = ice cream made with 50% soybean extract+50% cow milk.

SW3 = ice cream made with 25% soybean extract+75% cow milk.

SC1 = ice cream made with 75% soybean extract+25% coconut milk.

SC2 = ice cream made with 50% soybean extract+50% coconut milk.

SC3 = ice cream made with 25% soybean extract+75% coconut milk.

U = upward flow curve

D = downward flow curves

cfu = colony forming unit

MRS = de Man Rogosa and Sharpe agar

SGD = simulated gastrointestinal digestion

ANOVA = analysis of variance

rpm = revolution per minute

HCl = hydrochloric acid

NaOH = sodium hydroxide

L-Cys-HCl = Cystein hydrochloride

TA = titratable acidity

TS = total solid

dH_2O = distilled water

LC/MS = Liquid chromatography/mass spectrometry

TAA = total amino acid

σ = the shear stress

K = consistency index

γ = the shear rate

n = the flow behavior index.

MRD = maximum recovery diluents

T_0 = the onset temperatures

T_p = peak temperatures

T_f = freezing points

ΔH_f = enthalpies

ΔH_s = pure ice fusion latent heat

DSC = differential scanning calorimetry

OPM = optical polarizing microscope imaging

FF = functional foods

CHAPTER 1 INTRODUCTION

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1.1 Background

The consumption of functional foods (FF) is increasing rapidly worldwide because of increased consumers' awareness about the importance of diet and health (Salem *et al*., 2005). FF are foods considered to provide benefits beyond basic nutrition and may play a role in reducing or minimizing the risk of certain diseases and other health conditions. Examples of these foods include fruits and vegetables, whole grains, fortified foods and beverages and many processed food consumed as dietary supplements. New food products are being developed to include beneficial components such as probiotics and functional components isolated from plants (Grajek *et al*., 2005).

Ice cream is a delicious, wholesome and nutritious frozen dairy product widely cherished in many parts of the world. Ice cream has nutritional significance but encompasses no therapeutic properties (Salem *et al*., 2005). Ice cream is traditionally made from cows' milk and thus contains about 15–17% (w/w) lactose (Supavititpatana and Kongbangkerd, 2011). The demand for alternatives to cow' s milk is growing due to problems associated with its fat, cholesterol and lactose contents. Ice cream can be made functional by adding fruits, protein rich ingredients, partial or full replacement of cow milk using vegetable extract (e.g. coconut milk and soybean extract) and the addition of probiotics.

Increased utilization of soy ingredients in the food industries is encouraged by their high nutritional quality especially with respect to protein and amino acids (Gandhi *et al*., 2001). Frequent consumption of soy products offers health benefit including lowering the risk of getting breast and prostate cancers, diseases associated with arterial and

cardiovascular system, protective effects against obesity, diabetes, bone and kidney diseases (Dervisoglu *et al*., 2005). Soy protein may also be used for improving physical properties of foods and have been studied as successful replacers for animal proteins in food foams and emulsions (Mahdian *et al*., 2012). Soybean extract as cow milk alternative is known to be nutritionally helpful to address issues related to animal milk (Kolapo and Olubamiwa, 2012). Fortification of yogurt ice cream with soy protein can improve the quality of the product including texture, firmness and viscosity (Mahdian *et al*., 2012). Lecithin in the soy ingredient not only acts as emulsifiers but also helps increase the viscosity, stability, texture and extends the melting time of the ice cream (Samoto *et al*., 2007). Abdullah *et al*. (2003) experimented on improving the quality of ice cream by using different ratios of skim milk in soybean extract blend and found that large quantity of skim milk with soybean extract reduces the beany flavour of soybeans and increased the quality of ice cream. The options for other vegetable extract may increase in the future. Coconut milk is another vegetable extract that may be used to replace cow milk. It is a popular substitute for cow's milk in the tropics because it is simple to prepare, highly digestible and contains an abundance of nutrients (Wangcharoen, 2008). Coconut milk is rich in minerals (calcium, phosphorus and potassium), vitamins (vitamins C, E and many B vitamins) and antioxidants. The fatty acids (high oleic and lauric acid) in coconut milk are instrumental in preventing arteriosclerosis (Belewu and Belewu, 2007). A challenge in using coconut milk or soybean extract in ice cream is to stabilize the colloidal system unique to these vegetable extracts. For example, lecithin in the soybean extract is responsible for the formation of hard ice cream that makes this ice cream typically requires about 15 minutes to soften before serving (Wangcharoen, 2012). Thus it is important to establish to what extent the physical properties of ice cream may be affected by using coconut or soybean extract s as cow milk replacer.

Probiotic cultures may also be added into ice cream to produce ice cream with functional properties in the intestinal fact. Probiotics are defined ‘as live microorganisms which, when administered in adequate amounts confer several health benefits to the consumers’. These include improvement in intestinal microbiota, activation of the immune system, reduction in serum cholesterol and inhibition of the growth of potential pathogens (Grajek *et al*., 2005). The production of such probiotic ice cream may also involve a brief fermentation step (Favaro-Trindade *et al.,* 2007; Pandiyan *et al*., 2012a&b) that resulted in the formation of fermented ice cream that combines the physical characteristics of ice cream with the sensory and nutritional properties of fermented milk products (Pinto *et al.,* 2012). Fermented ice cream also provide the opportunity to mask too strong a yogurt flavour apart from benefitting this type of cultured milk product as a base for healthy ice cream (Salem *et al.,* 2005).

Soybean extract and coconut milks are rich media that can support the growth and reproduction of probiotic bacteria (Farnworth *et al*., 2007). Both milks contain carbohydrates (primarily sucrose and some starch), lipid, minerals (phosphorous, calcium, and potassium) and protein (Yuliana *et al*., 2010). Hence, ice creams made with vegetable extract can support the growth of probiotics by fulfilling the microbes growth requirement for amino acids and/or carbohydrates (Farnworth *et al*., 2007). Soybean extract may contribute to the unfavourable beany flavour but this may be reduced by fermenting soybean extract with *Lactobacillus acidophilus* (Desai *et al*., 2002). Thus the addition of probiotics into ice creams made with vegetable extracts may improve not only the growth and survival of probiotics but also the sensory properties of ice creams.

In order for probiotics to flourish in the intestine and exert their beneficial effects on the host, these microbes have to survive the passage through the host’s harsh digestive tract

environment (i.e., gastrointestinal tract, tolerating acid, bile and gastric enzymes; Maragkoudakis *et al*., 2006). The main factors detrimental to the viability of probiotics in the stomach are the low pH and antimicrobial action of pepsin. The pH of the stomach (typically 2.5-3.5) can reduce to as low as 1.5, or as high as 6.0 or even higher during periods immediately after food intake. Probiotic bacteria may also need to survive the small intestinal environment, i.e. exposure to pancreatin and bile salts with typical pH of around 8.0. Food generally remains in the stomach for 2–4 h prior to the 1-4 h intestinal transit through the small intestine. Thus it is important to understand the importance of increasing the chances of probiotic survival during the gastric intestinal transit.

The tolerance of probiotic bacteria to the stomach and small intestine conditions is influenced, amongst others by the carrier food, which may protect probiotic bacteria from acid conditions and enhance gastric survival (Huang and Adams, 2004). The protective effects on probiotic by food against the gastrointestinal stress are (i) the increase in the pH of the gastric tract due to food formulations with appropriate pH (>5) and high buffering capacity and (ii) reducing their physical exposure to the harsh gastrointestinal environment (Ranadheera *et al*., 2012). This study was demonstrated in earlier studies when probiotics were incorporated into cheese high in fat content (Stanton *et al*., 1998; Valerio *et al*., 2006), amylose enriched maize starch granules (Wang *et al*., 1999) and into two kinds of liquid vegetarian foods, So-Goodk original soybean extract Up & Go® liquid breakfast, and So-Good™ original soybean extract (Huang and Adams, 2004). Therefore, the use of suitable food matrices needs to be thoroughly evaluated to maximize probiotic efficacy (Huang and Adams, 2004). The focus of this thesis is to establish benefits of the presence of vegetable extract in ice cream on the survival of probiotics.

1.2 Problem statement

A profound understanding in the relationship between food and health is integral in the development of new functional foods (Bhat and Bhat, 2010). The dairy industry, in particular, has a vast potential to incorporate probiotic cultures into milk for the purpose of development of new functional products (Champagne *et al*., 2005). Probiotic food is defined as a food product that contains viable probiotic microorganisms in sufficient quantities (Saxelin *et al*., 2003). Some of the main health benefits related to probiotics are prevention and treatment of diarrhea, anti-microbial activity, relief of symptoms caused by lactose intolerance, anti-carcinogenic and anti-mutagenic activities, and stimulation of the immune system (Shah, 2007). The survival of probiotic bacteria is very important in relation to their therapeutic values (i.e. colonization of large intestine; Sanz, 2007). This means that their viability must be kept intact at all steps of the food processing operation: from the production, transportation, "shelf" storage until being ingested by the consumer, and to subsequently survive the gastrointestinal tract environment (Saxelin *et al*., 2003). The acidic nature of fermented milk (yogurt) may unfortunately cause loss of viable probiotic (Donkor *et al*., 2006). In this regard ice cream, due to its neutral pH, may be used to deliver the probiotics (Akın *et al*., 2007). However, the freezing process in ice cream making affects dramatically the number of live probiotic cells (Magarinos *et al*., 2007). As such the inclusion of ice cream ingredients which can provide additional freezing protection to cells are really needed to sustain viable probiotics.

The replacement of cow milk with soybean extract is known to improve the pH of probiotic ice cream for increased survival of probiotics (Heenan *et al*., 2004). The unique nutrient compositions in coconut and soybean extract s are expected to support the growth and survival of the lactic acid bacteria in ice cream and increase the nutritional components

and improve health benefits of probiotic ice creams. For instance the lecithin of soybean extract may act as emulsifier and thus provide physical protection against freezing damage and acidic gastric condition by encapsulating probiotics with their lecithin and proteins. The soy proteins are also able to form a stable network looks like a gel structure (Akesowan, 2009). The raw bean flavour limits the wide consumption of soybean extract and other soybean products (Wang *et al*., 2002). However this could be reduced by fermenting soybean extract with *Lactobacillus acidophilus* (Desai *et al*., 2002). The lactic acid bacteria fermentation of soybean extract also considerably increases soybean extract antioxidative activity (Stijepic *et al*., 2013), thus making the fermented soybean extract healthier than pure soybean extract. In addition, fermented dairy products play a functional role either directly through interaction with consumed microorganisms (probiotic effect) or indirectly as a result of action of microbial metabolites like vitamins, proteins, peptides, oligosaccharides and organic acids generated during the fermentation process (Bhat and Bhat, 2011). Thus fermented milk contains intrinsic milk nutritious properties, healthy bacteria and fermentation products (bioactive peptides, free fatty acids with healthy properties such as anti-diabetic and anti-hypertensive properties (Östman *et al*., 2001; Papadimitriou *et al*., 2007; Donkor *et al*., 2007). Another big challenge in using soybean extract in ice cream is to stabilize the colloidal system unique to these vegetable extracts. For example, lecithin in the soybean extract is known to be responsible for the formation of a relatively hard ice cream that requires about 15 minutes to soften before serving (Wangcharoen, 2012). Thus it is important to optimize the milk compositions in order to establish acceptable physical properties of ice cream without compromising the viability of added probiotics.

Vegetable extract contains unique nutrient composition with respect to protein, free amino acid, prebiotic, vitamin and minerals. It is hypothesized that the replacement of cow milk with vegetable extracts would improve probiotic growth in ice cream and their survival during frozen storage and exposure to gastrointestinal conditions. Studies using various milk combinations present unique opportunity to establish the differences in probiotics growth, survivability and metabolism apart from achieving better physicochemical properties and quality of ice cream with or without prior limited fermentation by probiotics.

1.3 Objectives of study

In the present study, the effects of cow milk and vegetable (soy and coconut) extracts and various milk mixes (cow and coconut milk with soybean extract) on the survival of probiotics *Lactobacillus acidophilus* (La-05) and *Bifidobacterium bifidum* (Bb-12) in non fermented and fermented ice cream were investigated.

The specific objectives were:

1. To determine the effects of replacement of cow milk with soybean extract or coconut milk on the colony forming units of La-05 and Bb-12 in non fermented probiotic ice cream.
2. To measure the effects of replacement of cow milk with vegetable extracts on the time taken required for fermentation of ice creams until pH = 5.50 by probiotics and growth rate of probiotics in this pH, the colony forming units of La-05 and Bb-12 in fermented probiotic ice cream during storage at -20 ^{0}C and after in vitro gastrointestinal digestion.

3. To determine the effects of replacement of cow milk with soybean extract or coconut milk on sensory and physical properties in non fermented and fermented probiotic ice cream.

1.4 Significant of study

This study would provide more information on the extent of improvement of survival of probiotics in ice cream during storage as a result of cow milk replacement with vegetable extracts. This information can be used to increase 1) the shelf life of probiotic ice cream and 2) the viability of probiotics in order to enhance the success in the real mentation of large intestine with highly viable friendly bacteria.

CHAPTER 2 LITERATURE REVIEW

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2.1 Aims and scope of the literature review

This literature review aims to present current understanding on the progress and application of vegetable extracts and probiotics in fermented and non fermented ice creams.

An overview of the history of probiotics will be initially presented. This is then followed by a discussion on the delivery of probiotics through foods and how to make a healthier ice cream incorporated with probiotics were attempted. A review of vegetable extracts properties and ice creams made using various milks together with the use of probiotics and their health properties were then presented. Attention is focused on the changes in milk components after fermentation such as metabolism sugar and proteolysis of milk protein. A general overview of human digestive system and the process of food digestion in the body will be described to lay foundation on the importance of finding means to sustain high viability of probiotics under these conditions. Since the protein fat and carbohydrate compositions are markedly different in these milks. The current knowledge on the impact of cow milk replacement with vegetable extracts on ice cream melting rate, fat globules size and rheology with or without fermentation will also be presented.

2.2 Concept of probiotics

Most people may have experienced at least once in their lifetime the efficient effects of antibiotics to cure bacterial infections. Antibiotics have been the “gold standards” in the management of bacteria borne diseases. However, the side effects of antibiotics over use such as hypersensitivity, induction of yeast vaginitis, and sometimes even death have made

supportive means to minimize the occurrence of these side effects a priority. The concept of probiotics came into existence around 1900 when the Elie Metchnikoff made a remarkable observation and hypothesized that the Bulgarian peasants lived longer and healthier lives as a result of their consumption of fermented dairy products containing *Lactobacillus* (Ross *et al.*, 2005). Ross *et al.* (2005) described probiotic as "living microorganisms, which upon ingestion in certain numbers exert health benefits above inherent basic nutrition". Probiotic organisms for human should have provable health benefits and have 'generally regarded as safe' (GRAS) status, with a demonstrated low risk of inducing or being associated with the etiology of disease. The food and pharmaceutical industry are increasingly spending research funds to understand and enhance probiotic actions so that it can deliver better health benefits. This is reflected in an upsurge in clinical research assessing the therapeutic benefits of probiotic bacteria as well as parallel growing commercial interest in food fortification with them (Czinn and Blanchard, 2009). There is now highly convincing findings in supporting the possibilities of a link between probiotics and prevention of human diseases (Oliveira *et al.*, 2001; Teitelbaum and Walker, 2002). Milk containing probiotics is expected to be widely available in the next 15-20 years.

Limited clinical studies showed several commercially available probiotic bacteria may provide one or several proposed health benefits (Shah, 2007) (See Table 2.1). It can be seen that the beneficial effects of probiotic bacteria do not tie to specific genus or species, but instead are strain-specific which is also demonstrated (Gorbach, 2000) and Figueroa-Gonzalez *et al.*, 2011).

Table 2.1 Probiotic strains and their specific clinically proven health benefits (Shah, 2007).

Probiotic Strain	Clinical Benefits
L. acidophilus NCFM	Lowers fecal enzyme activity, improves lactose absorption and produces bacteriocin
L. rhamnosus GG	Plays a role in prevention of antibiotic and rotavirus associated diarrhea
L. casei shirota	Helps in prevention of intestinal disturbance, balancing intestinal flora and lowering of fecal enzyme activity
L. reuteri	Colonizes the intestinal tract, shortens the duration of rotavirus diarrhea, and helps in immune enhancement
B. animalis Bb-12	Plays a role in treatment of rotavirus diarrhea and balancing intestinal flora

2.3 Probiotic bacteria and current scenario

No approved standard of identity for probiotics is in existence but it is generally accepted that an established suitable level of viable cells to be ingested for therapeutic benefits is 10^6 cfu/g or mL, representing a daily dose of 8 log (Cruz *et al*., 2009; Ding and Shah, 2007; Abghari *et al*., 2011). The apparent effective concentration of probiotic microorganisms needed for biological health benefits depends on the strain, the delivery medium and the desired health effect (Champagne *et al*., 2005). High dosage is likely required to compensate for the possible decline of the number of viable probiotic cells during processing and storage of probiotic containing products (Waterman and Small, 1998). Thus it is important to ascertain the viability of probiotic bacteria in a food matrix of interest throughout its shelf life and ensure that the viability is maintained at level much greater than 10^6 cfu/g at the time of product consumption (Tharani, 2012).

Various species of genera *Lactobacillus* and *Bifidobacterium* have been incorporated into dairy and non dairy products over the years to study the effect of food vehicle on the survivability and functionality of probiotic. The *Lactobacillus* and *Bifidobacterium* genera are most commonly studied genera and have played an extensive role as probiotics because of their association with healthy human intestinal tract and

specifically in the case of *Lactobacillus*, due to their association with fermented foods (Tharani, 2012).

2.3.1 Characteristics of genus *Bifidobacterium*

Bifidobacterium are Gram positive, anaerobic and branched rod-shaped bacteria, forming the 'y' shaped rods as shown in Figure 2.1a. At present, 30 species of the genus *Bifidobacterium* have been recognized, 10 of these species are from human sources and 17 from intestinal tracts of animal or rumen (Table 2.2). Of these, six species from human origins, *B. adolescentis, B. bifidum, B. breve, B lactis, B. longum* and *B. infantis* have been used in dairy products (Boylston *et al*., 2004).

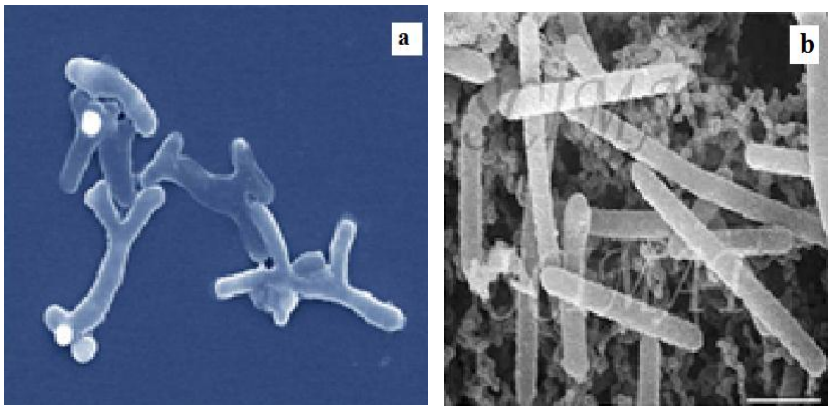


Figure 2.1 Micrograph of (a) *Bifidobacterium bifidum* (bar 1 μm) and (b) *Lactobacillus acidophilus* (bar 1 μm). Images are from SciMAT Photo Researchers, Inc.

Bifidobacterium are often posited in the lactic acid bacteria (LAB) family based on metabolic activities, even though they are phylogenetically distinct with a high guanine +cytosine (G+C) (42%-67%) content (Klein *et al*., 1998). *Bifidobacterium* are obligate anaerobes with optimum growth temperature of 37-41 °C and optimum growth pH of 6.5 to 7.0. Some *Bifidobacterium* strains can survive intestinal transit and persist transiently within the colon (Von Wright *et al.*, 2002). The isolation and growing of these bacteria is

often difficult in the laboratory because they are intransigent organisms and have special nutritional requirements (Shah, 2000a). *Bifidobacterium* is a saccharolytic organism and produces acetic acid and lactic acid without generation of CO_2. They are able to utilizing simple (glucose, fructose, galactose and lactose), as well as complex (stachyose and raffinose) carbohydrates. Fructose-6-phosphate phosphoketolase is the characteristic enzyme of this species, and is the most direct and reliable test used for assigning an organism to the genus *Bifidobacterium.*

The therapeutic roles of *Bifidobacterium* contain four major mechanisms including resistance to infectious diseases such as against rotavirus diarrhoea and enteropathogens, modulation of the host immune system, prevention of cancer and control of inflammatory bowel disease such as Crohn's disease, ulcerative colitis and pouchitis (Ong, 2007).

Table 2.2 List of species of the genera *Bifidobacterium* and *Lactobacillus* (Ong, 2007).

***Bifidobacterium* Species**		***Lactobacillus* Species**		
B. *adolescentes*	B. *indicum*	Lb. *acetotolerans*	Lb. *fermentum*	Lb. *murinus*
B. *angulatum*	B. *infantis*	Lb. *acidophilus*	Lb. *fructivorans*	Lb. *orisa*
B. *animalis*	B. bifidum	Lb. *agilis*	Lb. *fructosus*	Lb. *parabuchneri*
B. *asteroides*	B. *longum*	Lb. *alimentarius*	Lb. *gallinarum*	Lb. *paracasei*
B. *bifidum*	B. *magnum*	Lb. *amylolyticus*	Lb. *gasseri*	Lb. *pentosus*
B. *boum*	B. *merycicum*	Lb. *amylophilus*	Lb. *graminis*	Lb. *plantarum*
B. *breve*	B. *minimum*	Lb. *amylovorus*	Lb. *halotolerans*	Lb. *pontis*
B. *catenulatum*	B. *pseudocatenulatum*	Lb. *aviarius*	Lb. *amsteri*	Lb. *reuteri*
B. *choerinum*	B. *pseudolongum*	Lb. *bifermentans*	Lb. *helvesticus*	Lb. *rhamnosus*
B. *coryneforme*	B. *pullorum*	Lb. *brevis*	Lb. *hilgardii*	Lb. *ruminis*
B. *cuniculi*	B. *ruminantium*	Lb. *buchneri*	Lb. *jensenii*	Lb. *sakei*
B. *Pentium*	B. *saeculare*	Lb. *casei subsp. casei*	Lb. *johnsonii*	Lb. *salivarius*
B. *gallicum*	B. *subtile*	Lb. *collinoides*	Lb. *kandleri*	Lb. *sanfranciscensis*
B. *gallinarum*	B. *suis*	Lb. *coryniformis*	Lb. *kefiri*	Lb. *sharpeae*
B. *globosum*	B. *thermophilum*	Lb. *crispatus*	Lb. *kefiranofaciens*	Lb. *suebicus*
		Lb. *curvatus*	Lb. *malefermentans*	Lb. *vaccinostercus*
		Lb. *delbrueckii*	Lb. *mali*	Lb. *vaginalis*
		Lb. *farciminis*	Lb. *Minor*	Lb. *viridescense*
				Lb. *homohiochii*
				Lb. *intestinalis*

2.3.2 Characteristics of genus *Lactobacillus*

Lactobacillus is Gram positive, nonsporeforming, non-flagellated rods or coccobacilli. Some species are strictly anaerobic, while others are aerotolerant and can utilize oxygen by the presence of enzyme flavoprotein oxidase. Presently there are 56 species included in the genus *Lactobacillus* (Table 2.2; Ong, 2007).

Apart from a few heterofermenters *L. acidophilus* are mainly mandatory homofermenters by which the major end product is lactic acid. They occur naturally in the gastrointestinal (GI) tract of animals and humans, in the human vagina and mouth, and in some traditional fermented dairy products, such as kefir. They are either microaerophilic, anaerobic or aerotolerant and strictly fermentative with the G+C content of their DNA usually between 32 and 53 mol% (Salminen and Wright, 1998). *L. acidophilus* is a short Gram-positive rod (0.6-0.9 μm in width and 1.5-6.0 μm in length) with rounded ends that occurs as single cells, as well as in pairs or in short chains.

L. acidophilus are also non-motile and non-spore forming (Figure 2.1b). The surface growth on solid media is generally increased by reduced oxygen pressure or anaerobic condition because of their microaerophilic nature. Carbohydrates as energy and carbon source as well as nucleotides, amino acids and vitamins are essential for the growth of these organisms. Their complex nutritional requirements include amino acids, nucleotide bases, peptides, minerals, vitamins, carbohydrates and fatty acids (Axelsson, 2004). *L. acidophilus* utilizes sucrose as well as lactose. Most *L. acidophilus* strains require a medium supplementation with different micronutrients, such as oleic acid, manganese and esters especially Tween 80 for the growth. The optimum temperature and pH for the growth of *L. acidophilus* is between 35-40 ºC (with several at as high as 45 ºC) and 5.5-6.0, respectively.

The acid tolerance varies from 0.3 to 1.9% titratable acidity (Shah, 2000a). *L. acidophilus* tends to grow slowly in milk or soybean extract because of low content of available peptides and amino acids in these media. Moreover, due to low pH of fermented milk, most strains of *L. acidophilus* do not grow well in it (Ong, 2007).

The important health benefits of *L. acidophilus* include supporting the immune system, replacement of good bacteria in the intestinal tract following antibiotic therapy, reducing outbreak of diarrhea in humans (adults and children), lowering blood cholesterol, and improving the symptoms of lactose intolerance. The anti-tumor effect of *L. acidophilus* is thought to be delivered by the direct activation of the body's immune system and decreasing effects of azoreductase, nitroreductase, ß-glucuronidases and related bacterial enzymes instrumental in the conversion of procarcinogens to carcinogens. For instance supplementation with *L. acidophilus* in animal studies was found to decrease the number of colon cancer cells in a dose dependent manner (Ong, 2007).

2.4 Application of probiotics in foods

Growing consumer knowledge of roles of diet in health has aroused amongst others the demand for foods containing probiotic. A number of food products including frozen fermented dairy desserts (Ravula and Shah, 1998 a&b), yogurt (Kailasapathy and Rybka, 1997), cheeses (Stanton *et al.,* 2001), freeze-dried yogurt (Capela *et al*., 2006) ice cream (Haynes and Playne, 2002), coleslaw (Rodgers and Odongo, 2002), spray dried milk powder (Stanton *et al*., 2001), and fruit juices (Saarela *et al*., 2006) have been utilized as delivery vehicles for probiotic to consumer. Hence the selection and balancing of LAB is important to ensure food and dairy products maintain their desirable flavour, texture and nutritional value characteristics because these parameters may be affected by the initial

composition of the milk flora and starter culture (Ahmed and Kanwal, 2004). A number of health benefits associated with probiotic food products include treatment of diarrhea, alleviation of symptoms of lactose intolerance, reduction of blood cholesterol, anticarcinogenic properties, and improvement in immunity (Shah, 2000b). To elicit health effects, viable probiotic organisms must be viable large enough (~10^9 cfu/day) at the time of consumption (Ross *et al*., 2002). Therefore, it is important to minimize the decline in the numbers of viable bacteria during storage period. Dairy foods present ideal delivery system of food for probiotics to the human gut because it offers suitable environment and nutrients to promote growth or support viability of these cultures. The fermented milk and yogurt in particular are the most popular food delivery systems for probiotic. However the low pH of yogurts, the presence of H_2O_2 and inhibitory substances produced by the yogurt bacteria and the aerobic conditions of production and packaging may result in the decreases in the survival of probiotics in the final product. In fact the required level of viable cells of probiotic bacteria in many commercial yogurts cannot be guaranteed and therefore failed the prerequisite for successfully delivery of probiotics (Shah and Lankaputhra, 1997). For instance the colony forming units can decrease by two log cycles in a period of two weeks when *Lactobacillus casei* in fermented milk products were stored at room temperature (Magariňos *et al.,* 2007). More thermo-sensitive strains such as *L. acidophilus* and *B. bifidum* may even have more cell mortality at the same temperature (Salminen and Wright, 1998). For this reason ice creams may become an appropriate system to deliver viable probiotic to GI- tract by virtue of much lower storage temperature.

2.5 Ice cream

Ice cream is a frozen dairy product produced from a combination of several ingredients other than milk. The composition of ice cream varies depending upon the

ingredients used in its preparation. In many countries, the percentage composition of a good ice cream is 11–12% milk fat, 10–12% milk non-fat solids (MSNF), 12% sugar, 5% corn syrup solids, 0.3% stabilisers-emulsifiers (Guner *et al.*, 2007).

Ice cream is a delicious and nutritious frozen dairy dessert with high calorie food value (Guner *et al.*, 2007). It typically supplies approximately 200 calories, 3.99 g protein, 0.31 g calcium, 0.10 g phosphorus, 0.1 mg iron, 548 IU vitamin A, 0.038 mg thiamine and 0.23 mg riboflavin (Arbuckle, 1986). Ice cream has only nutritional significance but possesses no therapeutic value (Pandiyan *et al.*, 2012b). Recent consumers increasing preference for healthier and functional food has led to the production of ice cream with special ingredients with documented nutritional and physiological properties such as dietary fibers (Soukoulis *et al.*, 2009), probiotics (Akin *et al.*, 2007; Alamprese *et al.*, 2002), lactic acid bacteria (Hong and Marshall, 2001), alternative sweeteners (Soukoulis and Tzia, 2010), low glycemic index sweeteners (Whelan *et al.*, 2008), and natural antioxidants (Hwang *et al*., 2009).

2.6. Milk options for ice cream making

2.6.1 Animal milk (cow milk)

The main ingredient of ice cream is cow milk and this unfortunately may make dairy ice cream off limits to many consumers who suffer from lactose intolerance. The fermentation of milk can decrease lactose by approximately 30% (Supavititpatana and Kongbangkerd, 2011). Thus fermented milk products are more tolerable (Heyman and Ménard, 2002).

2.6.2 Vegetable extracts

Soy and coconut based products are suitable dairy product substitutes for lactose-intolerant or vegetarian individuals (Granato *et al*., 2010). In addition, the high nutrient composition of soybean extract and coconut milk over cow's milk certainly gives it numerous health advantages. In the present study, further improvement of healthier ice cream was attempted by allowing limited probiotic fermentation of ice cream mixes made using milk partially or fully replaced cow milk with vegetable (soy and coconut) extracts.

2.6.2.1 Soybean extract

The soybean seeds contain 13-25% oil, 30-50% protein, and 14-24% carbohydrates. The major fatty acids are linoleic acid (55%) followed by oleic acid (21%), palmitic acid (9%), stearic acid (6%) and other fatty acids (9%). The ratio of polyunsaturated fatty acid to saturated fatty acid (p/s ratio) is 82:18. Soy protein contains all the essential amino acids, most of which are present in amount that closely match with those required for humans or animals, soy protein digestibility of about 92%, also matches with that of animal protein such as egg white and casein (Feneslav and Schrezemeir, 2000). Apart from being highly nutritious soybean extract is a cost effective source of energy and protein, such that it has a great potential to solve the problem of protein energy malnutrition in many developing countries. The high proportion of unsaturated fatty acids makes soybean extract to contain healthful oil (Bisla *et al.*, 2011). Soybean extract can be effectively used for supplementing cereal based products because of the fact that it is a good source of vitamin and minerals (Khetarpaul and Goyal, 2008). In this regard, soy based diets are becoming popular due to its neutraceutical benefits that suit those who are lactose intolerant, hypercholesterolemic, diabetic, anemic and lactating mothers or postmenopausal women (Nsofor and Anyanwu,

1992). In fact, soybean extract is widely adopted as a substitute for milk in the parts of the world where milk production is low and dairy products prices are exorbitant (Nsofor and Osuji, 1997).

2.6.2.2 Coconut milk

Apart from coconut oil production, coconut is also used for the production of coconut milk (aqueous extract of the solid endosperm) for cooking and in the food industry. In fact 25% of the world's output of coconut is consumed as coconut milk (Seow and Gwee, 1997). The extraction of coconut milk begins with shelling and paring of fully mature coconuts. Paring removes the brown testa and the white coconut flesh or meat is then washed, drained and grated by machine (Seow and Gwee, 1997). The grated coconut is then pressed using a hydraulic or screw press and the extracted milk is then filtered through a cloth filter or centrifuged at low speed (using a basket centrifuge) to remove finely comminuted particles of coconut pulp without breaking the emulsion. The chemical composition of coconut milk may vary widely because of differences in factors such as variety, geographical location, cultural practices, maturity of the nut, method of extraction, and the degree of dilution with added water or liquid endosperm (Soler, 2005). The main carbohydrates present in the coconut milk are sugars (primarily sucrose) and some starch. The major minerals found in raw coconut milk consist of phosphorous, calcium, and potassium. Freshly extracted milk will also contain small amounts of water-soluble B vitamins and ascorbic acid (Seow and Gwee, 1997). Based on their solubility characteristics, at least 80% of proteins in coconut endosperm would be classified as albumins and globulins i.e. the predominant proteins in coconut milk. The protein content of undiluted milk ranges from 5 to 10% (on dry basis). Although coconut is high in saturated fat, most are made up of medium chain triglycerides (MTC's) which are more

efficiently catabolized for energy rather than stored as body fat. Approximately 50% of the fatty acids in coconut fat are lauric acid. Lauric acid has been recognized for its unique properties in food use by virtue of its antiviral, antibacterial, and antiprotozoal functions. Capric acid in coconut oil (6-7%) also has antimicrobial properties (Soler, 2005).

2.6.3 Comparison of milk composition

Whole soybean extract contains 90-93.81% moisture, 0.27–0.48% ash, 2.86–3.12% protein, 1.53-2% fat and 1.53–3.90 % carbohydrate (Rosenthal *et al.,* 2003; Yadav *et al.,* 2003). The major protein in cow milk is casein whereas soybean extract protein consists mainly of glycinin. Soybean extract is deficient in the essential sulphur amino acids–methionine and cysteine, but comparatively rich in lysine. The proteins of coconut milk (80%) can be classified as albumins and globulins, whereas only 30% of protein in the filtered milk is dissolved in the aqueous phase (Seow and Gwee, 1997). Coconut milk protein contains all essential amino acids except methionine and cysteine and it also contain relatively high levels of glutamic acid, aspartic and arginine acid (Seow and Gwee, 1997). Cow milk carbohydrate is particularly in the form of lactose whereas soybean extract carbohydrate is in the form of oligosaccharides particularly raffinose and stachyose (Saidu, 2005). The main carbohydrates of coconut milk are sucrose, and some starch. In contrast, it has high levels of phosphorus and calcium, but is extremely low in iron content. The fractions components could vary in coconut and soybean extracts depending on formulation, processing and solids contents of them (Seow and Gwee, 1997).

When compared on weight basis (100 g portions), coconut milk (230 kcal) contain the highest energy content followed by cow's milk (61 kcal) and soybean extract (33 kcal) (Saidu, 2005). Cow's milk has about 14 mg of cholesterol, lactose but no dietary fiber,

whereas coconut and soybean extract contain no cholesterol, no lactose but appreciable amount of fiber (2.2 and 1.3 g, respectively) (Saidu, 2005). While all milks contain protein and a full range of amino acids, coconut milk contains high amounts of glutamic acid, aspartic and arginine acid (Saidu, 2005) whereas soybean extract contains high levels of arginine, alanine, aspartic acid and glycine (Saidu, 2005). Adequate levels of amino acids are necessary to ensure health benefits of consuming these milks. Alanine aids in the metabolism of sugars, arginine slows the growth of cancers by strengthening the immune system, glycine is necessary for brain and nervous system function and muscle/energy metabolism (Kengen *et al*., 1996; Schoenen, 1996; Rodríguez and Augusto, 2008), whereas aspartic acid increases stamina and plays a vital role in metabolism by acting as an antioxidant (Saidu, 2005). Preparation of milk and subsequent pasteurization destroys vitamins C in cow, soy and coconut milk, but high amount of thiamin (4 times) and niacin (2 times) are retained in soybean extract compared to those in cow milk. Soybean extract also contains 42 times the manganese, 12 times the copper and more magnesium than cow's milk (Hajirostamloo, 2009). Freshly extracted coconut milk contains small amounts of water-soluble ascorbic acid and B vitamins (Seow and Gwee, 1997). The high nutrient composition of soy and coconut milks over cow's milk certainly gives it numerous health advantages (Saidu, 2005).

2.6.4 Soybean extract and coconut milk ice creams

Replacing cow's milk with vegetable extract in general would help address two nutritional issues related to cow's milk: lactose intolerance and cholesterol content. Vegetable extracts are at par with cow's milk in relation to certain micronutrients vitamins and minerals with the added advantage of the presence of phytonutrients.

Consumers do not in general like the taste of soybean extract or other soy products and they could limit more consumption of healthy soybean extract useful in reducing LDL cholesterol and plasma triglycerides, which are risk factors for cardiovascular disease (Clarkson, 2002). Therefore, the consumption of soybean extract ice cream instead of full fat ice cream could help intake and increase unsaturated fat, zero cholesterol and balance soy protein intake. Soy protein is also effective at reducing fractures in post-menopausal women (Zhang *et al*., 2005). Replacing cow's milk with coconut milk would result in the fortification of ice cream with oleic and lauric acid which are known for their unique properties in preventing arteriosclerosis and related illness (Belewu and Belewu, 2007).

There is little information on the effect of soybean extract or coconut milk replacement of cow's milk on ice creams on nutritious, rheology and consumer acceptability. Bisla *et al*. (2011) studied ice creams made using soybean extract and watermelon seeds milk and found that both type of ice creams are highly acceptable and free from beany flavour. Ice cream containing blended milk ice cream (50% soybean extract and 50% watermelon seed milk) with guava pulp-D had the highest overall acceptability. Ice creams made using these vegetable extracts are rich in protein and in mineral such as iron and vitamin C compared to those using cow's milk. Wangcharoen (2012) found that ice cream recipe with 7% sucrose and 4% ginger extract had the highest total acceptability ($p<0.05$). Total phenolic content of this recipe was 91.6±6.8 mg gallic acid equivalent per 100 g and antioxidant capacity values including ferric reducing/antioxidative power (FRAP), 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were 37.9+3.7, 13.4+1.2 and 49.0+5.1 mg vitamin C equivalent per 100 g, respectively. Abdullah (2003) investigated that the ice cream's taste, flavour and mouth feel improved tremendously with a decrease in

soybean extract content. Wangcharoen (2008) noted the nutrient contents of soybean extract ice cream and black sesame flavoured soybean extract ice cream are comparable to that of cow's milk. The antioxidant capacity of the samples was equal to 69.8 mg ascorbic acid equivalent/100 g for ABTS assay, and 7.2 mg ascorbic acid equivalent/100 g for DPPH assay. Significantly higher contents of protein, fat, ash (including calcium, phosphorus, iron and zinc), and significantly higher antioxidant capacity (2–4.5 times) were found ($p<0.05$) for black sesame flavoured soybean extract ice cream. Soybean extract ice cream and black sesame flavoured soybean extract ice cream in these studies could not meet the definition of health claims for soy protein, nutrient content and antioxidant nutrient content claims. However, the high antioxidant capacities of both products might be used to claim health benefits because these were found to be equivalent to about 10% DV of vitamin C for soybean extract ice cream and about 2 times or more for black sesame flavoured soybean extract ice cream.

2.7 Metabolic systems of probiotics

2.7.1 Sugar metabolism

Carbohydrate fermentation coupled with substrate level phosphorylation is the essential feature of lactic acid bacteria (LAB) metabolism. The produced ATP is subsequently used for biosynthetic purposes. LAB displays a great capacity to reduce the concentration of different carbohydrates and related compounds, with the accumulation of lactic acid as the predominant end-product (>50% of sugar carbon). As is common for microorganisms, LAB as can change their metabolism for adaptation in various conditions accordingly and this may lead to significantly different end-product patterns (Salminen and Wright, 1998):

1) **Fermentation of hexose:** The two major pathways for hexose (e.g., glucose) fermentation utilized by LAB are shown in Figure 2.2 (Donkor, 2007).

2) **Fermentation of disaccharides:**

Disaccharides enter the cell either as free sugars or sugar phosphates depending on the mode of transport. Free disaccharides are split by specific hydrolyses to monosaccharides, e.g. lactose to galactose and glucose (Figure 2.3) which then enter the major hexose pathways described above. However, when phosphotransferase systems (PTS) for uptake of sugar are involved, specific phosphohydrolases cleave disaccharide phosphates into monosaccharides and monosaccharide phosphates (Donkor, 2007).

3) **Lactose metabolism:** This is the most studied disaccharide metabolism in LAB (Figure 2.4; Donkor, 2007)

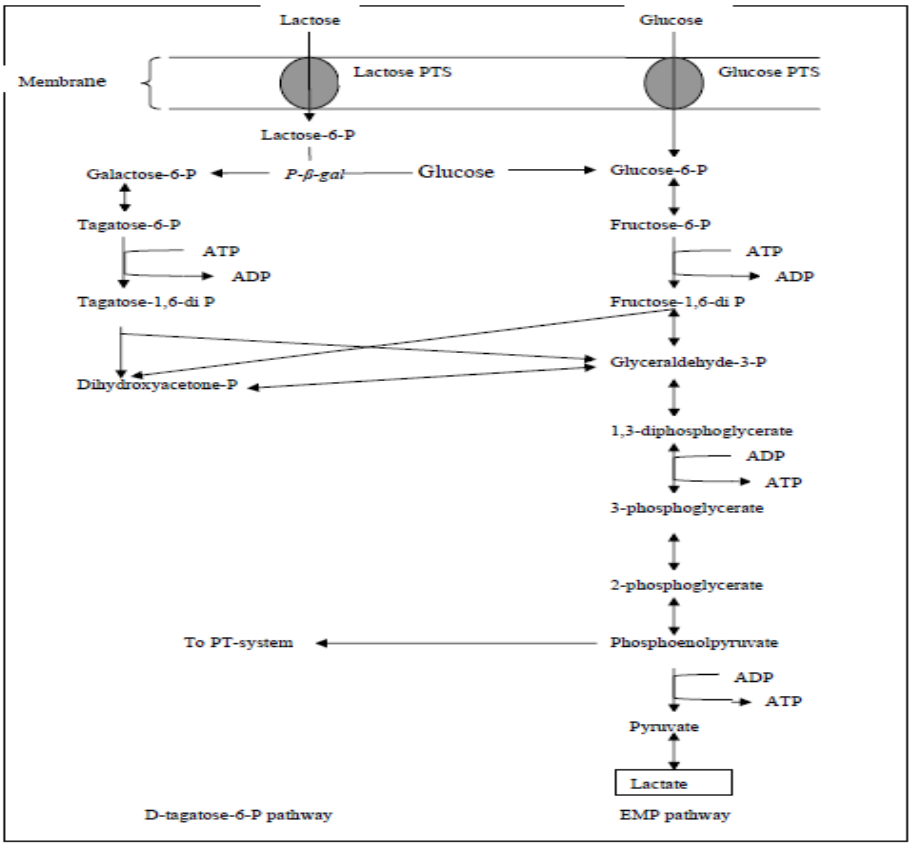


Figure 2.2 Fermentation pathways for lactose and glucose in LAB. Tagatose-6-phosphate pathway and EMP-glycolytic pathway (Donkor, 2007).

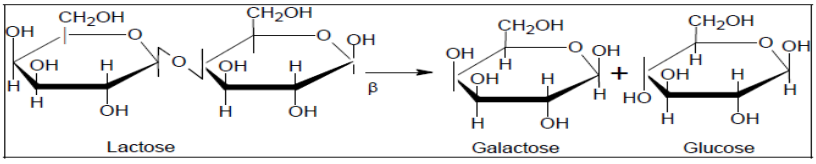


Figure 2.3 Hydrolysis of lactose.

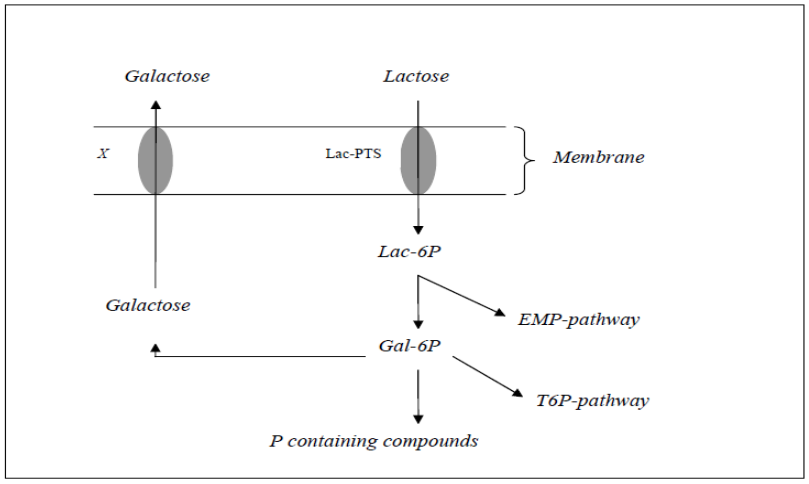


Figure 2.4 Schematic representation of dephosphorylation of Gal-6P and expulsion of galactose to the medium during lactose metabolism (Donkor, 2007).

2.7.2 Nitrogen metabolism

2.7.2.1 Proteolysis of milk protein

Lactic acid bacteria are fastidious microorganisms with regard to nutritional requirements (Guarner *et al*., 2005; Lee *et al*., 2001). They have limited biosynthetic ability hence the requirement for an exogenous source of amino acids (such as isoleucine, leucine, valine, histidine and methionine) or peptides for optimum growth (Vermeirssen *et al*., 2002; Donkor *et al*., 2005; Papadimitriou *et al*., 2007). Since milk is deficient in such low-molecular components the growth of the starter bacteria depends on their proteolytic systems to hydrolyze caseins (Ong and Shah, 2008). The amino acids released by the bacteria and accumulated in the milk affect the nutritional potential and biological value of the fermented product. Amino acids may not be directly contributory to the flavour and aroma of fermented milk. However, they act as precursors for a number of reactions that produce carbonyl compounds (Considine *et al*., 2000). The spectrum and level of free amino acids in fermented milk depend on several variables such as type of milk, composition of the starter, method of preparation and storage conditions. Caseins are the main source of amino acids ensuring 98% of LAB growth (Matsuura *et al*., 2005; Salami *et*

al., 2011). The contribution of caseins to the provision of essential amino acids depends on the type of proteinase (Salami *et al*., 2011). Proteinase is capable of initiating the degradation of casein to oligopeptides which are transported into the bacteria and afterwards degraded through a complex sequence of intracellular peptidases (Salami *et al*., 2011). The amino acid necessity and production activity in mixed cultures can be modified using selected strains of *Lactobacillus* (Lee *et al*., 2001) capable of intracellular splitting of oligopeptides or of attacking peptides and proteins in the nutrient medium by means of releasing proteolytic enzymes (Lee *et al*., 2001).

In the mixed yogurt culture, *Lactobacillus delbrueckii* ssp. *bulgaricus* has higher proteolytic activity than *S. thermophilus* and thus the free amino acids produced by *Lactobacillus delbrueckii* ssp. *bulgaricus* are also used by *S. thermophilus* (Gobbetti *et al*., 2002; Pescuma *et al*., 2011). The total amino acid content in yogurt reflects the balance between proteolysis and assimilation by bacteria (Gobbetti *et al*., 2002). The pathway of peptide hydrolysis in yogurt bacteria ensures the release of amino acids respectively and the growth relation between *S. thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Shihata and Shah, 2000; Robinson and Tamime, 2002; Pescuma *et al*., 2011). Proteolysis in fermented milk is mainly related to yogurt cultures which explain the high level of proteolysis in fresh biokefir after storage compared to other fermented milk (Gobbetti *et al*., 2002). The pathway of casein catabolism through yogurt organisms can be altered via endopeptidase activity as described for strains of *S. thermophilus* and *Lactococcus lactis* ssp. *lactis*, and aminopeptidase as described for *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus helveticus* (Gobbetti *et al*., 2002).

2.7.2.2 Proteolytic system (proteolytic activity)

LAB depend on preformed amino acids present in the growth medium as a nitrogen source because they have a limited capacity to synthesize amino acids using inorganic nitrogen sources. A central metabolic activity in LAB is the conversion of peptides to free amino acids and the subsequent utilization of these amino acids. There are species and strain variations within species with respect to requirement for amino acids. For example *Lactococcus (Lc). lactis* ssp. *cremoris* and *L. helveticus* strains may require 13-15 amino acids, whereas certain strains of *Lactococcus (Lc.) lactis* ssp. *lactis* are in fact prototrophic for most amino acids (Donkor, 2007).

LAB depends on rich environments with nitrogen sources because of slow growth on chemically defined minimal media. The peptidase system is involved in the hydrolysis of peptides formed by housekeeping proteinases and hydrolysis of exogenous peptides to obtain essential amino acids for growth. The amino acids formed by this system can be used for processes such as generation of metabolic energy, protein synthesis, and recycling of reduced cofactors (Salminen and Wright, 1998).

2.7.3 Metabolism of lactic acid bacteria (LAB) in soybean extract and coconut milk

The fermentation of soybean extract improved the health and acceptability properties of soybean extract. Lactic acid bacteria (LAB) in fermented soybean extract expressing α-galactosidase as a promising solution for the degradation of α-galactooligosaccharides (LeBlanc *et al.,* 2004), or the use of other bacteria strains to increase beverage quality (Wang *et al.*, 2004), increase or stimulate immunomodulatory properties of soy bioactive compounds–isoflavones (Saidu, 2005), and reducing indigestible oligosaccharides, like stachyose and raffinose, and beany flavour (which is

undesirable for most Western consumers). *L. fermentum* CRL 722 grown in commercial soybean extract and was shown to remove that raffinose and stachyose completely during fermentation because of its high α-galactosidase activity (LeBlanc *et al.,* 2004). In addition, rats fed with the fermented soybean extract had smaller caecums compared with rats fed with unfermented soybean extract and this suggested fermented soy reduced α-galactosidase concentrations in soybean extract, thus removing possible undesirable physiological effects of its consumption. Therefore, *L. fermentum* CRL 722 fermented soybean could prevent gastrointestinal disorders in sensitive individuals associated with the consumption of soya-based products. *L. fermentum* CRL 251 and *B. longum* CRL 849 in a mixed culture were able to continue growing on but their growth and acid production in soybean extract was decreased by reducing stachyose and α–galactosidase activity (LeBlanc *et al.,* 2004). Soybean extract inoculated with a mixture of *L. acidophilus, B. bifidum,* and *S. thermophillus* and supplemented with 2% sucrose showed increased acceptability considerably (Behrens *et al.,* 2004).

Lactic and acetic acid contents were reported to increase while the molar ratio of acetic and lactic acid was decreased during fermentation. Stachyose, sucrose and raffinose contents decreased, with stachyose demonstrating the largest magnitude of reduction. On the other hand, contents of fructose and glucose plus galactose contents were reported to increase during fermentation (Hou *et al*., 2000). However, such novel soy products have been reported to cause undesirable secondary effects such as animal weight loss and microbial translocation (LeBlanc *et al.,* 2004). Another advantage of fermentation process is that the total protein increased in soybean meal (SBM) from 47% to 50% because of the microbial proteolytic activity. SBM fermented with *S. cerevisae* increased its protein level to 58%. These different results in protein concentration may be explained by the

microorganism load during processing. With regards to levels of non-essential amino acids, unfermented SBM presented a large amount of glutamic acid followed by aspartic acid, arginine, alanine, glycine and serine, and proline. Among the essential amino acids, leucine presented the highest amount (2.3%), followed by lysine, isoleucine, valine, threonine, tyrosine, phenylalanine and histidine. In lower amounts were cysteine and methionine (0.54% and 0.48%, respectively). However, when SBM was subjected to fermentation with different microorganisms, most of the amino acids increased significantly ($p<0.05$) and only few of them showed a decrease depending on the type of fermentation. Methionine levels did not change significantly ($p>0.05$) under natural fermentation or when fermented with *L. plantarum* or *S. cerevisae*; while *B. bifidum* caused a reduction of 15%. Cysteine, however, decreased in naturally fermented SBM or under *B. bifidum* or *L. plantarum* fermentation but underwent a sharp rise from 0.54% to 0.84% after fermentation with *S. cerevisae*. Taking into consideration the limiting essential amino acids, the fermentation of SBM with *S. cerevisae* should be recommended since although methionine content was not significantly changed, cysteine showed a sharp increase (56%, $p<0.05$). Similarly, bacterial enzymatic proteolysis have shown enhanced bioavailability of protein, fat, and increased availability of free amino acids and short chain fatty acids (Saidu, 2005).

There is limited information about the incubation of coconut milk with probiotics. Yuliana *et al.* (2010) reported *L. acidophilus, Lactobacillus delbrueckii* ssp. *bulgaricus* and *S. thermophilus* could grow well in all of the coco milk drink prepared from mixture of coconut water and coconut milk combination. Among the three of lactic acid bacteria, *L. acidophilus* still continue its growth metabolism during 4 days of storage due probably to its end of logarithmic phase has not yet been attained and the sucrose in the coconut milk drink was still available. Storage of fermented coco milk drink at 5 °C for 16 day could

stabilize the quality of this drink with viability of *L. acidophilus* (log 10.201 (log cfu/mL)) retained at pH 3.58. Besides providing mineral for the LAB growth media, presence of mineral in coconut drink is a part of fortified cultured milk itself. Mineral fortification with calcium salts and calcium content is a usual attempt in some milk cultured for example in yogurt (Pirkul *et al.*, 1977; Khurana and Kanawjia, 2007).

2.8 Viability of probiotics in fermented and non fermented ice cream

The viability of the probiotic bacteria in ice cream after freezing is an important parameter to be determined to ensure compliance to the food industry standards and meeting consumer expectation. Early studies by Hagen and Narvhus (1999) showed that the survival of individually inoculated *B. bifidum*, *L. acidophilus*, *L. rhamnosus* and *L. reuteri* did not change significantly during 13 months of frozen storage in ice cream. Alamprese *et al.* (2002) found different sugar and fat concentrations did not have significant difference on *Lactobacillus johnsonii La1* viability during 8 months frozen storage at -28 °C. Hence their study has demonstrated that it is possible to produce unfermented ice cream containing probiotic bacteria with high survival for up to 240 days of storage regardless of the ice cream formulation. Turgut and Cakmakci (2009) investigated the possible use of *L. acidophilus* and *B. bifidum* in ice cream manufacture during 90 days and found that the counts of *L. acidophilus* and *B. bifidum* decrease during three months storage. *L. acidophilus* had the highest survival whereas *L. acidophilus* and *B. bifidum* in double-cultured samples had the lowest survival. Nevertheless all types of ice cream were found to preserve their probiotic property even after 90 days. Salem *et al*. (2005) found that the viability of *L. acidophilus*, *B. bifidum*, *L. reuteri*, *L. gasseri* and *L. rhamnosus* decreased until 2.23, 1.68, 1.54, 1.23 and 1.77 log cfu/g respectively during three months of frozen storage but the counts were still above the recommended minimum limit of 10^6 cfu/g after

90 days of storage at -26 °C. Pandiyan *et al.* (2012) found that incorporating fructo-oligosaccharides (FOS) into probiotic ice creams increased survival of *Lactobacillus acidophilus* and *Saccharomyces boulardii* under freezing and exposure to human gut conditions. The *L. acidophilus* and *S. boulardii* count were higher in the treatments when both bacteria were incorporated in combination than in isolation. The consumption of synbiotic and probiotic ice cream could significantly increase the gut flora and thereby improve the health of consumers. Hence, it is concluded that ice cream can effectively be used as a medium to deliver probiotic bacteria as well as prebiotic substance like FOS to enhance the human gut health. Criscio *et al.* (2010) found all their experimental ice creams (probiotic ice creams, a prebiotic ice cream containing inulin and a synbiotic ice cream containing probiotic bacteria and inulin) improved survival of probiotics during frozen storage for 4 months and the best results obtained with *Lb. casei* and 2.5% inulin. *Bifidobacterium* Bb-12 with different contents of reconstituted skim milk and inulin protected probiotics during 90 days of storage and they preserved unchanged in their counts whereas in control treatment showed a decrease of about 34%. Akalin and Erisir (2008) improved the survivability of *L. acidophilus* La-05 and *B. animalis* Bb-12 in low-fat probiotic ice cream by adding inulin and oligofructose in ice cream during storage at –18 °C for 3 months. Akin *et al*. (2005) noted inulin and sugar levels affected probiotic viability in ice cream during 3 months frozen storage. The ice creams with 18% sugar showed highest number of probiotics. Ice cream supplemented with inulin showed increased probiotics survival. Hence inulin can improve the survival of *L. acidophilus* and *B. bifidum* during frozen storage at -18 °C. Miguel *et al*. (2004) studied the health beneficial effects of soy yogurt fermented with *E. faecium* and *L. jugurti* and their sensory properties and found that it is possible to have a probiotic product with good sensory characteristics even after 180 days of frozen storage. This is despite the development of oxidation process and an

increase in the concentrations malondialdehyde. Also *E. faecium* and *L. jugurti* can survive about 10^6 cfu/g in frozen soy yogurt during 180 days at -23 °C. Hermanto and Masdiana (2011) found yogurt bacteria in the presence of soy extract powder (SEP) could grow in ice cream mix before incubation and increased in numbers after incubation (8.30 log cfu/mL) in comparison to the probiotic ice cream with the standard formula without the addition of SEP (7.5 log cfu/mL). The best quality functional ice cream contained 8.8% fat, 38.2 mg lysine, 6.3 mg methionine, 5.1 mg cystine, 3.14% fibre and 8.30 log cfu/mL of probiotic bacteria, was produced by the addition 8% SEP. SEP as prebiotic could therefore promote the growth of yogurt bacteria in the frozen product.

Recent studies on probiotic survival during frozen storage have focused on the protective effects of encapsulation and supplemented ice creams with prebiotics. Microencapsulation of *Lactobacillus casei* (Lc-01) and *B. bifidum* (Bb-12) using resistant starch showed increased survival of these bacteria in ice cream during 180 days freezing at -20 ^{0}C. The survival of probiotics encapsulated in calcium alginate could even increased this survival to 30% higher during storage at -20 ^{0}C (Homayouni *et al*. 2008). Karthikeyan *et al*. (2013) also indicated that microencapsulation of *Lactobacillus casei (NCDC-298)* and *Bifidobacterium animalis* ssp. *Lactis* (Bb-12) along with calcium alginate and whey protein increased the survival of probiotics until above 30% in contrast to when probiotics as free use in ice cream during 6 months storage at -23 °C. Sahitya *et al.* (2013) noted the co-encapsulated *Lactobacillus helveticus* 194 and *Bifidobacterium bifidum* 231 along with prebiotics (3% FOS) increased probiotic viability during 90 days of storage at -20 °C.

Probiotic microorganisms were routinely incorporated into non fermented, vegetarian frozen soy dessert at initial populations greater than 10^6 cfu/g (Heenan *et al.*, 2004). Probiotics such as *L. acidophilus* MJLA1, *L. rhamnosus* 100-C, *L. paracasei* ssp.

paracasei 01, *B. bifidum* BBDB2, *B. bifidum* Bb-12 may all survived the 6 month storage (populations > 10^7 cfu/g). The frozen soy dessert can be used as a suitable food for the delivery of bacterial probiotic strains with excellent viability and acceptable sensory characteristics. However other studies (Hekmat and McMahon, 1992; Akalin and Erisir, 2008) reported fermentation may cause a decrease in *L. acidophilus* counts after storage for 17 weeks at -29 ^{o}C and 13 weeks at -18 ^{o}C, respectively. The sensory properties of ice cream may also be negatively affected due to acidification of the ice cream mix causing less preference for fermented probiotic yogurt like products (Hekmat and McMahon, 1992; Christiansen *et al.,* 1996). In addition all fermented ice cream scored slightly lower values in melting quality and colour attributes than control treatment (Salem *et al.,* 2005). This indicates that fermentation of ice cream may result in adverse effects on colony forming unit and sensory qualities.

2.9 Gastric condition

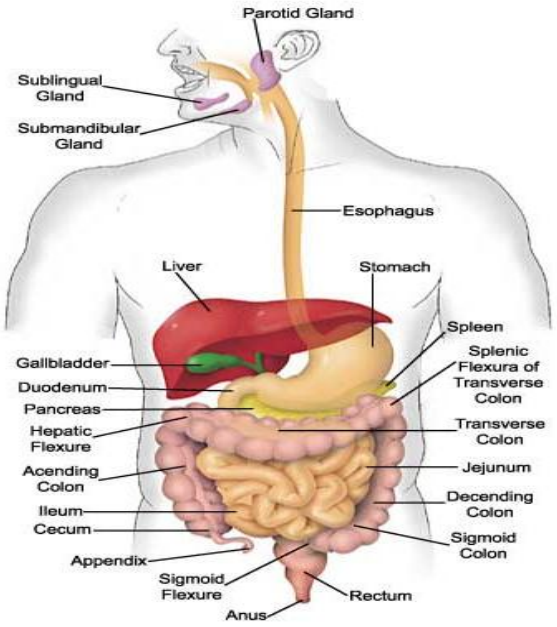


Figure 2.5 Human digestive system.

Human digestive system contains a multipart series of organs and glands (Figure 2.5), which digest food via physical and chemical means. An adult human has approximately 5 meters of upper and lower human gastrointestinal (GI) tracts. Most of the digestive organs are tube-like such as stomach and intestine, and this GI tract releases hormone such as gastrin, secretin, cholecystokinin and ghrelin to help the regulation of the digestion process (Shetzline and Liddle, 2002).

The process of digestion starts in the mouth. The food that had been eaten is broken down by the process of chewing and also chemical action of salivary enzymes which resulted in the break down starch into smaller molecules. The process will then proceed to the esophagus on the way to the stomach. Stomach is a large sack-like organ that sank the food in a very strong acid called gastric acid. The volume of stomach can be as low as 50 mL when empty and up to 4 L when full and the pH inside stomach could be as low as pH

1.5 (Shetzline and Liddle, 2002) or as high as pH 6 or above after the digestion (Shetzline and Liddle, 2002). This partly digested food mixed with the acid is called chyme. The food will subsequently enter the duodenum, which is the first part of small intestine. There are 3 regions that make up the small intestine, which are duodenum, jejunum and ileum (Cilla *et. al*., 2009). The food will pass through the jejunum and then ileum which is the final part of small intestine. In this small intestine, the ingested food will be mixed with bile (produced in the liver and stored in gall bladder), pancreatic enzymes, and others digestive enzymes produced by the wall of small intestine which help in the breaking down of food. The presence of villi and microvilli in the small intestine increase the surface area for better absorption. The critical condition of small intestine is due to the presence of bile salts and also pancreatin (Cilla *et. al*., 2009). In the large intestine, most water and electrolytes (such as sodium) will be reabsorbed into the blood. Many microbes like *Bacteroides, L. acidophilus, Escherichia coli*, and *Klebsiella* which are present in large intestine support the digestion process. At the end of the digestion process, the water content of the undigested materials in the large intestine is reabsorbed and the solid waste is kept in the rectum until it is excreted through the anus (Shetzline and Liddle, 2002).

Various structural design of food-based delivery systems has been formulated to encapsulate, protect and release bioactive components believed to benefit the human gastrointestinal (GI) tract health (McClements *et al*., 2009). These delivery systems may depend on the release of bioactive components at a particular location in the GI tract under environmental trigger (pH, ionic strength or enzyme activity; Hur *et al*., 2011). The simulation of the complex physicochemical and physiological actions occuring in the human GI tract is important in the testing of the efficacy of designed delivery systems models. Animals or humans *in vivo* method provide a realistic environment to study these

models but unfortunately they are time consuming and expensive (Vosloo, 2005). Thus, *in vitro* digestion models provide a useful alternative for rapidly screening food ingredients (Coles *et al*., 2005).

2.9.1 Viability of probiotics during digestion process

Probiotics are viable microorganisms that are beneficial to the host when consumed in sufficient quantities. Benefits include reduction in the incidence of constipation, diarrhea and bowel cancer, and stimulation of the immune system (Grajek *et al.,* 2005). In order to exert their beneficial effects on the host, they have to be able to survive passage through the host's digestive tract i.e., gastrointestinal tract, tolerating acid, bile and gastric enzymes (Maragkoudakis *et al*., 2006). The main factors detrimental to the viability of probiotics in the stomach are the low pH and antimicrobial action of pepsin. The pH range of the stomach generally is from 2.5 to 3.5, although can be as low as pH 1.5, or as high as pH 6 or above after food intake. Another barrier the probiotic bacteria need face to overcome is to survive the small intestinal environment, where they are exposed to pancreatin, and bile salts with a pH of around 8.0. Food generally remains in the stomach for 2–4 h and then transit through the small intestine between 1 and 4h. The tolerance to stomach and small intestine conditions of probiotic bacteria may also be influenced by the carrier food. A common delivery system for probiotic is food, food and other food ingredients present may also protect probiotic bacteria from acid conditions and enhance gastric survival (Huang and Adams, 2004). Two roles of food for probiotic protection from the gastrointestinal stress are (i) the increase in the pH of the gastric tract due to food formulations with appropriate pH (>5) and high buffering capacity; and (ii) reducing their physical exposure to the harsh gastrointestinal environment (Ranadheera *et al.,* 2012).

Lactobacillus and *Bifidobacterium* can be protected during passage through the gastrointestinal tract, and hence improve their viability, by incorporating them in cheese with a high-fat content (Valerio *et al.*, 2006), or amylose maize starch granules (Wang *et al.*, 1999), or two liquid vegetarian foods: Up & Go® liquid breakfast, and So-Good™ original soybean extract (Huang and Adams, 2004). Therefore, delivery in a suitable food matrix is one of the most appropriate means to maximise probiotic efficacy (Huang and Adams, 2004).

Carrier food matrix had a significant influence on the *in vitro* gastrointestinal tolerance of probiotics. This was demonstrated in *L. acidophilus* LA-5, *B. animalis* subsp. *lactis* BB-12 and *Propionibacterium jensenii* 702 when these bacteria were exposed to both highly acidic conditions (pH 2.0) and 0.3% bile. Exposure to conditions of lower pH (pH 2.0) resulted in a significant reduction in probiotic viability during simulated gastric transit tolerance compared to pH levels of 3.0 and 4.0. However, ice cream was generally found to improve the acid and bile tolerance of the probiotics compared to plain and stirred fruit yogurts. The *in vitro* adhesion ability of probiotics was also found to be influenced by the carrier food matrix, with fruit yogurt providing the most favorable outcomes, although in all cases a substantial number of viable bacteria (10^5–10^6 cfu/g) were able to attach to the Caco-2 cells (Ranadheera *et al*., 2012).

Low fat non fermented ice cream can sustain high viable numbers of *L. acidophilus* La-5 throughout its tested shelf life of 90 days (Tharani, 2012). In addition, protective effect of ice cream on the viability of *L. acidophilus* (La-05) against harsh stomach conditions was observed, but this effect was not as a result of viscosity of ice cream. It was also found that an ice cream supplemented with 10^6 cfu/g would result in a similar overall log reduction of *L. acidophilus* (La-05) at the end of 2 h simulated digestion compared to

an ice cream supplemented with 10^8 cfu/g. The aggressive stomach conditions had a negative impact on the survivability of *L. acidophilus* (La-05) during digestion of all the ice cream samples, but this detrimental effect can be reduced by incorporating *L. acidophilus* (La-05) into an ice cream matrix which would increase the opportunity of bacteria to reach the small intestine and provide the desired health benefit (Tharani, 2012).

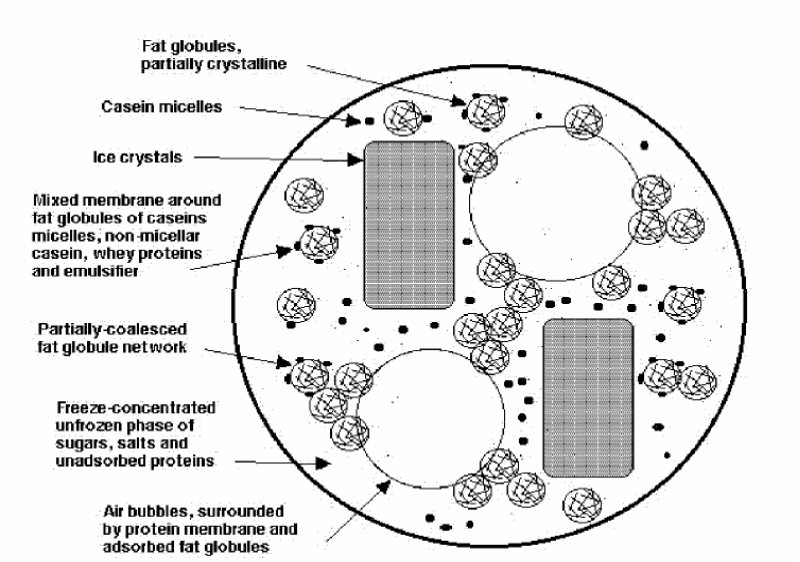
2.10 Ice cream structure characterization

Ice cream is a four-phase system containing air cells, ice crystals, emulsified fat and a continuous serum phase consisting dissolved and/or colloidal sugars, salts, proteins and stabilizers. The microscopic images of freeze fractured ice cream samples along with a schematic sketch of its structure (Figure 2.6a) showed that. Air cells appear spherical and smooth, while ice crystals are more polygons like with a network like surface structure caused by the etching process (Figure 2.6b). Thin serum lamellae separate these two disperse phases from each other (Figure 2.6c, d). Partially coalesced fat globules coat part of the air bubble surfaces (Figure 2.6e), but are also present in the serum phase (Figure 2.6f). Ice crystals grow from nuclei during manufacture and can also form networked structures by accretion (Figure 2.6g; Eisner, 2006).

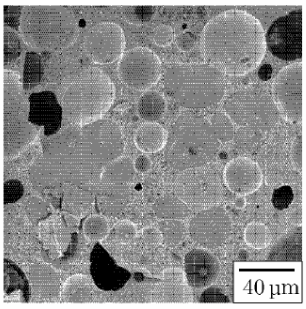
Many properties of ice cream are related to agglomerated and partially coalesced fat, like slow meltdown, good shape retention, and resistance to shrinkage, but also undesired properties like poor whipping properties, a watery serum or a buttery structure. Fat structures can be controlled by ingredients and process parameters (Eisner, 2006). These are investigated in the present studies.

Instabilities of the fat phase in ice cream can be broadly classified as creaming, coalescence and flocculation/agglomeration. Creaming plays only a minor role in

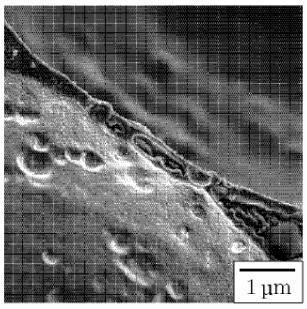
homogenised ice cream mixes and is not relevant for the frozen product. Coalescence involves the complete merging liquid of fat droplets and results in the irreversible loss of the dispersed state, as does creaming. If fusing of the droplets is obstructed the identity of the individual entity is preserved. Such aggregation can be triggered by a perikinetic or orthokinetic mechanism, the former is based on the Brownian motion, while the latter is shear induced and up to six orders of magnitude faster. It results either in flocculates or agglomerates. The first are held together reversibly (with minor energy input) either by surfactants (e. g. proteins) shared between two droplets or by hydrophobic interactions while the fat globule membrane prevents coalescence. If the fat droplets contain fat crystals and liquid fat total coalescence is obstructed even without protecting layer. Fat droplets bound together by partially solid fat bridges are referred to as fat agglomerates or partially coalesced fat. The emulsified fat droplets in ice cream usually contain liquid and crystallised fat during processing, and these are denoted as fat globules (Eisner, 2006).



(a) ice cream structure[a]

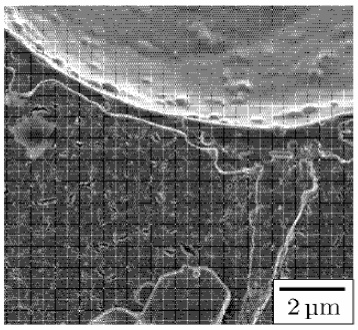


(b) overview

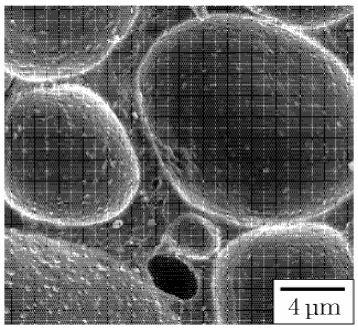


(c) lamella between two air bubles

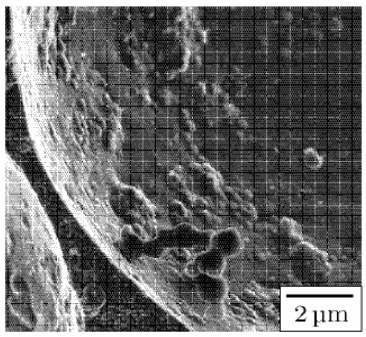
Figure 2.6 The structure of ice cream drawn schematically (a) and depicted by LT-SEM micrographs (b) to (f) at 500 × to 20000 × magnification (Eisner, 2006).



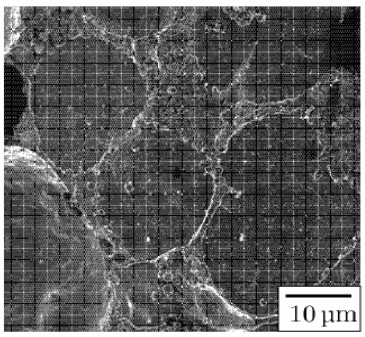
(d) serum lamellae between ice with visible growth patterns



(e) air bubbles partially covered with fat globules



(f) fat agglomerates at an air bubble surface



(g) partially accreted ice crystals crystals and an air cell

Figure 2.6 The structure of ice cream drawn schematically (a) and depicted by LT-SEM micrographs (b) to (f) at 500 × to 20 000 × magnification (continued) (Eisner, 2006).

A higher fat content in general increases creaminess and mouth coating characteristics in ice cream, while the perception of iciness is reduced and improves the products resistance to melting and heat shock (Eisner, 2006).

A network of partially coalesced fat globules in the final product is essential to stabilize air bubbles and thus foam structure (Udabage and Augustin, 2003). Partial coalescence requires the presence of fat crystals and liquid fat as the fat crystals obstruct the complete merging of two globules into a spherical shape which underlines the

importance of the solid fat content (SFC) at processing conditions (Boode and Walstra, 1993; Boode *et al*, 1993; Aken, 2001).

The milk solids nonfat (MSNF) includes mainly whey protein, micellar casein, lactose and minerals (ash). Both the source of MSNF and their treatment during processing influence the properties of the final ice cream product. They also have inherent water-holding capacities and enhance the viscosity of the mix and later of the unfrozen matrix phase (Eisner, 2006).

Proteins play an important role in stabilizing the emulsion, as they are surface active and can adsorb to both the fat globule surface and the air interface formed later on during whipping. Proteins decrease the interfacial tension of the fat droplets and form a viscoelastic and thick film at the interface that contributes to the stabilization of the fat droplets (Botega, 2012).

The main functions of sugars in ice cream are to impart a sweet taste, enhance flavour and improve shelf live. They also reduce firmness and enable the combined whipping and freezing of the ice cream mix by depressing the freezing point. The most commonly used sugars are sucrose and hydrolysed corn starch and these are blended in order to adjust relative sweetness, freezing point depression and their contribution to the total solids content of the mix (Udabage and Augustin, 2003).

Stabilisers for ice cream, typically hydrocolloids, are added in order to increase mix viscosity for improved whippability and reduced ice crystal growth. Beside this they can improve smoothness of body, structure uniformity, melt resistance and handling properties (Chang and Hartel, 2002; Udabage and Augustin, 2003).

Emulsifiers are used to lower the interfacial tension between the fat phase and the aqueous phase of emulsion systems and thus permit a finer dispersion. In ice cream mix, emulsifiers are added to destabilize the protein membranes around the fat globules in order to allow for partial coalescence (Eisner, 2006).

2.10.1 Standard methods for dissecting ice cream structure

2.10.1.1 Overrun

Overrun is commonly used by the industry to measure the amount of air incorporated in the frozen ice cream. It is expressed by the percentage increase in volume that the initial ice cream mix undergoes during whipping (batch process) or injection of air (continuous process) (Marshall *et al.*, 2003).

The light and soft texture of ice cream is directly related to its ability to incorporate and stabilize air cells. The destabilization of fat droplets is responsible for the stabilization of air cells and consequently to obtaining a high overrun. Therefore, overrun measurements become an easy way to measure the development of the structure of ice cream. Parameters such as meltdown resistance of ice cream, among others, have been associated with the overrun obtained during freezing (Muse and Hartel, 2004), such that an increase in overrun would lead to the formation of smaller air cells in the final ice cream (Rosalina and Hartel, 2004).

2.10.1.2 Meltdown rate

The meltdown rate of ice cream can be determined by placing a known amount of ice cream over a mesh grid at room temperature, and allowing it to melt. The meltdown rate of the ice cream is defined by the percentage of serum melted over time (Marshall *et al.*,

2003). The ability of an ice cream to resist meltdown is one of the most obvious attributes related to the structure of ice cream. This is because the destabilization of fat and the formation of a fat network that wraps the air cells is believed to be one of the most important factors affecting meltdown stability. However, some other factors may also affect the meltdown rate of ice cream such as the presence of a high volume of air in samples with higher overrun. The insulating effect caused by the presence of air seems to affect the heat transfer and consequently the meltdown rate of ice cream (Muse and Hartel, 2004). Muse and Hartel (2004) have also found in their study that ice crystal size and the viscosity of the mix also have an influence in the melting rate of frozen ice cream. The meltdown stability test includes evaluation of other factors besides the meltdown rate. The shape retention also characterizes the fat network formation around the air cells that gives it structure and support to overcome melting, and roughly, keep the shape of the ice cream. Visual and physical analyses of the retained and dripped phases provide important information on the extent of fat destabilization and structure formation (Bolliger *et al.*, 2000; Muse and Hartel, 2004).

2.10.1.3 Light scattering

As the emulsion is formed, controlling and monitoring its stability against aggregation and separation of the fat is important. It is also of interest to characterize the mix in terms of fat droplet size distribution to verify the level of dispersion. A stable emulsion, with small particle size, will lead to a satisfactory destabilization. Light scattering is one of the most common methods used to characterize the particle size of an emulsion (Dalgleish, 2004). Two different light scattering techniques, dynamic and integrated light scattering, are widely used to measure particle size. In the framework of

this thesis, the integrated light scattering (ILS) method was considered more appropriate than dynamic light scattering (Botega, 2012).

The ILS method consists of the application of a laser beam that traverses a clear cell containing a highly diluted solution of the emulsion. The particles in the solution scatter the light in different angles that are detected by the equipment. Software collects the information, and in conjunction with the optical properties of the particle, transforms it into particle size distribution data (Dalgleish, 2004; Aguilera and Stanley, 1999; Murphy, 1997). ILS has the ability to measure a large range of scattering angles, which facilitate the analysis of a broader range of particle sizes. In addition of new equipment which includes backscatter and large angle detectors and a blue light source with a different wave length may improve resolution of the analysis by offering a wider detection range of particle sizes (Malvern Instruments, 2010).

2.10.1.4 Differential scanning calorimetry (DSC)

This technique compares the energy required (or liberated) to increase (or decrease) the temperature of a DSC pan that contains a small amount of sample, against an empty pan. The energy is exchanged, between the equipment and the pan, in the form of heat. DSC is used to determine specific enthalpy data for food. This method is based on a differential heat fluxes measurement between the sample cell and an empty reference cell. The DSC's main advantages rely on rapid and relatively simple measurement. In addition more valuable information can be obtained by a single thermogram, namely the specific enthalpy, the apparent heat capacity and the frozen water fraction (Cogné *et al.*, 2003).

2.10.1.5 Ice cream rheology

Rheological properties of ice cream are important since they govern the quality development throughout the manufacturing process. Rheology of ice cream systems can be divided into ice cream mix and the frozen product and covers the range from a low viscous fluid (ice cream mix) to a nearly solid body (hardened ice cream). Most existing models of ice cream flow properties focus on unfrozen mix or molten ice cream. Both, mix and frozen product show a shear thinning behavior. In the mix this is mainly caused by macromolecular stabilizers and emulsifiers rather than by the dispersed fat phase in the concentration range relevant as long as no flocculation of the fat occurs. Frozen ice cream contains high volume fractions of air (about 50%) and ice crystals (about 25%) which cause pronounced shear thinning flow characteristics comparable to those observed in foams and ice slurries (Eisner, 2006). The viscosity of unfrozen mix or molten ice cream can be described by a power law model:

$$\sigma = K(\gamma)^n$$

Where: σ=the shear stress (Pa); K=consistency index (Pa s^n); γ = the shear rate (s^{-1}); and n=the flow behavior index.

which reduces to Newtonian behavior if the flow index n equals unity. For ice cream mix at 5 °C with varying stabilizers and sweeteners, the consistency coefficient K to be 0.8 Pa^{-s} and the flow index n as 0.8 on average (Eisner, 2006). With increasing temperature the viscosity decreases to an average consistency coefficient of 0.14 Pa^{-s} (n fixed to 0.7) for different fat, sweetener and MSNF contents at pasteurization (Goff *et al*., 1994). The consistency coefficient strongly depends upon the kind and amount of stabilizer added to

the mix (0.015 Pa s^n to 0.25 Pa s^n) as does the flow index (0.38 to 0.98). Often the apparent viscosity at a given shear rate is used as characteristic value (Eisner, 2006).

2.11 The structure characterization of vegetable extract and fermented ice cream

A challenge in using coconut or soybean extract in ice cream is to stabilize the colloidal system unique to these vegetable extracts. For instance the melting resistance of coconut ice cream is low due to the poor emulsifying properties of the coconut proteins (Tangsuphoom, 2008). In contrast, the soybean extract ice cream is a hard ice cream resulting in the requirement of about 15 minutes of standing at room temperature to soften before serving (Wangcharoen, 2012). Lecithin in the soy ingredient acts as emulsifier whereas the proteins of soybean extract bind with the water molecules, the resulting effects of which restrict excessive free movement among molecules in the ice cream which helps the formation of to form a stable gel network (Akesowan, 2009). As a whole both soy lecithin and proteins contribute to increase viscosity, stability, texture and extend the melting time of the ice cream (Samoto *et al.*, 2007). It is important to establish the extent of improvement in the physical properties of ice cream as a result of adding coconut or soybean extracts.

Abdullah *et al.* (2003) improved the quality of ice cream by using different ratios of skim milk in soybean extract blend and found that large quantity of skim milk with soybean extract reduces the beany flavour of soybeans and increased the quality of ice cream. In the attempt to improve physical and sensory properties of low fat coconut milk ice cream, it was found that the addition of sugar and replacement of skim milk powder with WPC in low fat coconut milk ice cream increased ice cream mix viscosity and reduced melting rate of ice cream (Kerdchouaym and Surapat, 2008). Supavititpatana and Kongbangkerd (2011)

mentioned the partial replacement of non-fat dry milk with sodium caseinate improved physical (such as melting rate) and sensory properties and also viable yogurt bacteria counts in yogurt ice cream from coconut milk. Akesowan (2009) found the replacement of skimmed milk powder with soy protein isolate (SPI) has significant effects on texture, viscosity, melting rate and sensory properties of ice cream samples, such that ice cream with 50% SPI and 50% skimmed milk powder had the highest overall acceptability.

As a result of fermentation associated with probiotics metabolism, pH milk decreased and its proteins form a gel with a sponge like structure (very small pores from microstructure of the protein network) which can retain all the water present in the milk. However this network is not very strong for holding water in yogurt and the liquid soaks back into the body of the yogurt as soon as the yogurt is stirred (Farnworth *et al*., 2007). Despite this it is known that the texture and firmness of fermented products is strongly dependent on protein content, type of protein and total solids content (Oliveira *et al.*, 2001). Hence, the fortification of yogurt ice cream with soy protein improves the textural quality of the product including firmness and viscosity (Mahdian *et al.*, 2012). These vegetable extracts properties can be extended when coconut milk is used as milk replacer. Coconut milk or coconut/soybean extract combinations are thus used to explore these possibilities in the present studies.

MATERIALS AND METHODS

CHAPTER 3 MATERIALS AND METHODS

3.1 Substrates and chemicals

Fresh cow milk, soybean, soy oil, butter, skim milk powder (Dutch Lady, Malaysia), sugar and vanilla were purchased from local grocery. Freshly pressed coconut milk was purchased from local markets. Cremodan SE 734 veg (Danisco AS, Copenhagen, Denmark, a complex of stabilizer and emulsifier containing mono- and diacyl-glycerols of fatty acid, cellulose gum, guar gum, carrageenan) was used as stabilizer. Sugar was used as sweetener whereas vanilla was added to enhance aroma development. *Bifidobacterium bifidum* (Bb-12) and *Lactobacillus acidophilus* (La-05) were obtained as pure freeze-dried probiotic culture from CHR-Hansen (Horsholm, Denmark). The de Man Rogosa and Sharpe (MRS) agar, M17 agar, buffered peptone water, yeast extract, glucose, hydrochloric acid, sodium hydroxide, phenolphthalein, petroleum ether, ammonium formate (≥99.0%), phenolphthalein, amino acid standards (99%) (including alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, hydroxyproline, leucine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan and valine), sugar standards (including lactose, glucose, fructose, galactose, sucrose, stachyose and raffinose), pepsin (1:10,000, ICN), bile salts, pancreatin (P-1500), and NaCl were purchased from Sigma Chemical Company (St Louis, MO USA) and cystein hydrochloride (L-Cys-HCl) and Anaerocult A sachets, formic acid (98%), acetic acid, sulphuric acid, catalyst ($CuSO_4.5H_2O+Na_2SO_4$; 1G (1+10)), ammonium sulphate, boric acid and bromocresol green indicator solution were obtained from Merck Company (New Jersey, USA). Maximum recovery diluent (MRD) was purchased from Oxoid Company (Australia).

3.2 Experimental design

The present study examined the effect of soybean extract or cow or coconut and composite milks on physic chemical and sensory properties of non fermented and fermented ice creams, the time taken required for fermentation of ice creams until pH = 5.50 by probiotics and growth rate of probiotics in this pH, the survival of probiotics in non fermented and fermented probiotic ice cream during 30 and 90 days of storage at -20 °C, respectively, and the viability of probiotics after subjecting fermented probiotic ice creams to simulated gastrointestinal digestion. Nine groups of set ice cream were prepared using soybean extract or cow (control) or coconut milks and various combinations of coconut or cow milks with soybean extract. The ice cream mixture was inoculated with the intermediate culture (La-05 or Bb-12) and the inoculated mixture was then equally divided into two portions. The first portion (non fermented ice cream) was immediately subjected to freezing in a batch ice cream maker and then stored (−20 °C) in a freezer. The second portion (fermented ice cream) was initially incubated in a water bath at 42 °C until the pH reached 5.50. The fermented mixes were cooled to 4 °C followed by the freezing process in a batch ice cream maker and then stored in a freezer (−20 °C). The parameters evaluated include chemical properties (pH changes, titratable acidity, total solid content, fat amount, free amino acids and sugars), physical properties (melting rate, reological, particle size, zeta potential, optical polarizing microscope (OPM) imaging and thermal properties), bacteria cell counts, time required for fermentation of probiotics in ice creams, viable bacteria cell counts in fermented ice creams (after stomach and intestinal digestion and during 90 days of storage at −20 °C) and sensory analysis. All analyses were carried out in triplicate.

3.3 Preparation of inoculated probiotic cultures

3.3.1 Starter culture

Each strain (La-05 or Bb-12) (1 g) was cultured in 250 mL sterile screw-capped glass jars containing 100 mL of sterilized skimmed milk (10 w/v). To facilitate the activation of these cultures, 0.05% (w/v) L-Cys-HCI was added to the milk in order to diminish the redox potential of the medium and thereby stimulate microbial growth. Two% (w/v) glucose and 1% (w/v) of yeast extract were also added. The incubation was carried out under aerobic condition in a still water bath (42 ^{0}C) (Julabo, Haake Model SWD 20, Germany) until pH was reduced to 5.0 (Magarinos *et al*., 2007).

3.3.2 Culture for inoculation (intermediate culture)

Inoculation culture for each strain (La-05 or Bb-12) was prepared fresh by inoculating sterilized skimmed milk in 100 mL sterile screw-capped glass jars with 4% (v/v) starter culture that were entirely filled (to minimize the presence of air). Anaerobic conditions were created using anaerocult A sachets, anaerobic jar and anaerotest® strip (Merck) prior to incubation in a still water bath (Julabo, Haake Model SWD 20, Germany) at 42 ^{0}C until pH has reduced to 5.0 (Magarinos *et al*., 2007). Bacteria colony forming unit in intermediate culture in pH = 5.0 were 5×10^{9} cfu/g for Bb-12 and 6×10^{9} cfu/g for La-05.

3.4 Preparation of vegetable extracts with 12% (w/w) total solid content

3.4.1 Preparation of soybean extract with 12% (w/w) total solid content

Soybeans (100 g) were washed three times using tap water, one time rinsing using de-ionized water, followed by soaking in de-ionized water (1 L) for 14 h at room

temperature. Excess water was then drained off and the shells were removed. The swollen beans were blended with 250 mL of boiling water in a laboratory blender (Waring, New Hartford, CT, USA) at low speed (3500 rpm) followed by boiling for 5 min. The blended soybean was then passed through 4 layers of cheesecloth. The soybean extract fat content (1.86% w/w) was corrected to 3.4% (w/w) using 1.54 g soy oil/100g soybean extract. The soybean extract was reheated to 80 °C for 10 min and immediately chilled (4 °C) prior to making ice cream.

3.4.2 Preparation of coconut milk 12% (w/w) total solid content

The brown hard coconut shell was cracked open and the white copra was grated followed by mechanical pressing to obtain the milk. To achieve coconut milk with 12% (w/w) total solid content, 300 g of fresh coconut milk (after sieving with double layers of cheesecloth) was mixed with 700 g of distilled water. The diluted coconut milk was heated at 80 °C for 10 min prior to chilling (4 °C) and was used within 1 h.

3.5 Preparation of ice cream

In many countries, the fat and total solid content in ice cream ranged 8-18% (w/w) and 35-44% (w/w), respectively (Goff and Hartel, 2013). Hyvoen *et al.* (2003) reported that different types of fat (dairy and vegetable fats) had no significant effect on physical properties of ice creams, although fat amount did affect of ice cream physical properties of ice creams. In the present studies, ice cream was prepared by using soybean extract, or cow, or coconut milks and various combinations of coconut or cow milks with soybean extract. The fat content in cow milk, soy bean extract and coconut milk were different when total solid was adjusted to 12% (w/w) (see Table 3.1). To achieve ice creams with the same fat amount (fat of ice cream mix = 10.52% (w/w)), butter was added.

Table 3.1 Chemical parameters of soybean extract, coconut and cow milks

Sample	Total solid (% w/w)	Fat (% w/w)
Cow milk	12±0.08	3.4±0.05
Soybean extract	12±0.07	3.4±0.04
Coconut milk	12±0.09	8.0±0.05

The amount of butter needed to adjust the fat of ice cream mixes (10.52% w/w) was calculated using following formula (Goff and Hartel, 2013):

$$\text{The amount of butter needed} = \frac{\text{Fat needed (g)} - [\text{milk needed (g)} \times (\text{Fat in milk (\%)}/100)}{\text{Fat in butter (\%)}} \times 100$$

Fat content in other ingredients (skim milk powder, sugar, stabilizer–emulsifier, vanilla and water) is 0% (w/w), hence they were not mentioned in the formula.

For example the amount of butter needed for ice cream with 100% coconut milk is determined as:

$$\text{The amount of butter needed} = \frac{10.52\ (\text{g}) - [55.4\ (\text{g}) \times (8\ (\%)/100)]}{83.3\ (\%)} \times 100 = 7.31$$

Fat content in butter = 83.3% (w/w)

Fat content in coconut milk = 8% (w/w)

Milk needed = 55.4 g

Fat in ice cream = 10.52 g

Hence ice cream mixes with fat content of 10.52% (w/w) and total solids of 40-43% (w/w) for a total batch of 100 g, formulated according to Table 3.2 (Goff and Hartel, 2013):

Table 3.2 The content of components used in ice cream mix formulations (percentage by weight)

Sample[A]	Ingredient						
	Milk formula (% w/w)	Butter (% w/w) (Fat = 83.3%w/w)	Skim milk powder (% w/w)	Sugar (% w/w)	Stabilizer (% w/w)	Vanilla (% w/w)	Water (% w/w)
W	55.40	10.37	7	17	0.6	0.1	9.62
C	55.40	7.31	7	17	0.6	0.1	9.62
S	55.40	10.37	7	17	0.6	0.1	9.62
SW1	55.40	10.37	7	17	0.6	0.1	9.62
SW2	55.40	10.37	7	17	0.6	0.1	9.62
SW3	55.40	10.37	7	17	0.6	0.1	9.62
SC1	55.40	9.60	7	17	0.6	0.1	9.62
SC2	55.40	8.84	7	17	0.6	0.1	9.62
SC3	55.40	8.08	7	17	0.6	0.1	9.62

[A]W: ice cream with 100% cow milk; C: ice cream with 100% coconut milk; S: ice cream with 100% soybean extract; SW1: ice cream with 75% soybean extract+25% cow milk; SW2: ice cream with 50% soybean extract+50% cow milk; SW3: ice cream with 25% soybean extract+75% cow milk; SC1: ice cream with 75% soybean extract+25% coconut milk; SC2: ice cream with 50% soybean extract+50% coconut milk; SC3: ice cream with 25% soybean extract+75% coconut milk.

The milk or milk combinations with butter were heated to 50 °C prior to mixing with the skim milk powder, sugar, vanilla, water and stabilizer. The mixes were subjected to two homogenization stages (16,000 rpm, 70 °C, 5 min; Ika Homogenizer T-25 basic Ultra Turrax, Germany). The mixes were pasteurized at 80 °C for 10min in a water bath and then cooled to 4 °C prior to overnight aging at 4 °C. Each mixture was inoculated with 4% (w/w) intermediate culture followed by thorough gentle mixing. The inoculated ice cream mixture was then equally divided into two portions.

The first portion was immediately frozen in a 1.5 L batch ice cream maker (Baumatic gelato1ss, UK; rotor speed 50 round/min, 40 min, -30 °C) and packed in 100 mL plastic cups. The cups were covered using the lids prior to storage at −20 °C in a freezer. The ice creams made from the first portion are called non fermented ice creams.

The second portion was fermented by incubating ice cream in a water bath at 42 °C for varying lengths of time until pH was reduced to 5.50. After fermentation, the ice cream mixes were cooled to 4 °C in an ice bath followed by freezing in a 1.5 L batch ice cream

maker and packing in 100 mL plastic cups. All cups were covered using the lids prior to storage at −20 °C in a freezer. The ice creams made from the second portion are called fermented ice creams.

3.6 Chemical analysis

3.6.1 Measurement of pH and titratable acidity (TA)

The pH change was monitored by determining the free H^+ concentration in ice cream using a digital pH meter (Mettler Toledo 320, Switzerland). The pH meter was calibrated to pH 4.0 and 7.0 using standard solution and the electrode was rinsed with distilled water before and after pH determination. Titratable acidity (TA; % lactic acid equivalent) was determined by titration using 0.1 N NaOH. Ice cream samples (1 mL) were transferred into an Erlenmeyer flask containing 9 mL dH_2O, followed by the addition of a few drops of 0.1% phenolphthalein. NaOH (0.1 N) was titrated into the sample subjected to continuous stirring until a definite pink colour lasting for 30 seconds was obtained. The volume of NaOH required to neutralize the acid in ice cream was used to calculate the content of TA (Sadler and Murphy, 1998) by using the following formula:

$$\text{TA (\% lactic acid)} = \frac{\text{d.f.} \times \ V_{NaOH} \ \times 0.009\text{g} \ \times 0.1}{\text{W (g)}} \times \ 100\%$$

Dilution factor (d.f.) = 10

V_{NaOH} = Volume of NaOH used to neutralize the lactic acid

0.009= conversion factor, 1 mL NaOH (0.01 N) neutralizes 0.009 g of lactic acid

0.1 = Normality of NaOH

W = weight of yogurt sample for titration

3.6.2 Total solid

Total solid (TS) is a measure of the quantity of solids dissolved or suspended in the sample. Total solid measurement in milks and ice creams was adapted from Akin *et al*. (2007). Approximately 10 g of milk or ice cream sample was placed in pre-dried dish of known weight (Adventure Ohaus) and kept in an air oven at 100±1 ^{0}C (Memmert) for 3.5 h. The sample was then cooled in the desiccator containing cobalt (II) chloride anhydrous for 15 min prior to re-weighing. The sample was again reheated in the oven for another 1 h, cooled and re-weighed. This was repeated until the dried sample showed constant weight. The total solids content were calculated as follows:

$$\text{Total solids (\% w/w)} = \frac{\text{weight of dried sample plus dish} \ - \ \text{weight of dish}}{\text{Weight of sample}} \times \ 100\%$$

3.6.3 Fat analysis

Dried sample (1-4 g, see 3.6.2) was added into a thimble. The thimble was inserted into the soxhlet apparatus, and the hot plate was turned on under a round bottom flask (150

mL) filled with the petroleum ether until its 2/3 full. Once the solvent was boiling at a steady rate, the sample was left to "run" through seven refluxes for 6 h. The flasks were allowed to dry in an oven (102 ºC) for 2 h. They were then weighed and the percent fat extracted was calculated (AOAC, 2005).

$$\text{Fat (\% w/w)} = \frac{\text{W2} - \text{W1}}{\text{W3}} \times 100\%$$

W1=Weight of empty flask (g) before reflux

W2=Weight of flask (g) after reflux and drying in oven

W3=Weight of sample (g)

3.6.4. Protein

I. *Digestion*: A prepared dried sample containing approximately 0.5 g is weighted on a piece of greaseproof paper tared on an analytical balance (Denver analytical company, USA). The paper is folded around the sample by tweezers and placed into 100 mL kjedahl flask. Catalyst mixture ($CuSO_4.5H_2O+Na_2SO_4$; 1G (1+10)) was added to kjeldahl flask with 10 mL of the concentrated sulphuric acid and mixed by swirling. The acid was used to wash down any catalyst or sample left on the neck of the flask. Each kjedahl flask was heated on the digestion apparatus, very gently at first, taking care to prevent the black froth from entering the neck of the flask. When the initial frothing had ceased and copious white vapour appeared, it was boiled vigorously until no black particles remained and until the digest became a clear pale blue-green in colour. On reaching this stage, the heating was adjusted to give gentle boiling and continued for two hours.

II. *Dilution*: at least 12 h were needed for more refractory materials unless it could be demonstrated that equal nitrogen is converted to ammonium sulphate in less time. The kjedahl flask was allowed to cool before 50 mL of distilled water was added. The contents were then mixed thoroughly to ensure any crystals, which separate out, were dissolved. Next, the contents were transferred into a 250 mL boiling flask, using 200 mL of distilled water to rinse thoroughly the contents from kjedahl flask into the boiling flask.

III. *Distillation*: sodium hydroxide solution (70 mL; 30 % w/v) was poured into the boiling flask. Immediately after this step, each flask was connected to the distillation apparatus, which had the tip of its condenser outlet tube immersed in 50 mL of 2% boric acid solution with a few drops of the indicator solution (bromocresol green) added in a 250 mL Erlenmeyer flask. The contents of each boiling flask were swirled to mix completely and were boiled gently at first to prevent excessive frothing. After 125 mL of distillate have been collected and colour had varied from green to red, the receiver flask was lowered until the tip of the condenser outlet tube was approximately 40 mm above the 200 mL mark. Heat treatment was terminated instantly.

IV. *Titration*: total nitrogen in the sample was now presumably held as ammonia in the boric acid indicator solution and titrated with standard volumetric sulphuric acid solution (0.2 N). It is delivered from burette graduated to 0.01 mL unit the colour matches that of a previously prepared solution before digestion. A blank titration was carried out following the procedure except for addition of the sample. The crude protein in the sample was calculated using the following formula (AOAC, 2005):

$$\text{Crude protein (\%)} = \left[\frac{(V - b)] \times N}{W}\right] \times 1.401 \times 6.38$$

Where 6.38 is the general factor

W: Weight of sample (g)

V: Titration value for the sample (mL)

N: Normality of sulphuric acid

b: Titration value for the blank test (mL)

Assumption: 100 g protein = 16 g nitrogen

3.6.5 Analysis of free amino acids by LC/MS

Free amino acid amounts were determined in accordance with a method as described by Ozcan and Senyuva (2006).

Stock solutions of 1000 μg/mL amino acids were prepared by dissolving 25 mg of each in 25 mL of distilled water. Working standards were prepared by diluting the stock solution of amino acids to concentrations of 0.05-5.00 μg/mL with 0.2 mM acetic acid. Stock solutions were kept at 4 °C for a week for daily use and kept at -18 °C for longer term storage. Working standards were prepared daily before analysis. For amino acid analysis in samples, ice creams were homogenized and the homogenate was stored at 20 ^{o}C. Subsequently, 1 g of the homogenized sample was transferred into a 10 mL capped glass centrifuge tube. Ten ml of 0.2 mM acetic acid was added to the sample. After mixed for 2 min by a vortex mixer, the mixture was centrifuged at 5000 rpm (10 min at -5 ^{o}C). The supernatant was filtered through a 0.22 μm-pore diameter filter and applied into the device

for analysis. Liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (LC/APCI-MS) analysis was used for the screening and quantification of different free amino acids. For this purpose, an HPLC system combining an autosampler, temperature-controlled column oven, and a binary pump coupled to an MS detector equipped with APCI was used. The analytical separation of samples was performed on Zorbax Bonus-RP, Narrow Bore (100 mm 2.1 mm, 3.5 mm) using an isocratic mixture of 0.01mM acetic acid in 0.2% aqueous solution of formic acid at a flow rate of 0.2 mL/min. Data acquisitions were performed in the selected ion monitoring (SIM; positive ion mode) mode using the interface parameters. Other conditions were drying gas (N2) flow of 4 L/min, drying gas and vaporizer temperatures of 320 °C, nebulizer capillary voltage of 3 kV, corona current of 8 mA, fragmentor voltage of 55 eV, and pressure of 55 psig. Full scan analyses were performed in the mass range of 50–500 Da for the spectral identification of amino acids and sample co-extractives, respectively.

3.6.6 Analysis of sugars by LC/MS

Sugars contents were determined in accordance with a method as described by Kumaguai (2001). Ice creams homogenized by ultra turrax and the pH of homogenized samples were determined. Subsamples of the homogenate were stored at -20 °C in high density polyethylene bottles with plastic screw cap lids. Finely homogenized sample (1 g) was weighed (fresh weight) into a 10 mL glass centrifuge tube with cap. Ten ml of 0.2 mM acetic acid was added to the sample. After mixing in a vortex mixer for 2 min, the mixture was centrifuged at 10000 rpm for 5 min at -5 ^{o}C. The clear supernatant was quantitatively transferred into a vial, avoiding the top oil layer if present. The supernatant was filtered through 0.22 µm nylon syringe filter prior to LC/MS analysis. The LC/MS analytical system consists of an HPLC system combining an autosampler, temperature-controlled

column oven, and a binary pump coupled to an MS/MS detector equipped with ESI was used. (Perkin Elmer UHPLC Flexar 15 with AB Sciex QTrap 3200 MS/MS detector). The analytical separation of samples was performed on Agilent Zorbax RP C18, (150 mm × 4.6 mm, 5 um) using a gradient elution of water and acetonitrile with 0.1% formic acid and 5 mM ammonium formate, at a flow rate of 0.8 mL/min. Data acquisitions were performed in the multiple reaction monitoring (MRM) mode using the interface parameters. Other Conditions were drying gas (N_2) flow of 40 psi, drying gas and vaporizer temperatures of 500 °C, nebulizer capillary voltage of 4.5 kV, selective collision energy and declustering potential for each sugar compounds. Multiple reactions monitoring (MRM) scan in negative ionisation which is a highly selective and sensitive method was used to analyse each sugar with their mass and fragments. Identified peaks were quantified using authentic standards.

3.7 Physical analysis

3.7.1 Meltdown

The ice cream melting rate was determined as described by Mahdian *et al.* (2012). Tempered ice cream samples (spherical shape, -20 °C, 30 g) were prepared by scraping the surface of ice cream using a stainless steel table spoon and these were placed on a 0.2 cm wire mesh screen above a beaker at room temperature (25 °C). The weight of the melted material was measured after 20 min and declared as percentage weight of ice cream melted.

3.7.2 Rheological measurements

Rheological measurements of melted ice cream samples were determined using a Physica MCR 301 rheometer (Anton-Paar GmbH, Graz, Austria; Figure 3.1) with a concentric cylinder geometry (Figure 3.2) coupled with a circulating cooling bath at

4.0±0.1 °C. Melted ice creams (about 20 g) were left to equilibrate at 4.0 °C for 15 min. The samples flow behavior was generated by linearly increasing the shear rate from 19.6 to 67.3 s^{-1} in 20 min followed by returning to 19.6 s^{-1} over a further 20 min.

The hysteresis of ice creams was evaluated by calculating the area between the shear stress/shear rate curves.

The consistency index and the flow behavior were explained by the Power Law model.

$$\sigma = K(\gamma)^n$$

σ=the shear stress (Pa)

K=consistency index (Pa s^n)

γ = the shear rate (s^{-1})

n = the flow behavior index.

Apparent viscosity of ice creams was estimated as a function of time under a constant shear rate of 20 s^{-1} (Rossa *et al*., 2012).

Figure 3.1 Physica MCR 301 rheometer (Anton-Paar GmbH, Graz, Austria).

Figure 3.2 Cup and geometry used for measuring rheology properties of ice creams.

3.7.3 Size and zeta potential

The average particle size and zeta potential of fat globules of ice cream mixes were determined by using Malvern Zetasizer Nano Series (Malvern Instruments, UK) at a constant temperature of 25 °C. Measurements were carried out with the dilution of the ice cream mixes with deionized water (1×10^{-4}). The zeta potential and size of ice cream mixes were monitored after the aging step (Tan and Misran, 2012).

3.7.4 Optical polarizing microscope imaging (OPM)

Light polarizing microscope from Leica model PM RXP by Leica Microsystems GmbH, Germany was used to observe the emulsion droplets formed of ice cream mixes (after aging step). Polarizing microscope unit was equipped with high voltage beam, polarizing unit and a JVC Colour Video camera with model KY F550, interfaced with personal computer with Leica QW in image analysis software. All measurements were carried out at room temperature (25 °C) (Tan and Misran, 2012).

3.7.5 Differential scanning calorimetry (DSC) of ice cream

The thermal properties of ice cream mixes (after aging step) were measured by a differential scanning calorimeter (DSC) by Mettler Toledo (model DSC822e) according to the method reported by Hwang *et al*. (2009). Sample of ice cream mixes (about 5 mg) was placed in a pre-weighed aluminum sample pan and the pan was sealed using a Quick Press pan crimper (Perkin Elmer) and the thermal data were recorded from -30 ^{o}C to +30 ^{o}C in nitrogen atmosphere with a heating rate of 5 °C min^{-1}. An empty pan served as the reference. The flow rates of nitrogen gas for cooling and heating were 110 and 40 cc/min, respectively.

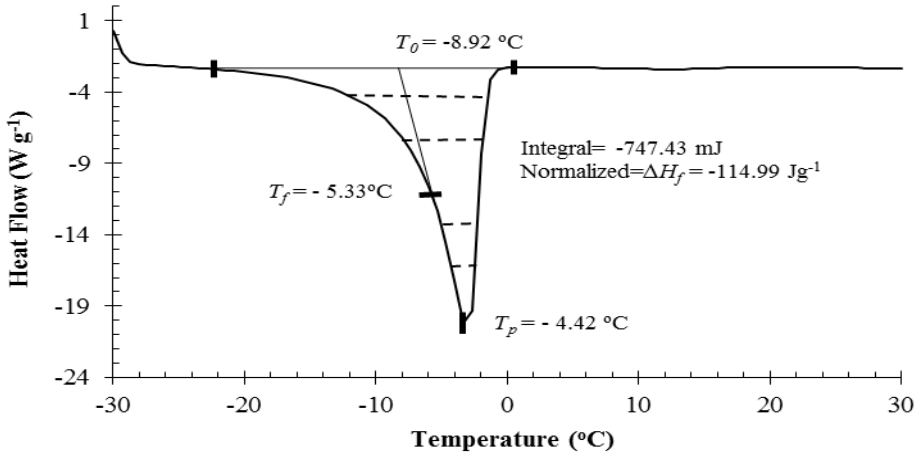


Figure 3.3 A typical DSC thermogram to determine the freezing point and ΔH_f of ice cream.

The onset temperatures (T_0), peak temperatures (T_p), freezing points (T_f) and enthalpies (ΔH_f) of the transitions of ice formation and ice melting were recorded. The onset temperatures are considered as the intersection of the tangent and base line to the left side of the melting peak. Freezing points were calculated by determining the temperature at which the steepest slope was observed (the temperature at maximum slope of the endotherm or the extra-plotted peak onset temperature (T_0) of the ice melting (point T_f in Figure 3.3; Rahman, 2008). The enthalpy of the phase transition (ΔH_f = enthalpy of fusion) was determined by extrapolating the baseline under the peak by connecting the flat baseline before and after the melting peak and integrating the peak above the baseline, as indicated in Figure 3.3. The amount of ice formed per gram of sample (freezable water) was determined by integrating the melting curves and dividing the melting enthalpy with the pure ice fusion latent heat (ΔH_s = 334 J g^{-1}) (Soukoulis *et al.*, 2009).

3.8 Microbial analysis

3.8.1 Enumeration of *Lactobacillus acidophilus*

The *Lactobacillus acidophilus* was enumerated using MRS agar by pour plate count method. One milliliter of aliquot dilution was transferred onto sterile petri dishes followed by gentle pouring of 15 mL of sterile MRS culture. The contents in the petri dishes were evenly stirred by gently tilting and swirling the dishes. Then they were left for 15 min at room temperature to allow the MRS agar to solidify. Parafilm was used to seal the petri dishes prior to incubation in an incubator (Revco Ultima, USA) under aerobic condition (5% CO_2; Ashraf and Shah, 2011) at 37 ^{0}C for 48-72 h. The colony forming unit of La-05 in the sample was expressed as colony forming units per milliliter sample (cfu/mL) using the following formula (Magarinos *et al*., 2007):

$$\text{cfu/mL} = \frac{\text{Number of colonies formed } \times \text{ dilution factor of sample}}{\text{1 mL of sample}}$$

*cfu: colony forming unit

3.8.2 Enumeration of *Bifidobacterium bifidum*

The *Bifidobacterium bifidum* was enumerated using MRS-L-Cys-HCl agar. Cystein hydrochloride (L-Cys-HCl) was added to the agar medium in order to diminish its redox potential (Magarinos *et al*., 2007). The formulation of MRS-L-Cys-HCl was prepared according to Magarinos *et al*., (2007) where MRS agar (62 g/ 930 L dH2O, 45 ^{o}C) was supplemented with 0.05% (w/v) L-Cys-HCl. Diluted ice cream (1 mL) was pour plated with 15 mL of sterilized MRS- L-Cys-HCl media (see section 3.2.10.2). The media plates were incubated anaerobically. Anaerobic conditions were created using anaerocult A

sachets, anaerobic jar and anaerotest® strip (Merck) prior to incubation in an incubator (Revco Ultima, USA) at 37 ^{0}C for 48-72 h. The results were expressed as colony-forming units per mililiter (cfu/mL) of sample and were calculated (Magarinos *et al*., 2007) as follows:

$$\text{cfu/mL} = \frac{\text{Number of colonies formed} \times \text{dilution factor of sample}}{\text{1 mL of sample}}$$

*cfu: colony forming unit

3.8.3 Survival of probiotics in ice cream during frozen storage

Colony forming unit was determined immediately after inoculating the probiotic cultures and after 1 and 30 days of frozen storage in nonfermented probiotic ice creams and immediately after inoculating the probiotic cultures, after fermentation and again after 1, 30, 60 and 90 days of frozen storage in fermented probiotic ice creams. Ice cream samples (1 mL) were mixed with 9 mL of sterile buffered peptone water (20 g/L dH_2O) and serially diluted with sterile peptone water (20 g/L dH_2O) before enumeration of colony forming unit of probiotics in ice creams (see sections 3.8.1 and 3.8.2).

3.8.4 Tolerance assay to gastrointestinal media

3.8.4.1 Preparation of simulated gastric and intestinal juices

The simulated gastric and intestinal juices were freshly prepared according to the protocols described by Ranadheera *et al.* (2012). Simulated gastric juices (SGJ) were prepared by suspending pepsin (1:10,000, ICN) (Sigma-Aldrich, USA) in sterile filtered 0.5% (w/v) NaCl solution to a final concentration of 3 g/L, with the pH adjusted to 2.0 with concentrated HCl or sterile 0.1 mol/L NaOH. Simulated small intestinal juices were

prepared by suspending pancreatin USP (P-1500, Sigma-Aldrich, USA) in filter sterilized 0.5% NaCl (w/v) solution to a final concentration of 1 g/L, with 0.3% bile salts (Oxoid, Australia) and adjusting pH to 8.00 with sterile 0.1 mol/L NaOH. Both solutions were filtered for sterilization through a sterile nylon 0.22 µm membrane.

3.8.4.2 Cell tolerance to gastrointestinal

Ice cream samples (1 g) were transferred into sterile 15 mL falcon tubes containing either gastric or small intestinal juices (9 mL). The mixture was then homogenized using a vortex mixer (Ratek Instruments Pty Ltd., Australia) at maximum setting for 10 s and incubated at 37 °C. Aliquots of 1 mL were removed from tubes (after 1, 30 and 120 min in order to assess acid tolerance and after 1, 60 and 120 min in order to determine bile tolerance) for the determination of total colony forming units.

3.8.4.3 Determination of total viable cell

Ice cream samples (1 mL) were mixed with 9 mL of sterile maximum recovery diluents (MRD) (20 g/L dH_2O) and serially diluted with sterile diluted with maximum recovery diluents (MRD) (20 g/L dH_2O). Colony forming unit was determined as described in section 3.7.

3.9 Sensory analysis

The ice creams were organoleptically evaluated by forty-two consumer panelists (25–30 year; twenty-two males, twenty females), from students and staff members of the Institute of Biological Sciences, Faculty of Science, University of Malaya, using a sensory rating scale of 1-10 for taste and flavour, and 1-5 for consistency and 1-5 for appearance and colour (Akin *et al*., 2007).

The defect properties evaluated are as followed: (a) four attributes for flavour and taste (cooked flavour, sweetness, lack of flavour, acidic/sour). For each criterion, sample was ranked from 1 to 10 (1–2 = low intensity, 5–6 = moderate, 9–10 = high intensity); (b) six characteristics of body and texture (crumbly, coarse, weak, gummy, fluffy, sandy). For each criterion, sample was ranked from 1 to 10 (1–2 = low intensity, 5–6 = moderate, 9–10 = high intensity); (c) two terms describing colour and appearance (dull colour, unnatural colour). For each criterion, sample was ranked from 1 to 10 (1–2 = low intensity, 5–6 = moderate, 9–10 = high intensity) (Table 3.3; Lin, 2012). The evaluation form was given to each panel with 3 groups of ice cream (cow, soy and coconut milk ice creams) with each group consisting of 3 coded ice cream samples served in plastic cups (10 g for each). The first group contained La-05 and Bb-12-cow milk ice creams. The second group contained La-05 and Bb-12-soybean extract ice creams. The third group contained La-05 and Bb-12-coconut milk ice creams. Water was available for panel members to rinse their mouth between samples eating.

Table 3.3 Sensory attributes and definitions (Goff and Hartel, 2013).

Categories	attribute	Definition
Taste and flavour (1-10)	no criticism	
	cooked flavour	Cooked: Caused by using milk products heated to too high a temperature or by using excessively high temperatures in mix pasteurization. It can dissipate with time, the same as cooked defect in fluid milk. Sulfhydryl flavour: Caramel-like, scalded milk, oatmeal-like.
	lack of sweetness and too sweet	
	lack of flavour	
	rancid and oxidized	Oxidized: Caused by oxidation of the fat or lipid material such as phospholipid, similar to fluid milk oxidation. Induced by the presence of copper or iron in the mix or from the milk itself. Mono-and-di-glyceride or Polysorbate 80 can also oxidize. Various stages - cardboardy, metallic (also described as painty, fishy). Rancid: Caused by rancidity (high level of free butyric acid from lipolysis) of milk fat. May be due to use of rancid dairy products (pumping or excessive foaming of raw milk or cream) or to insufficient heat before homogenization of mix. See description of Lipolysis, especially the release of free butyric acid.
	other	
Texture and body (1-5)	coarse	Due to the presence of ice crystals of such a size that they are noticeable when the ice cream is eaten.
	crumbly	A flaky or snowy characteristic
	weak	Ice cream lacks "chewiness" and melts quickly into a watery liquid.
	fluffy	A spongy/marshmallowy characteristic
	gummy	This defect is the opposite of Crumbly in that it imparts a pasty or putty-like body.
	sandy	One of the most objectionable texture defects but easiest to detect. It is caused by Lactose crystals, which do not dissolve readily and produce a rough or gritty sensation in the mouth. This can be distinguished from "iciness" because the lactose crystals do not melt in your mouth.
	no criticism	
Appearance and colour (1-5)	no criticism	
	dull colour	
	unnatural colour	-Wrong shade of colour used for flavourd ice cream. -Too much yellow colouring used in vanilla ice cream. -Grayish colour due to neutralization.

3.10 Statistics

The statistical analysis was performed using SAS statistical software, Version 6.12 edition (SAS, 1996) followed by Duncan's multiple range method for mean comparison. The criterion for statistical significance was $p<0.05$ (Homayouni *et al*., 2008). The experiments were assayed in triplicates, and the results were expressed as mean±S.E.M

(standard mean error) values. Principal component analysis (PCA) was performed by XLSTAT software version 2014 (Addinsoft SARL, Paris, France) on the covariance matrix for all sensory attributes.

CHAPTER 4 RESULTS

CHAPTER 4 RESULTS

4.1 Chemical properties

4.1.1 Composition and chemical properties (pH, TA, TS and fat)

The chemical compositions of the ice creams are presented in Tables 4.1 and 4.2. Total solid and fat in both non fermented and fermented ice creams were unchanged by partial replacement of cow milk with soybean extract or coconut milk. The pH and titratable acidity (TA) were unchanged in fermented ice creams. But in non fermented ice creams, the pH of ice cream with cow milk (W = 6.80±0.01) were lower than those made with vegetable extracts while TA values were unchanged.

Table 4.1 Chemical properties (pH and TA) of experimental ice creams

Samples	Fermented ice cream								Non fermented ice cream	
	Fermented ice cream by La-05				Fermented ice cream by Bb-12					
	pH (Value)			TA	pH (Value)			TA	pH	TA
	Fermented ice creams	In simulated gastric juice	In simulated intestinal juice	(% lactic acid)	Fermented ice creams	In simulated gastric juice	In simulated intestinal juice	(% lactic acid)	(Value)	(% lactic acid)
W	5.51±0.01[a]	4.47±0.01[a]	5.91±0.01[a]	0.27±0.004[a]	5.50±0.01[a]	4.46±0.01[a]	5.90±0.01[a]	0.27±0.006[a]	6.80±0.01[f]	0.158±0.006[a]
C	5.50±0.01[a]	4.46±0.01[a]	5.91±0.01[a]	0.27±0.003[a]	5.50±0.01[a]	4.47±0.01[a]	5.91±0.01[a]	0.27±0.004[a]	7.38±0.01[a]	0.164±0.004[a]
S	5.50±0.01[a]	4.38±0.02[a]	5.90±0.01[a]	0.27±0.006[a]	5.51±0.01[a]	4.45±0.01	5.89±0.01[a]	0.27±0.003[a]	6.930.01±[e]	0.160±0.003[a]
SW	5.51±0.01[a]	4.42±0.01[a]	5.91±0.01[a]	0.27±0.004[a]	5.50±0.02[a]	4.44±0.02[a]	5.90±0.01[a]	0.27±0.006[a]	7.04±0.02[d]	0.161±0.006[a]
SW2	5.50±0.02[a]	4.44±0.01[a]	5.91±0.01[a]	0.27±0.002[a]	5.49±0.01[a]	4.42±0.01[a]	5.91±0.01[a]	0.27±0.004[a]	7.08±0.01[d]	0.160±0.004[a]
SW3	5.49±0.03[a]	4.43± 0.01[a]	5.90±0.01[a]	0.27±0.007[a]	5.50±0.01[a]	4.44± 0.01[a]	5.91±0.01[a]	0.27±0.003[a]	7.14±0.01[c]	0.160±0.003[a]
SC1	5.51±0.02[a]	4.46±0.03[a]	5.91±0.01[a]	0.27±0.008[a]	5.52±0.03[a]	4.44±0.03[a]	5.93±0.01[a]	0.27±0.009[a]	7.12±0.03[c]	0.162±0.009[a]
SC2	5.50±0.00[a]	4.45±0.01[a]	5.92±0.01[a]	0.27±0.008[a]	5.50±0.01[a]	4.45±0.01[a]	5.91±0.01[a]	0.27±0.008[a]	7.22±0.01[b]	0.162±0.008[a]
SC3	5.50±0.01[a]	4.47±0.01[a]	5.90±0.01[a]	0.27±0.009[a]	5.51±0.01[a]	4.43±0.01[a]	5.90±0.01[a]	0.27±0.005[a]	7.35±0.01[a]	0.160±0.005[a]

[A]W: ice cream with 100% cow milk; C: ice cream with 100% coconut milk; S: ice cream with 100% soybean extract; SW1: ice cream with 75% soybean extract+25% cow milk; SW2: ice cream with 50% soybean extract+50% cow milk; SW3: ice cream with 25% soybean extract+75% cow milk; SC1: ice cream with 75% soybean extract+25% coconut milk; SC2: ice cream with 50% soybean extract+50% coconut milk; SC3: ice cream with 25% soybean extract+75% coconut milk.
[B]means values±standard deviation.
[a-f] Means in the same column followed by different letters were significantly different($p < 0.05$).

Table 4.2 Chemical properties (TS and fat) of experimental ice creams

Samples[A]	Fermented ice cream				Non fermented ice cream	
	Fermented ice cream by La-05		Fermented ice cream by Bb-12			
	TS ($g\ 100g^{-1}$)[B]	Fat ($g\ 100g^{-1}$)[B]	TS ($g\ 100g^{-1}$)[B]	Fat ($g\ 100g^{-1}$)[B]	TS ($g\ 100g^{-1}$)[B]	Fat ($g\ 100g^{-1}$)[B]
W	43.89±0.09[a]	10.40±0.05[a]	43.91±0.08[a]	10.50±0.04[a]	43.90±0.07[a]	10.50±0.04[a]
C	43.18±0.06[a]	10.40±0.04[a]	43.16±0.07[a]	10.40±0.05[a]	43.17±0.05[a]	10.40±0.05[a]
S	43.90±0.07[a]	10.40±0.03[a]	43.94±0.08[a]	10.50±0.02[a]	43.93±0.07[a]	10.50±0.02[a]
SW	43.21±0.14[a]	10.50±0.05[a]	43.23±0.15[a]	10.40±0.04[a]	43.24±0.12[a]	10.40±0.04[a]
SW2	43.45±0.18[a]	10.40±0.04[a]	43.42±0.17[a]	10.30±0.05[a]	43.43±0.19[a]	10.30±0.05[a]
SW3	43.68±0.16[a]	10.30±0.05[a]	43.66±0.15[a]	10.50±0.02[a]	43.69±0.18[a]	10.50±0.02[a]
SC1	43.63±0.11[a]	10.40±0.03[a]	43.62±0.10[a]	10.30±0.02[a]	43.65±0.13[a]	10.30±0.02[a]
SC2	42.78±0.14[a]	10.50±0.02[a]	42.79±0.12[a]	10.50±0.01[a]	42.80±0.14[a]	10.50±0.01[a]
SC3	43.20±0.10[a]	10.50±0.02[a]	43.21±0.11[a]	10.40±0.01[a]	43.22±0.10[a]	10.40±0.01[a]

[A]W: ice cream with 100% cow milk; C: ice cream with 100% coconut milk; S: ice cream with 100% soybean extract; SW1: ice cream with 75% soybean extract+25% cow milk; SW2: ice cream with 50% soybean extract+50% cow milk; SW3: ice cream with 25% soybean extract+75% cow milk; SC1: ice cream with 75% soybean extract+25% coconut milk; SC2: ice cream with 50% soybean extract+50% coconut milk; SC3: ice cream with 25% soybean extract+75% coconut milk.
[B] means values±standard deviation.
[a-f] Means in the same column followed by different letters were significantly different($p< 0.05$).

4.1.2 Sugar amounts in ice creams

The content of sugars in non fermented ice creams and ice creams fermented by La-05 and Bb-12 are presented in Tables 4.3 and 4.4. In ice creams containing composite milks, the stachyose and sucrose amounts increased with higher soybean extract amount in non fermented and fermented ice creams ($p<0.05$). However, the lactose content increased with decreasing soybean extract proportion in composite milks containing cow milk ($p<0.05$; Tables 4.3 and 4.4). There were no differences in lactose content in non fermented ice creams containing coconut milk with increasing soybean extract amount ($p>0.05$; Table 4.3) but it decreased in fermented kind of them with increasing soybean extract amount ($p<0.05$; Table 4.4).

Table 4.5 shows the change rate of sugars ($mg.mL^{-1}/h$) due to fermentation until pH = 5.50 (positive amount (+) = appearance; negative amount (-) = disappearance). Lactose and sucrose were the primary sugars being catabolized by the bacteria during the fermentations of ice creams. Regardless of the starter culture used, the change rate of stachyose and sucrose increased with higher soybean extract amount in ice creams by fermentation ($p>0.05$). Bb-12 was found to disappear stachyose content more than La-05 can ($p>0.05$; Table 4.5). The change rate of lactose by both probiotics in composite milk ice creams containing cow milk was higher with increasing cow milk amount ($p<0.05$; Table 4.5). Fermentation increased glucose, galactose and fructose in ice creams fermented by both probiotics ($p>0.05$) (Table 4.5). The change rate content of monosaccharides increased with higher soybean extract proportion in composite milk ice creams as a result of fermentation ($p>0.05$). The change rate amount of total sugar in these ice creams due to fermentation by both probiotics increased in ice creams containing coconut milk with higher soybean extract contents ($p<0.05$). However, for ice creams containing cow milk, it

increased with increasing cow milk content when fermented by La-05 but not when fermented by Bb-12 ($p<0.05$).

Table 4.3 Sugar contents (mg/mL) in non fermented ice creams.

Samples	Sugars							
	Raffinose (mg/mL)	**Stachyose (mg/mL)**	**Sucrose (mg/mL)**	**Lactose (mg/mL)**	**Galactose (mg/mL)**	**Glucose (mg/mL)**	**Fructose (mg/mL)**	**Total (mg/mL)**
S	<LoD	0.192±0.04[a]	5.70±0.07[a]	2.37±0.08[c]	0.026±0.01[a]	0.018±0.01[a]	0.034±0.02[a]	8.34±0.05[a]
C	<LoD	<LoD	2.80±0.03[e]	2.38±0.06[c]	<LoD	0.011±0.01[a]	<LoD	5.19±0.03[d]
W	<LoD	<LoD	2.53±0.04[f]	4.80±0.07[a]	0.020±0.01[a]	0.016±0.01[a]	0.017±0.01[a]	7.38±0.08[b]
SC1	<LoD	0.077±0.04[b]	4.91±0.08[b]	2.42±0.17[c]	0.013±0.01[a]	0.021±0.02[a]	0.022±0.01[a]	7.46±0.07[b]
SC2	<LoD	0.045±0.03[d]	2.95±0.04[d]	2.10±0.07[c]	0.018±0.01[a]	0.020±0.03[a]	0.024±0.01[a]	5.16±0.04[d]
SC3	<LoD	0.041±0.02[d]	2.42±0.07[f]	1.94±0.12[c]	<LoD	0.001±0.01[a]	<LoD	4.40±0.05[e]
SW1	<LoD	0.058±0.04[d]	3.28±0.04[c]	1.99±0.07[c]	0.015±0.01[a]	0.017±0.01[a]	0.027±0.01[a]	5.39±0.06[c]
SW2	<LoD	0.020±0.04[d]	2.90±0.05[d]	2.18±0.02[c]	<LoD	0.022±0.01[a]	0.027±0.01[a]	5.15±0.03[d]
SW3	<LoD	<LoD	1.71±0.02[g]	3.47±0.07[b]	<LoD	0.003±0.01[a]	<LoD	5.18±0.06[d]

[a-g] Means in the same column followed by different letters were significantly different($p< 0.05$).

Table 4.4 Sugar contents (mg/mL) in fermented ice creams.

Samples	Sugars [A]							Total (mg/mL)
	Raffinose (mg/mL)	Stachyose (mg/mL)	Sucrose (mg/mL)	Lactose (mg/mL)	Galactose (mg/mL)	Glucose (mg/mL)	Fructose (mg/mL)	
SB	<LoD	0.122±0.06^{a}	4.88±0.08^{a}	1.22±0.11^{h}	0.159±0.07^{a}	0.174±0.08^{a}	0.171±0.08^{a}	6.73±0.07^{a}
CB	<LoD	<LoD	2.29±0.07^{f}	2.00±0.07^{b}	0.074±0.04^{a}	0.073±0.04^{a}	0.090±0.06^{a}	4.53±0.08^{c}
WB	<LoD	<LoD	2.05±0.04^{g}	2.42±0.09^{a}	0.078±0.03^{a}	0.089±0.06^{a}	0.111±0.07^{a}	4.75±0.07^{c}
SC1B	<LoD	0.027±0.01^{c}	3.11±0.09^{c}	1.19±0.08^{h}	0.023±0.02^{b}	0.031±0.02^{a}	0.034±0.03^{a}	4.41±0.09^{c}
SC2B	<LoD	0.015±0.01^{c}	1.17±0.17^{k}	1.70±0.06^{d}	0.030±0.01^{a}	0.036±0.02^{a}	0.034±0.0 ^{2a}	2.99±0.06^{e}
SC3B	<LoD	0.021±0.02^{c}	1.92±0.11^{h}	1.62±0.09^{d}	0.060±0.02^{a}	0.080±0.04^{a}	0.083±0.06^{a}	2.78±0.05^{e}
SW1B	<LoD	0.018±0.01^{c}	2.03±0.04^{g}	1.59±0.07^{e}	0.021±0.01^{b}	0.030±0.01^{a}	0.032±0.02^{a}	3.72±0.08^{d}
SW2B	<LoD	0.004±0.00^{d}	1.38±0.06^{j}	1.83±0.07^{c}	0.023±0.01^{b}	0.026±0.01^{b}	0.032±0.01^{a}	3.29±0.09^{d}
SW3B	<LoD	<LoD	0.88±0.08^{k}	2.42±0.08^{a}	0.029±0.01^{b}	0.038±0.02^{a}	0.045±0.03^{a}	3.41±0.08^{d}
SL	<LoD	0.172±0.07^{a}	3.71±0.07^{b}	1.46±0.17^{f}	0.079±0.03^{a}	0.081±0.04^{a}	0.087±0.04^{a}	5.59±0.07^{b}
CL	<LoD	<LoD	2.64±0.08^{d}	1.57±0.07^{e}	0.015±0.01^{a}	0.024±0.01^{b}	0.031±0.02^{a}	4.28±0.06^{c}
WL	<LoD	<LoD	2.46±0.07^{e}	2.02±0.07^{b}	0.025±0.01^{a}	0.036±0.01^{a}	0.038±0.03^{a}	4.58±0.07^{c}
SC1L	<LoD	0.047±0.02^{b}	2.17±0.06^{f}	1.34±0.07^{g}	0.093±0.06^{a}	0.104±0.06^{a}	0.106±0.08^{a}	3.86±0.07^{d}
SC2L	<LoD	0.015±0.01^{c}	1.90±0.05^{h}	1.10±0.07^{i}	0.085±0.05^{a}	0.101±0.06^{a}	0.111±0.07^{a}	3.31±0.06^{d}
SC3L	<LoD	0.038±0.01^{b}	1.70±0.07^{i}	1.87±0.07^{c}	0.069±0.04^{a}	0.082±0.05^{a}	0.091±0.06^{a}	3.85±0.07^{d}
SW1L	<LoD	0.038±0.01^{b}	2.66±0.08^{d}	1.85±0.07^{c}	0.143±0.07^{a}	0.093±0.04^{a}	0.163±0.07^{a}	4.95±0.09^{c}
SW2L	<LoD	0.016±0.01^{c}	2.87±0.09^{c}	1.53±0.04^{e}	0.082±0.05^{a}	0.148±0.07^{a}	0.097±0.06^{a}	4.74±0.08^{c}
SW3L	<LoD	<LoD	1.70±0.06^{i}	2.37±0.07^{a}	0.010±0.01^{b}	0.017±0.07^{b}	0.018±0.01^{a}	4.11±0.10^{c}

[a-j] Means in the same column followed by different letters were significantly different($P< 0.05$).
LoD= limit of detection.

Table 4.5 Change rates in sugar contents (mg.mL^{-1}/h) resulting from fermentations of ice creams until pH 5.50 by La-05 and Bb-12.

Samples	Sugars							Total (mg.mL^{-1}/h) [A]
	Raffinose (mg.mL^{-1}/h) [A]	Stachyose (mg.mL^{-1}/h) [A]	Sucrose (mg.mL^{-1}/h) [A]	Lactose (mg.mL^{-1}/h) [A]	Galactose (mg.mL^{-1}/h) [A]	Glucose (mg.mL^{-1}/h) [A]	Fructose (mg.mL^{-1}/h) [A]	
SB	Na	-0.008^{e}	-0.097^{h}	-0.136^{h}	0.0157^{a}	0.0180^{a}	0.0160^{a}	-0.191^{d}
CB	Na	Na	-0.050^{e}	-0.037^{c}	Na	0.0061^{a}	Na	-0.081^{b}
WB	Na	Na	-0.077^{e}	-0.384^{l}	0.009^{b}	0.0118^{a}	0.0152^{a}	-0.425^{g}
SC1B	Na	-0.006^{d}	-0.219^{m}	-0.150^{j}	0.001^{b}	0.0012^{a}	0.0015^{a}	-0.371^{f}
SC2B	Na	-0.004^{b}	-0.240^{m}	-0.054^{e}	0.002^{b}	0.0022^{a}	0.0013^{a}	-0.292^{e}
SC3B	Na	-0.003^{a}	-0.081^{g}	-0.052^{e}	Na	0.0127^{a}	na	-0.123^{c}
SW1B	Na	-0.005^{c}	-0.147^{j}	-0.047^{d}	0.0007^{b}	0.0015^{a}	0.0006^{a}	-0.196^{d}
SW2B	Na	-0.002^{a}	-0.164^{k}	-0.038^{c}	Na	0.0004^{a}	0.0005^{a}	-0.203^{d}
SW3B	Na	Na	-0.090^{h}	-0.113^{i}	Na	0.0038^{a}	na	-0.199^{d}
SL	Na	-0.002^{a}	-0.179^{l}	-0.082^{g}	0.005^{b}	0.0057^{a}	0.0048^{a}	-0.247^{e}
CL	Na	Na	-0.009^{d}	-0.045^{d}	Na	0.0007^{a}	na	-0.050^{b}
WL	Na	Na	-0.007^{c}	-0.265^{k}	0.0005^{b}	0.0019^{a}	0.0020^{a}	-0.267^{e}
SC1L	Na	-0.003^{a}	-0.261^{m}	-0.103^{i}	0.0076^{b}	0.0079^{a}	0.0080^{a}	-0.343^{f}
SC2L	Na	-0.003^{a}	-0.100^{i}	-0.095^{h}	0.0064^{b}	0.0077^{a}	0.0083^{a}	-0.176^{c}
SC3L	Na	-0.003^{a}	-0.068^{f}	-0.007^{a}	Na	0.0077^{a}	na	-0.070^{b}
SW1L	Na	-0.002^{a}	-0.055^{e}	-0.012^{b}	0.0113^{a}	0.0067^{a}	0.0120^{a}	-0.039^{a}
SW2L	Na	-0.003^{a}	-0.002^{b}	-0.048^{d}	Na	0.0093^{a}	0.0052^{a}	-0.038^{a}
SW3L	Na	Na	-0.0006^{a}	-0.068^{f}	Na	0.0009^{a}	na	-0.068^{b}

[A] Change rates (mg.mL^{-1}/h) = the differences between the initial (Table 4.3) and final (Table 4.4) concentration of sugars in ice creams (changes in sugar amount = sugar contents (mg/mL) in non fermented ice creams (Table 4.3) - sugar contents (mg/mL) in fermented ice creams (Table 4.4)) divided by the time required for pH reduction to 5.50 (Table 4.21).

[a-i] Means in the same column followed by different letters were significantly different($p < 0.05$).

na = not applicable.

4.1.3 Free amino acid amounts in ice creams

Table 4.6 presents free amino acids contents (mg/mL) in non fermented ice creams. All types of amino acids were higher in ice creams containing vegetable extracts than ice cream containing 100% cow milk (control). Coconut milk (100%) ice cream (C) showed higher amino acid concentration for glutamic acid, aspartic acid, alanine, serine, proline, isoleucine, leucine, valine, lysine and methionine than S and W ice creams (Table 4.6). Hence, the amounts for these amino acids increased with increasing coconut milk content in ice creams containing coconut milk. For other amino acids, ice cream containing 100 % soybean extract (S) showed higher amino acid content for arginine, histidine, threonine, tyrosine and phenylalanine than C and W ice cream. Hence, the amounts for these amino acids increased with increasing soybean extract content in ice creams ($p<0.05$; Table 4.6). The highest total free amino acid (TFAA) was in SC1 ice cream (50.45±0.24 mg/mL), whereas 100% cow milk ice cream contains the lowest TFAA (14.71±0.19 mg/mL) ($p<0.05$). In composite milk ice creams, ice creams containing coconut milk showed higher total amino acid (40.99-50.45 mg/mL) than ice creams containing cow milk (24.15-39.65 mg/mL) ($p<0.05$). The TFAA increased with increasing soybean extract content in ice creams ($p<0.05$).

Tables 4.7 and 4.8 present the concentration of free amino acids contents (mg/mL) in fermented ice creams with La-05 and Bb-12, respectively. Regardless of the probiotic used, all types of amino acids were higher in ice creams containing vegetable extracts than ice cream containing 100% cow milk (control). In fermented ice creams inoculated by La-05, ice cream with 100% coconut milk showed higher amino acid concentration for glutamic acid, alanine, proline, isoleucine, leucine and valine than S and W ice creams

(Table 4.7). Hence, the amounts for these amino acids increased with increasing coconut milk content in ice creams containing coconut milk. For other amino acids, ice cream containing 100 % soybean extract (S) showed higher amino acid content for arginine, histidine, threonine, tyrosine, methionine, lysine and phenylalanine than C and W ice cream. Hence, the amounts for these amino acids increased with increasing soybean extract content in ice creams ($p<0.05$; Table 4.7). In fermented ice creams inoculated by Bb-12, coconut milk (100%) ice cream (C) showed higher amino acid concentration for alanine, proline, isoleucine, and lysine than S and W ice creams (Table 4.8). Hence, the amounts for these amino acids increased with increasing coconut milk content in ice creams containing coconut milk. For other amino acids, ice cream containing 100 % soybean extract (S) showed higher amino acid content for arginine, threonine, tyrosine, valine and phenylalanine than C and W ice cream. Hence, the amounts for these amino acids increased with increasing soybean extract content in ice creams ($p<0.05$; Table 4.8).

The free amino acid content in fermented ice cream reflects the balance between proteolysis and assimilation by probiotics (Tables 4.9 and 4.10; Donkor *et al*., 2007). Tables 4.9 and 4.10 show the change rate of free amino acids due to fermentation until pH = 5.50 (positive amount (+) = appearance; negative amount (-) = disappearance). The change rate of amino acids during fermentation by both probiotics was higher in ice creams containing vegetable extracts than in those containing cows' milk ($p<0.05$; Tables 4.9 and 4.10). In fermented ice creams with La-05, the amounts of alanine, arginine, leucine, isoleucine, proline and lysine increased after fermentation due to proteolysis activity of La-05. Alanine, proline, lysine and arginine amounts increased in fermented ice creams with Bb-12. Threonine, tyrosine, valine and phenylalanine disappearance more than other amino acids in all ice creams by both probiotics ($p<0.05$; Tables 4.9 and 4.10). TFAA content

decreased after fermentation in all ice creams except ice creams made using 100% coconut and La-05 (CL) ($p<0.05$; Tables 4.9 and 4.10). The change rate of TFAA was increased in ice creams with increasing soybean extract content and higher TFAA change rate was also recorded in ice creams containing coconut milk than in ice creams containing cow milk ($p<0.05$; Tables 4.9 and 4.10).

Table 4.6 The free amino acid concentration (mg/mL) in non fermented ice creams.

Amino acids (mg/mL)	Samples								
	S	**C**	**W**	**SC1**	**SC2**	**SC3**	**SW1**	**SW2**	**SW3**
Alanine	1.16 ±0.08^{c}	1.53±0.11^{a}	1.32±0.06^{b}	1.29±0.08^{b}	1.06±0.05^{d}	1.03±0.09^{d}	1.30±0.09^{b}	1.18±0.04^{c}	1.04±0.06^{d}
Arginine	4.76±0.21^{a}	1.32±0.31^{c}	<LoD	2.69±0.29^{b}	2.08±0.09^{c}	1.48±0.09^{c}	2.43±0.35^{b}	2.44±0.31^{b}	1.67±0.09^{c}
Aspartic acid	<LoD	0.32±0.09^{a}	<LoD	<LoD	<LoD	0.18±0.08^{a}	<LoD	<LoD	<LoD
Cysteine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Glutamic acid	<LoD	1.2±0.08^{a}	0.08±0.03^{c}	<LoD	0.29±0.09^{b}	0.91±0.09^{a}	<LoD	<LoD	0.06±0.04^{c}
Histidine	0.06±0.03^{a}	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Leucine	3.47±0.21^{c}	4.20±0.18^{b}	0.36±0.05^{d}	4.29±0.41^{b}	4.37±0.24^{b}	5.15±0.09^{a}	4.64±0.34^{b}	4.35±0.09^{b}	3.42±0.18^{c}
Isoleucine	2.99±0.51^{b}	4.05±0.09^{a}	<LoD	2.64±0.49^{b}	2.25±0.63^{b}	2.98±0.71^{b}	2.47±0.56^{b}	2.30±0.49^{b}	1.67±0.08^{c}
Lysine	0.56±0.10^{b}	0.87±0.09^{a}	0.61±0.11^{b}	0.59±0.10^{b}	0.59±0.08^{b}	0.62±0.07^{b}	0.54±0.11^{b}	0.39±0.05^{c}	0.32±0.06^{c}
Methionine	1.91±0.09^{d}	2.66±0.41^{c}	<LoD	4.25±0.20^{a}	3.05±0.09^{b}	3.44±0.50^{b}	2.34±0.34^{c}	2.36±0.35^{c}	1.06±0.09^{d}
Proline	0.28±0.19^{b}	0.83±0.09^{a}	<LoD	0.45±0.39^{b}	0.26±0.15^{b}	1.11±0.09^{a}	0.04±0.02^{c}	0.09±0.05^{c}	<LoD
Serine	<LoD	0.21±0.09^{a}	<LoD	<LoD	<LoD	0.03±0.01^{b}	<LoD	<LoD	<LoD
Threonine	9.00±0.76^{a}	5.00±0.65^{c}	4.00±0.09^{d}	10.2±0.89^{a}	9.55±0.72^{a}	5.75±0.80^{c}	7.60±0.78^{b}	7.60±0.63^{b}	4.05±0.08^{d}
Tyrosine	8.20±0.66^{a}	5.00±0.09^{d}	5.00±0.07^{d}	8.70±0.51^{a}	7.40±0.32^{b}	5.90±0.09^{c}	7.25±0.41^{b}	7.20±0.29^{b}	4.65±0.18^{e}
Valine	5.45±0.42^{b}	6.25±0.29^{a}	0.83±0.08^{e}	5.70±0.53^{b}	6.45±0.32^{a}	6.60±0.41^{a}	4.08±0.53^{c}	3.85±0.48^{c}	2.57±0.46^{d}
Phenylalanine	10.00±0.89^{a}	3.80±0.31^{f}	2.50±0.11^{g}	9.65±0.92^{a}	7.95±0.72^{c}	5.80±0.09^{e}	6.95±0.47^{d}	8.15±0.08^{b}	3.63±0.22^{f}
Total	47.85±0.44^{b}	37.25±0.28^{e}	14.71±0.19^{g}	50.45±0.24^{a}	45.31±0.63^{c}	40.99±0.96^{d}	39.65±0.86^{d}	39.92±0.76^{d}	24.15±0.09^{f}

$^{a-g}$ Values with different letters in the same row are significantly different (P<0.05) (Tukey test).

LoD= limit of detection.

Table 4.7 The free amino acid concentration (mg/mL) in ice creams fermented by La-05.

Amino acids (mg/mL)	Samples								
	SL	**CL**	**WL**	**SC1L**	**SC2L**	**SC3L**	**SW1L**	**SW2L**	**SW3L**
Alanine	2.25±0.19^{b}	3.46±0.11^{a}	1.58±0.21^{c}	1.61±0.08^{c}	1.89±0.11^{c}	2.22±0.09^{b}	1.95±0.09^{c}	1.66±0.32^{c}	1.82±0.42^{c}
Arginine	7.30±0.51^{a}	1.31±0.41^{e}	<LoD	4.23±0.23^{b}	3.13±0.11^{c}	1.8±0.76^{e}	3.38±0.53^{c}	2.31±0.10^{d}	1.18±0.11^{e}
Aspartic acid	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Cysteine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Glutamic acid	<LoD	1.02±0.20^{a}	<LoD	<LoD	0.38±0.09^{b}	1.21±0.11^{a}	<LoD	<LoD	<LoD
Histidine	0.04±0.02^{a}	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Leucine	8.60±0.39^{b}	11.20±0.72^{a}	7.75±0.63^{c}	6.25±0.42^{d}	6.19±0.33^{d}	6.70±0.56^{d}	2.38±0.11^{g}	3.41±0.32^{f}	5.20±0.23^{e}
Isoleucine	8.55±0.09^{b}	11.45±0.13^{a}	<LoD	2.66±0.09^{e}	2.81±0.09^{e}	5.40±0.09^{c}	4.96±0.09^{d}	2.70±0.09^{e}	1.05±0.09^{f}
Lysine	1.24±0.11^{a}	1.05±0.09^{a}	1.03±0.04^{a}	1.14±0.11^{a}	0.75±0.20^{b}	0.5±0.14^{c}	0.55±0.16^{c}	0.58±0.11^{c}	0.80±0.09^{b}
Methionine	2.15±0.054^{a}	0.94±0.31^{b}	<LoD	<LoD	<LoD	1.00±0.24^{b}	0.13±0.35^{c}	0.12±0.29^{c}	<LoD
Proline	1.31±0.76^{b}	2.58±0.47^{a}	<LoD	0.88±0.44^{c}	0.92±0.84^{c}	2.24±0.53^{a}	0.80±0.64^{c}	0.73±0.36^{c}	<LoD
Serine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Threonine	3.11±0.65^{a}	2.71±0.49^{a}	0.96±0.09^{b}	2.70±0.72^{a}	2.64±0.48^{a}	1.11±0.55^{b}	0.13±0.22^{d}	0.17±0.07^{d}	0.69±0.09^{c}
Tyrosine	2.28±0.11^{a}	0.47±0.09^{c}	1.06±0.23^{b}	1.12±0.31^{b}	1.00±0.46^{b}	<LoD	<LoD	1.09±0.26^{b}	<LoD
Valine	1.40±0.22^{b}	2.20±0.11^{a}	0.71±0.08^{c}	0.89±0.07^{c}	2.25±0.65^{a}	2.69±0.42^{a}	1.09±0.19^{b}	1.50±0.64^{b}	1.62±0.25^{b}
Phenylalanine	3.00±0.09^{a}	2.34±0.23^{b}	0.32±0.66^{d}	0.69±0.72^{d}	0.48±0.84^{d}	0.71±0.23^{c}	1.89±0.10^{c}	3.29±0.44^{a}	0.53±0.10^{d}
Total	41.235±0.89^{a}	40.74±0.79^{a}	13.41±0.09^{d}	22.175±0.96^{b}	22.438±0.68^{b}	23.36±0.81^{b}	17.26±0.32^{c}	17.56±0.46^{c}	12.89±0.21^{d}

$^{a-e}$ Values with different letters in the same row are significantly different ($p<0.05$) (Tukey test).

LoD = limit of detection.

Table 4.8 The free amino acids concentration (mg/mL) in ice creams fermented by Bb-12.

Amino acids (mg/mL)	Samples								
	SB	**CB**	**WB**	**SC1B**	**SC2B**	**SC3B**	**SW1B**	**SW2B**	**SW3B**
Alanine	1.42±0.22^{d}	2.00±0.20^{a}	1.62±0.18^{d}	1.42±0.26^{b}	1.70±0.32^{c}	1.80±0.35^{b}	1.41±0.42^{d}	1.29±0.34^{d}	1.40±0.21^{d}
Arginine	5.15±0.23^{a}	1.41±0.65^{d}	<LoD	3.39±0.32^{b}	2.35±0.11^{c}	2.51±0.09^{c}	3.56±0.29^{b}	1.52±0.46^{d}	0.78±0.27^{e}
Aspartic acid	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Cysteine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Glutamic acid	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	0.12±0.04^{a}	<LoD
Histidine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Leucine	3.30±0.24^{a}	3.10±0.08^{a}	0.31±0.33^{d}	1.42±0.08^{c}	2.95±0.09^{b}	2.13±0.46^{b}	1.84±0.22^{c}	1.72±0.41^{c}	0.58±0.10^{d}
Isoleucine	0.62±0.09^{d}	2.44±0.09^{a}	<LoD	1.50±0.20^{b}	1.30±0.32^{b}	1.83±0.19^{b}	1.17±0.09^{c}	1.07±0.09^{c}	0.80±0.09^{d}
Lysine	0.65±0.20^{c}	1.02±0.11^{a}	1.00±0.09^{a}	0.80±0.21^{b}	0.49±0.31^{c}	0.92±0.39^{b}	0.58±0.21^{c}	0.52±0.27^{c}	0.31±0.10^{c}
Methionine	<LoD	<LoD	0.004±0.00^{a}	<LoD	<LoD	<LoD	0.02±0.01^{a}	0.06±0.02^{a}	<LoD
Proline	0.54±0.32^{b}	0.93±0.31^{a}	0.11±0.23^{d}	0.53±0.19^{b}	0.61±0.18^{b}	0.99±0.21^{a}	0.49±0.18^{b}	0.36±0.17^{c}	0.27±0.17^{c}
Serine	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD	<LoD
Threonine	5.30±0.52^{a}	1.12±0.11^{c}	0.82±0.31^{d}	1.18±0.17^{b}	1.31±0.32^{b}	1.14±0.21^{b}	1.70±0.41^{b}	1.70±0.10^{b}	0.97±0.09^{d}
Tyrosine	6.15±0.67^{a}	0.71±0.09^{d}	0.00±0.00^{e}	1.41±0.10^{b}	1.31±0.13^{b}	1.03±0.08^{c}	1.60±0.07^{b}	1.35±0.34^{b}	0.95±0.21^{d}
Valine	1.28±0.26^{a}	1.08±0.04^{b}	0.19±0.10^{c}	0.42±0.02^{c}	0.49±0.21^{c}	0.27±0.11^{c}	1.25±0.10^{a}	1.05±0.42^{b}	0.23±0.14^{c}
Phenylalanine	5.25±0.57^{a}	1.05±0.23^{d}	0.29±0.11^{e}	1.48±0.24^{d}	1.32±0.26^{d}	1.15±0.31^{d}	2.36±0.25^{c}	4.89±0.32^{b}	0.82±0.14^{e}
Total	29.66±0.22^{a}	14.86±0.34^{c}	4.34±0.21^{f}	16.33±0.39^{b}	13.83±0.32^{d}	13.77±0.41^{d}	15.98±0.45^{b}	15.53±0.52^{b}	7.11±0.61^{e}

$^{a-e}$ Values with different letters in the same row are significantly different ($p<0.05$) (Tukey test).

LoD = limit of detection.

Table 4.9 Change rates in free amino acids concentration (mg.mL^{-1}/h) resulting from fermentations of ice creams until pH 5.50 by La-05.

Amino acids (mg.mL^{-1}/h) [A]	**Samples**								
	SL	CL	WL	SC1L	SC2L	SC3L	SW1L	SW2L	SW3L
Alanine	0.098 [c]	0.107 [b]	0.025 [h]	0.031 [g]	0.079 [d]	0.113 [a]	0.058 [e]	0.035 [g]	0.048 [f]
Arginine	0.228 [a]	-0.00 [g]	Na	0.147 [b]	0.100 [c]	0.030 [e]	0.084 [d]	-0.010 [f]	-0.030 [f]
Aspartic acid	na	na	Na	na	na	na	na	na	na
Cysteine	na	na	Na	na	na	na	na	na	na
Glutamic acid	na	-0.010 [c]	Na	na	0.009 [b]	0.029 [a]	na	na	na
Histidine	-0.002 [a]	na	Na	na	na	na	na	na	na
Leucine	0.461 [b]	0.387 [c]	0.704 [a]	0.187 [d]	0.173 [d]	0.148 [d]	-0.200 [e]	-0.069 [e]	0.110 [d]
Isoleucine	0.499 [a]	0.409 [a]	Na	0.002 [d]	0.053 [c]	0.230 [b]	0.220 [b]	0.029 [c]	-0.038 [e]
Lysine	0.061 [a]	0.010 [e]	0.040 [c]	0.052 [b]	0.015 [e]	-0.011 [g]	0.001 [f]	0.014 [e]	0.030 [d]
Methionine	0.022 [a]	-0.095 [b]	Na	na	na	-0.232 [d]	-0.196 [c]	-0.165 [c]	na
Proline	0.092 [b]	0.097 [b]	Na	0.041 [d]	0.063 [c]	0.108 [a]	0.067 [c]	0.047 [d]	na
Serine	na	na	Na	na	na	na	na	na	na
Threonine	-0.529 [d]	-0.126 [a]	-0.289 [b]	-0.714 [f]	-0.658 [e]	-0.442 [c]	-0.661 [e]	-0.548 [d]	-0.207 [b]
Tyrosine	-0.532 [d]	-0.250 [a]	-0.375 [b]	-0.722 [f]	-0.609 [e]	na	na	-0.451 [c]	na
Valine	-0.364 [d]	-0.224 [c]	-0.011 [a]	-0.458 [e]	-0.400 [e]	-0.372 [d]	-0.265 [c]	-0.173 [b]	-0.059 [b]
Phenylalanine	-0.629 [f]	-0.081 [a]	-0.208 [c]	-0.853 [h]	-0.711 [g]	-0.485 [e]	-0.448 [e]	-0.358 [d]	-0.191 [b]
Total	-0.594 [c]	0.193 [a]	-0.124 [b]	-2.693 [h]	-2.178 [g]	-1.679 [e]	-1.981 [f]	-1.649 [e]	-0.695 [d]

[A] Change rates (mg.mL^{-1}/h) = the differences between the initial and final concentration of amino acids in ice creams (changes in amino acid amount = amino acids contents (mg/mL) in non fermented ice creams (Table 4.6) − amino acids contents (mg/mL) in fermented ice creams (Table 4.7)) divided by the time required for pH reduction to 5.50 (Table 4.21).

[a-i] Means in the same row followed by different letters were significantly different($p < 0.05$).

na = not applicable.

Table 4.10 Changes in free amino acids concentration (mg.mL^{-1}/h) resulting from fermentations of ice creams until pH 5.50 by Bb-12.

Amino acids (mg.mL^{-1}/h) [A]	Samples								
	SB	CB	W	SC1B	SC2B	SC3B	SW1B	SW2B	SW3B
Alanine	0.023 [c]	0.026 [c]	0.029 [c]	0.012 [d]	0.061 [b]	0.073 [a]	0.010 [d]	0.008 [e]	0.022 [c]
Arginine	0.035 [c]	0.005 [e]	na	0.067 [b]	0.026 [d]	0.098 [a]	0.100 [a]	-0.068 [f]	-0.055 [f]
Aspartic acid	na	Na	na	na	na	na	Na	na	na
Cysteine	na	Na	na	na	na	na	Na	na	na
Glutamic acid	na	Na	na	na	na	na	Na	na	na
Histidine	na	Na	na	na	na	na	Na	na	na
Leucine	-0.015 [b]	-0.061 [c]	-0.005 [a]	-0.009 [a]	-0.135 [d]	-0.288 [e]	-0.248 [e]	-0.194 [d]	-0.175 [b]
Isoleucine	-0.213 [e]	-0.089 [b]	0	-0.109 [c]	-0.090 [b]	-0.109 [c]	-0.115 [d]	-0.090 [b]	-0.054 [a]
Lysine	0.008 [c]	0.008 [b]	0.0371 [b]	0.020 [b]	-0.009 [b]	0.028 [b]	0.003 [b]	0.009 [b]	-0.001 [b]
Methionine	na	Na	na	na	na	na	-0.205 [b]	-0.170 [a]	na
Proline	0.023 [b]	0.005 [c]	na	0.008 [c]	0.0333 [a]	-0.011 [d]	0.0398 [a]	0.020 [b]	na
Serine	na	Na	na	na	na	na	Na	na	na
Threonine	-0.332 [c]	-0.214 [b]	-0.303 [c]	-0.859 [g]	-0.785 [f]	-0.439 [d]	-0.522 [e]	-0.435 [d]	-0.190 [a]
Tyrosine	-0.184 [a]	-0.237 [b]	-0.476 [c]	-0.694 [f]	-0.580 [e]	-0.464 [c]	-0.500 [d]	-0.431 [b]	-0.228 [b]
Valine	-0.375 [d]	-0.286 [c]	-0.061 [a]	-0.503 [e]	-0.568 [e]	-0.603 [f]	-0.250 [c]	-0.206 [c]	-0.144 [b]
Phenylalanine	-0.427 [c]	-0.152 [a]	-0.210 [b]	-0.778 [f]	-0.631 [e]	-0.443 [c]	-0.406 [c]	-0.240 [b]	-0.173 [a]
Total	-1.634 [d]	-1.237 [c]	-0.987 [a]	-3.249 [i]	-2.998 [h]	-2.592 [g]	-2.094 [f]	-1.799 [e]	-1.052 [b]

[A] Change rates (mg.mL^{-1}/h) = The differences between the initial and final concentration of amino acids in ice creams (changes in amino acid amount = amino acids contents (mg/mL) in non fermented ice creams (Table 4.6) - amino acids contents (mg/mL) in fermented ice creams (Table 4.8)) divided by the time required for pH reduction to 5.50 (Table 4.21).

[a-i] Means in the same row followed by different letters were significantly different($p < 0.05$).

na = not applicable.

4.2 Physical properties of non fermented and fermented probiotic ice creams

4.2.1 Melting rate of ice creams

In non fermented ice cream, the melting rate of ice creams containing cow milk (SW1 = 22.25±5.50; SW2 = 30.20±6.70; SW3 = 33.36±11.10% w/w) were higher than ice creams containing coconut milk (SC1 = 18.11±8.90; SC2 = 23.50±7.50; SC3 = 26.50±10.10% w/w) (Table 4.11). The melting rate of W (35.88±10.16%) was higher than S (16.27±7.00%) and C (27.00±4.16% w/w) ice creams ($p<0.05$).

In both type of fermented ice creams (both of La-05 and Bb-12), the melting rate of ice creams containing cow milk were higher than ice creams containing coconut milk (Table 4.11). The melting rate of fermented ice creams containing 100% cow milk (30.51±0.01 and 35.51±0.04% w/w in inoculated with La-05 and Bb-12, respectively) was higher than fermented ice creams containing 100% soybean extract and 100% coconut milk (0.00±0.02 and 0±0.04 in inoculated with La-05 and Bb-12, 27.82±0.02 and 29.82±0.02% w/w in inoculated with La-05 and Bb-12, respectively). No significant effects ($p>0.05$) were observed between samples with respect to the kind of probiotic (La-05 and Bb-12).

Table 4.11 Melting rate of non fermented and fermented probiotic ice creams.

Samples [A]	Melting rate (% w/w melted after 20 min) [B]		
	Non fermented ice cream	Fermented ice cream	
		Fermented ice cream by La-05	Fermented ice cream by Bb-12
W	35.88±10.16^{a}	30.51±0.01^{b}	35.51±0.04^{a}
C	27.00±4.16^{bc}	27.82±0.02^{d}	29.82±0.02^{bc}
S	16.27±7.00^{f}	0.00±0.02^{j}	0.00±0.04^{j}
SW1	22.25±5.50^{d}	0.23±0.03^{j}	0.53±0.02^{j}
SW2	30.20±6.70^{b}	18.32±0.02^{f}	21.32±0.04^{e}
SW3	33.36±11.10^{ab}	28.78±0.02^{dc}	30.78±0.03^{b}
SC1	18.11±8.90^{e}	0.10±0.01^{j}	0.41±0.04^{j}
SC2	23.50±7.50^{cd}	9.14±0.01^{i}	10.14±0.02^{i}
SC3	26.50±10.10^{c}	12.99±0.02^{h}	16.20±0.03^{g}

[A]W: ice cream with 100% cow milk; C: ice cream with 100% coconut milk; S: ice cream with 100% soybean extract; SW1: ice cream with 75% soybean extract+25%cow milk; SW2: ice cream with 50% soybean extract+50% cow milk; SW3: ice cream with 25% soybean extract+75% cow milk; SC1: ice cream with 75% soybean extract+25% coconut milk; SC2: ice cream with 50% soybean extract+50% coconut milk; SC3: ice cream with 25% soybean extract+75% coconut milk.
[B] means values±standard deviation.
[a-f] Means in the same column followed by different letters were significantly different ($p<0.05$).

4.2.2 Rheological measurements

All non fermented and fermented ice creams demonstrated non-Newtonian behavior, i.e. their viscosity decreases with increasing shear rate (Figures 4.1, 4.2 and 4.3).

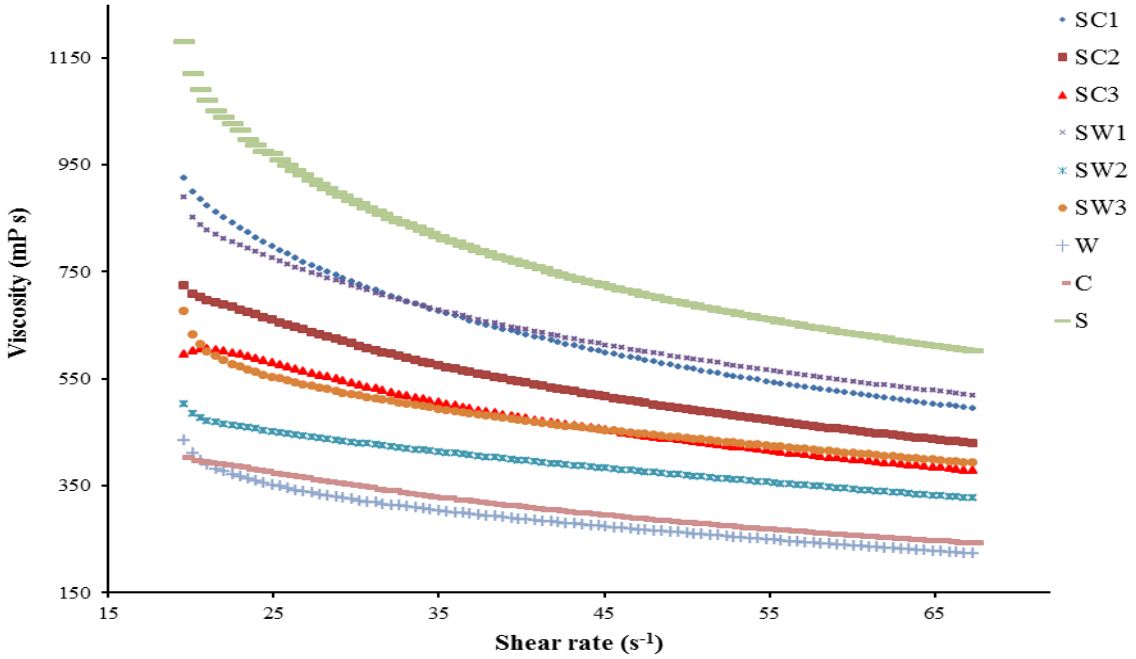


Figure 4.1 Effect of shear rate on the apparent viscosity of non fermented ice creams.

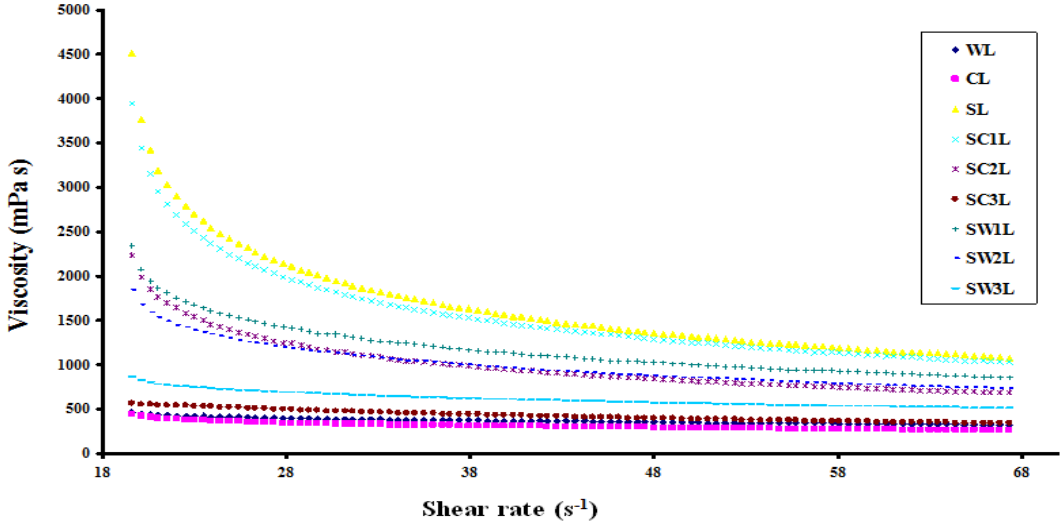


Figure 4.2 Effect of shear rate on the apparent viscosity of fermented ice cream inoculated with La-05.

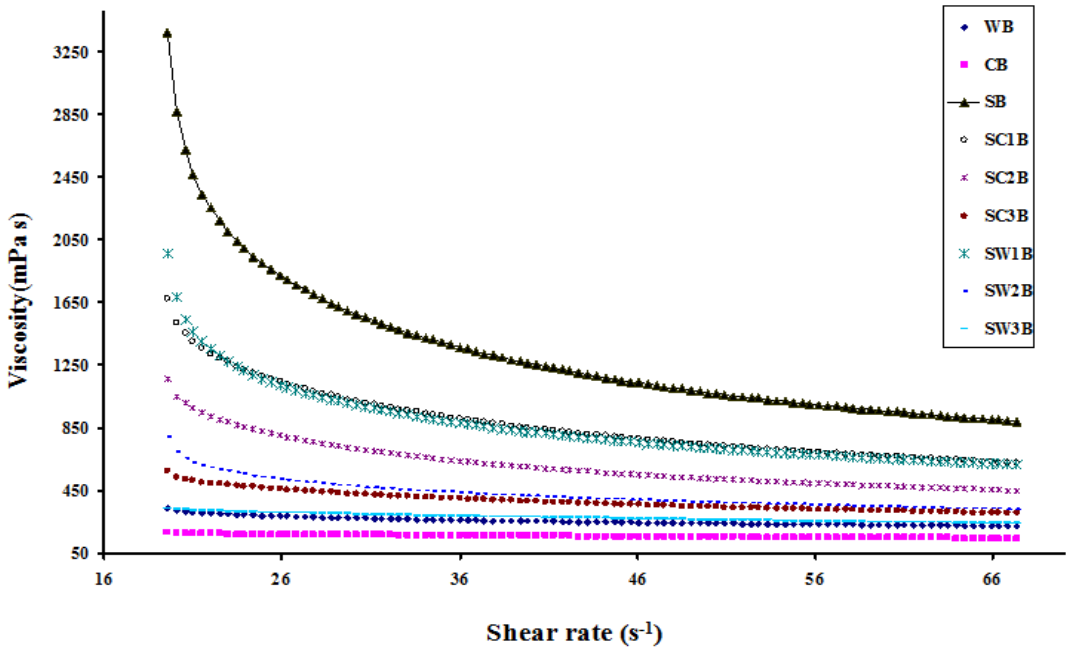


Figure 4.3 Effect of shear rate on the apparent viscosity of fermented ice cream inoculated with Bb-12.

In non fermented ice creams, the apparent viscosity value of C, W and S ice creams were 363±1.16, 289±0.80 and 1120±1.06 mPa s, respectively. The apparent viscosity value of ice creams containing coconut milk (SC1 = 982±1.30, SC2 = 739±0.91 and SC3 = 603±1.80 mPa s) were higher than ice creams containing cow milk (SW1 = 818±1.20, SW2 = 488±2.01 and SW3 = 398±1.01 mPa s) ($p<0.05$) in upward curves (Table 4.12). The apparent viscosity value of C, W and S were 294±1.16, 287±1.07 and 1012±0.91 mPa s, respectively. The apparent viscosity value of ice creams containing coconut milk (SC1 = 817±1.09, SC2 = 667±1.03 and SC3 = 577±2.06 mPa s) were higher than ice creams containing cow milk (SW1 = 784±1.11, SW2 = 536±0.87 and SW3 = 391±0.96 mPa s) ($p<0.05$) in downward curves (Table 4.12). The consistency index (K) of ice creams containing cow milk (SW1 = 3.10±0.01, SW2 = 1.30±0.03 and SW3 = 1.18±0.02 Pa s^n) were seen lower than ice creams containing coconut milk (SC1 = 4.81±0.01, SC2 = 2.89±0.03 and SC3 = 2.17±0.02 Pa s^n). The K values of C, W and S ice creams were 1.29±0.01, 0.87±0.01 and 4.67±0.01 Pa s^n in upward curves ($p<0.05$). In the downward

curves, the consistency index (*K*) of ice creams containing cow milk (SW1 = 2.66±0.01, SW2 = 1.83±0.03 and SW3 = 1.22±0.01 Pa s^n) were seen lower than ice creams with containing coconut milk (SC1 = 2.43±0.01, SC2 = 1.87±0.02 and SC3=1.62±0.01 Pa s^n). The *K* value of C, W and S were 0.76±0.01, 0.71±0.02 and 3.61±0.01 Pa s^n, respectively ($p<0.05$). No significant effects ($p>0.05$) were observed between all samples with respect to the flow behavior index (*n*) in upward and downward curves.

Table 4.12 Rheological parameters of the non fermented ice creams obtained using the Power Law model.

Samples[A]	Apparent viscosity (mPa s)[b]	*K* (Pa s^n)[b]	*n*[b]	R^{2c}
	upward curves			
W	289±0.80[h]	0.87±0.01[g]	0.65±0.01[a]	0.994
C	363±1.16 [g]	1.29±0.01[f]	0.56±0.01[a]	0.996
S	1120±1.06[a]	4.67±0.01[b]	0.51±0.01[a]	0.996
SW1	818±1.20[c]	3.10±0.01[c]	0.55±0.01[a]	0.999
SW2	488±2.01[f]	1.30±0.03[f]	0.68±0.01[a]	0.998
SW3	398±1.01[g]	1.18±0.02[f]	0.63±0.01[a]	0.997
SC1	982±1.30[b]	4.81±0.01[a]	0.47±0.01[a]	0.999
SC2	739±0.91[d]	2.89±0.03[d]	0.55±0.01 [a]	0.999
SC3	603±1.80[e]	2.17±0.02[e]	0.59±0.01 [a]	0.993
	Downward curves			
W	287±1.07[h]	0.71±0.02[f]	0.69±0.01 [a]	0.997
C	294±1.16[h]	0.76±0.01[f]	0.68±0.01 [a]	0.998
S	1012±0.91[a]	3.61±0.01[a]	0.57±0.01 [a]	0.997
SW1	784±1.11[c]	2.66±0.01[b]	0.58±0.01 [a]	0.996
SW2	536±0.87[f]	1.83±0.03[c]	0.58±0.01 [a]	0.997
SW3	391±0.96[g]	1.22±0.01[e]	0.62±0.01 [a]	0.998
SC1	817±1.09[b]	2.43±0.01[b]	0.63±0.01 [a]	0.995
SC2	667±1.03[d]	1.87±0.02[c]	0.65±0.01 [a]	0.996
SC3	577±2.06[e]	1.62±0.01[d]	0.647±0.01 [a]	0.996

[a] *K* = consistency index; *n* = flow behavior index.
[b] Mean values±standard deviation. Values with different letters in the same column are significantly different ($p<0.05$) (Tukey test).
[c] Coefficient of determination.

In fermented ice creams with La-05, the apparent viscosity value of WL, CL and SL were 450±2.01, 420±1.76 and 3770±0.89 mPa s, respectively. The apparent viscosity value of ice creams containing coconut milk (SC1L = 3440±1.1, SC2L = 1990±1.32 and SC3L =

556±1.03 mPa s, respectively) was higher than ice creams containing cow milk (SW1L = 2080±1.02, SW2L = 1680±1.66 and SW3L = 818±1.32 mPa s) ($p<0.05$) in upward curves (Table 4.13). The apparent viscosity value of WL, CL and SL were 437±1.52, 373±0.83 and 1720±1.07 mPa s, respectively. The apparent viscosity value of ice creams with coconut milk (SC1L = 1550± 2.01, SC2L = 990± 1.43 and SC3L = 500± 1.62 mPa s) was higher than ice creams with cow milk (SW1L = 1370±1.32, SW2L = 1120±0.94 and SW3L = 760±0.87 mPa s) ($p<0.05$) in downward curves (Table 4.13). The consistency index (*K*) of ice creams with cow milk (SW1L = 12.61±0.01, SW2L = 9.56±0.02 and SW3L = 2.32±0.02 Pa s^n) were seen lower than ice creams with coconut milk (SC1L = 36.41±0.01, SC2L = 17.25±0.01 and SC3L = 1.95±0.02 Pa s^n). The *K* value of WL, CL and SL were 0.90±0.01, 1.09±0.02 and 43.12±0.02 Pa s^n, respectively in upward curves. In the downward curves, the consistency index (*K*) of ice creams with cow milk (SW1L = 4.33±0.02, SW2L = 3.01±0.02 and SW3L = 2.07±0.02 Pa s^n) were seen lower than ice creams with coconut milk (SC1L = 4.16±0.02, SC2L = 2.43±0.02 and SC3L = 1.17±0.02 Pa s^n). The *K* value of WL, CL and SL were 0.93±0.02, 0.87±0.02 and 5.24±0.02 Pa s^n, respectively. The flow behavior index (*n*) of ice creams with cow milk (SW1L = 0.35±0.02, SW2L = 0.38±0.01 and SW3L = 0.64±0.02) were seen lower than ice creams with coconut milk (SC1L = 0.14±0.02, SC2L = 0.22±0.02 and SC3L = 0.59±0.01). The *n* value of WL, CL and SL were 0.76±0.010, 66±0.020.11±0.01, respectively in upward curves. In the downward curves, the flow behavior index (*n*) of ice creams with cow milk (SW1L = 0.61±0.02, SW2L = 0.66±0.01 and SW3L = 0.66±0.02) were seen lower than ice creams with coconut milk (SC1L = 0.66±0.02, SC2L = 0.69±0.02 and SC3L = 0.70±0.01). The *n* value of WL, CL and SL were 0.74±0.010.71±0.01 and 0.61±0.02, respectively (Table 4.13).

Table 4.13 Rheological parameters of the fermented ice creams inoculated with La-05 obtained using the Power Law model.

Samples	Apparent viscosity (mPa s)[b]	K (Pa s^n)[b]	n[b]	R^2[c]
		upward curves		
WL	450±2.01[h]	0.90±0.01[i]	0.76±0.01[a]	0.998
CL	420±1.76[i]	1.09±0.02[h]	0.66±0.02[a]	0.990
SL	3770±0.89[a]	43.12±0.02[a]	0.11±0.01[c]	0.600
SW1L	2080±1.02[g]	12.61±0.01[d]	0.35±0.02[b]	0.920
SW2L	1680±1.66[c]	9.56±0.02[e]	0.38±0.01[b]	0.950
SW3L	818±1.32[f]	2.32±0.02[f]	0.64±0.02[a]	0.990
SC1L	3440±1.1[b]	36.41±0.01[b]	0.14±0.02[c]	0.420
SC2L	1990±1.32[d]	17.25±0.01[c]	0.22±0.02[bc]	0.740
SC3L	556±1.03[g]	1.95±0.02[g]	0.59±0.01[a]	0.990
		Downward curves		
WL	437±1.52[h]	0.93±0.02[g]	0.74±0.01[a]	0.996
CL	373±0.83[i]	0.87±0.02[g]	0.71±0.01[a]	0.997
SL	1720±1.07[a]	5.24±0.02[a]	0.61±0.02[a]	0.990
SW1L	1370±1.32[c]	4.33±0.02[b]	0.61±0.02[a]	0.994
SW2L	1120±0.94[d]	3.01±0.02[c]	0.66±0.01[b]	0.993
SW3L	760±0.87[f]	2.07±0.02[e]	0.66±0.02[a]	0.997
SC1L	1550±2.01[b]	4.16±0.02[b]	0.66±0.02[a]	0.990
SC2L	990±1.43[e]	2.43±0.02[d]	0.69±0.02[a]	0.996
SC3L	500±1.62[g]	1.17±0.02[f]	0.70±0.01[a]	0.995

K = consistency index; n = flow behavior index; Noted: ice cream inoculated with La-05 and made with 100% cow milk: WL; 100% coconut milk: CL; 100% soybean extract: SL; 75% soybean extract+25% cow milk: SW1L; 50% soybean extract+50% cow milk: SW2L; 25% soybean extract+75% cow milk: SW3L; 75% soybean extract+25% coconut milk: SC1L; 50% soybean extract+50% coconut milk: SC2L; 25% soybean extract+75% coconut milk: SC3L.
[b] Mean values±standard deviation. Values with different letters in the same column are significantly different ($p<0.05$) (Tukey test).
[c] Coefficient of determination.

In fermented ice creams with Bb-12, the apparent viscosity value of WB, CB and SB was 323±1.02, 179±1.10 and 2860±0.91 mPa s, respectively. The apparent viscosity value of ice creams with coconut milk (SC1B = 1520±1.03, SC2B = 1050±0.78 and SC3B = 537±2.01 mPa s) were higher than ice creams with cow milk (SW1B = 1680±1.02, SW2B = 697±0.88 and SW3B = 330±0.91 mPa s) ($p<0.05$) in upward curves (Table 4.14). The apparent viscosity value of WB, CB and SB were 233±1.03, 182±0.97 and 1430±2.01 mPa s, respectively. The apparent viscosity value of ice creams with coconut milk (SC1B =

965±2.10, SC2B = 655±1.50, SC3B = 427±1.43 mPa s) were higher than ice creams with cow milk (SW1B = 968±1.76, SW2B = 479±1.43, SW3B = 322±1.02 mPa s) ($p<0.05$) in downward curves (Table 4.14). The consistency index (*K*) of ice creams with cow milk (SC1B = 10.37±0.01, SC2B = 6.61±0.02 and SC3B = 1.94±0.01 Pa s^n, respectively) were seen lower than ice creams with coconut milk (SW1B = 11.74±0.02, SW2B = 3.00±0.00 and SW3B = 0.70±0.02 Pa s^n). The *K* value of WB, CB and SB were 0.71±0.02, 0.29± 0.02 and 28.54± 0.02 Pa s^n in upward curves. In the downward curves, the consistency index (*K*) of ice creams with cow milk (SW1B = 2.47±0.01, SW2B = 1.14±0.02 and SW3B = 0.49±0.02 Pa s^n) were seen lower than ice creams with coconut milk (SC1B = 2.71±0.01, SC2B = 1.58±0.02 and SC3B = 0.94±0.02 Pa s^n). The *K* values of WB, CB ad SB were 0.25±0.02, 0.32±0.02 and 4.48±0.02 Pa s^n, respectively. The flow behavior index (*n*) of ice creams with cow milk (SW1B = 0.29±0.02, SW2B = 0.47±0.02 and SW3B = 0.75±0.02) were seen lower than ice creams with coconut milk (SC1B = 0.33±0.02, SC2B = 0.35±0.02 and SC3B = 0.56±0.02). The *n* value of WB, CB and SB were 0.72±0.01, 0.83±0.02 and 0.16±0.02, respectively in upward curves. In the downward curves, the flow behavior index (*n*) of ice creams with cow milk (SW1B = 0.67±0.00, SW2B = 0.70±0.02 and SW3B = 0.84±0.01) were seen lower than ice creams with coconut milk (SC1B = 0.64±0.01, SC2B = 0.69±0.02 and SC3B = 0.72±0.02). The *n* value of WB, CB and SB were 0.96±0.01, 0.79±0.02 and 0.60±0.01, respectively in downward curves (Table 4.14).

Table 4.14 Rheological parameters of the fermented ice creams inoculated with Bb-12 obtained using the Power Law model

Samples	Apparent viscosity (mPa s)[b]	K (Pa s^n)[b]	n[b]	R^2[c]
		upward curves		
WB	323±1.02[g]	0.71±0.02[g]	0.72±0.01[ab]	0.998
CB	179±1.10[h]	0.29±0.02[h]	0.83±0.02[a]	0.999
SB	2860±0.91[a]	28.54±0.02[a]	0.16±0.02[f]	0.509
SW1B	1680±1.02[b]	11.74±0.02[b]	0.29±0.02[ef]	0.796
SW2B	697±0.88[e]	3.00±0.00[e]	0.47±0.02[dc]	0.954
SW3B	330±0.91[g]	0.70±0.02[g]	0.75±0.02[a]	0.999
SC1B	1520±1.03[c]	10.37±0.01[c]	0.33±0.02[df]	0.945
SC2B	1050±0.78[d]	6.61±0.02[d]	0.35±0.02[de]	0.947
SC3B	537±2.01[f]	1.94±0.01[f]	0.56±0.02[bc]	0.996
	Downward curves			
WB	233±1.03[g]	0.25±0.02[h]	0.96±0.01[a]	0.998
CB	182±0.97[h]	0.33±0.02[gh]	0.79±0.02[ac]	0.996
SB	1430±2.01[a]	4.48±0.02[a]	0.60±0.01[c]	0.990
SW1B	968±1.76[b]	2.47±0.01[b]	0.67±0.00[dc]	0.985
SW2B	479±1.43[d]	1.14±0.02[e]	0.70±0.02[bc]	0.993
SW3B	322±1.02[f]	0.49±0.02[g]	0.84±0.01[ab]	0.984
SC1B	965±2.10[b]	2.71±0.01[c]	0.64±0.01[dc]	0.991
SC2B	655±1.50[c]	1.58±0.02[d]	0.69±0.02[bc]	0.991
SC3B	427±1.43[e]	0.94±0.02[f]	0.72±0.02[bc]	0.994

[a] K = consistency index; n = flow behavior index; note: ice cream inoculated with Bb 12 and made with 100% cow milk: WB; 100% coconut milk: CB; 100% soybean extract: SB; 75% soybean extract+25% cow milk: SW1B; 50% soybean extract+50% cow milk: SW2B; 25% soybean extract+75% cow milk: SW3L; 75% soybean extract+25% coconut milk: SC1B; 50% soybean extract+50% coconut milk: SC2B; 25% soybean extract+75% coconut milk: SC3B.
[b] Mean values±standard deviation. Values with different letters in the same column are significantly different ($P<0.05$) (Tukey test).
[c] Coefficient of determination.

The presence of flow curves hysteresis, as shown in Figures 4.4, 4.5, 4.6, 4.7, 4.8, and 4.9. Table 4.15 shows the hysteresis areas of ice creams with coconut milk were seen higher than ice creams with cow milk in all non fermented and fermented ice creams.

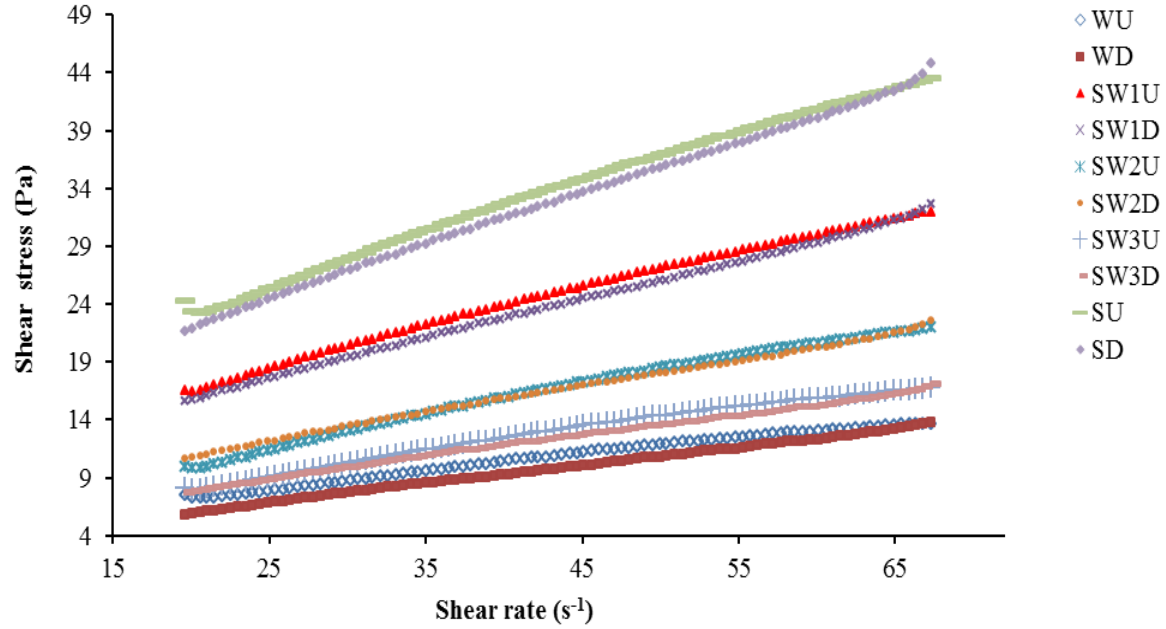


Figure 4.4 Shear stress & shear rate relationship upward (U) and downward (D) flow curves and hysteresis areas for non fermented ice creams. Noted: ice cream made using 100% cow milk: WU & WD; ice cream with 100% soybean extract: SU & SD; ice cream with 75% soybean extract+25% cow milk: SW1U & SW1D; ice cream with 50% soybean extract+50% cow milk: SW2U & SW2D; ice cream with 25% soybean extract+75% cow milk: SW3U & SW3D.

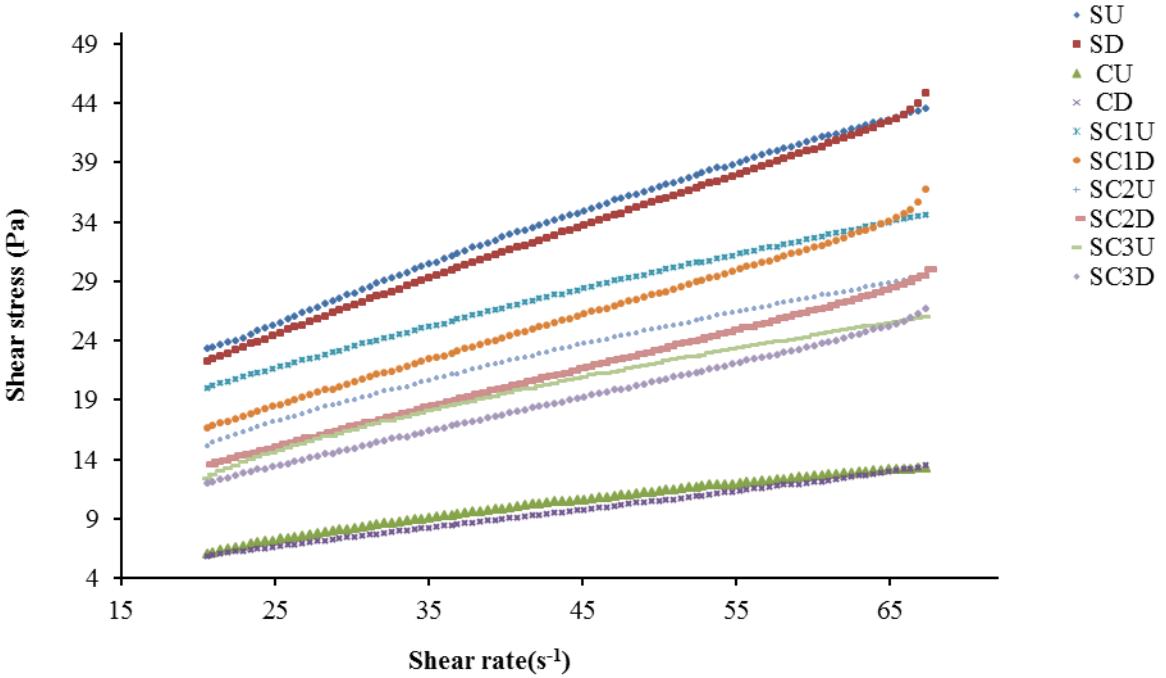


Figure 4.5 Shear stress & shear rate relationship upward (U) and downward (D) flow curves and hysteresis areas for non fermented ice creams. Noted: ice cream made using with 100% coconut milk: CU & CD; ice cream with 75% soybean extract+25% coconut milk: SC1U & SC1D; ice cream with 50% soybean extract+50% coconut milk: SC2U & SC2D; ice cream with 25% soybean extract+75% coconut milk: SC3U & SC3D.

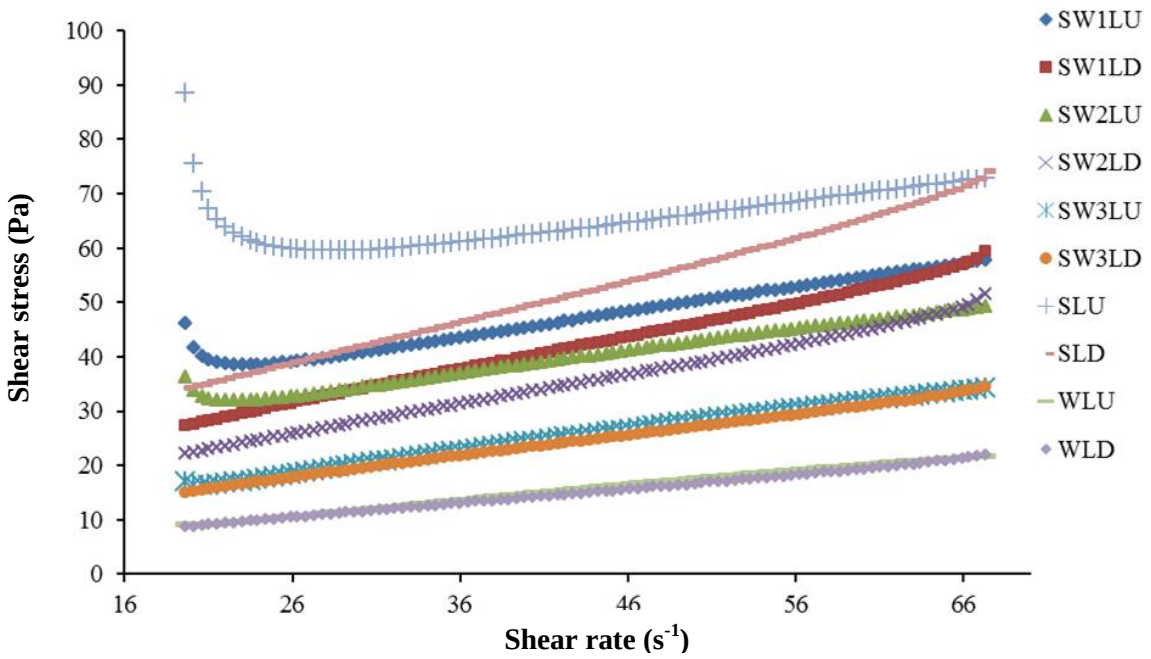


Figure 4.6 Shear stress & shear rate relationship upward (U) and downward (D) flow curves and hysteresis areas for fermented ice creams. Noted: ice cream inoculated with La-05 made with 100% cow milk: WLU & WLD; ice cream with 100% soybean extract: SLU & SLD; ice cream with 75% soybean extract+25% cow milk: SW1LU & SW1LD; ice cream with 50% soybean extract+50% cow milk: SW2LU & SW2LD; ice cream with 25% soybean extract+75% cow milk: SW3LU & SW3LD.

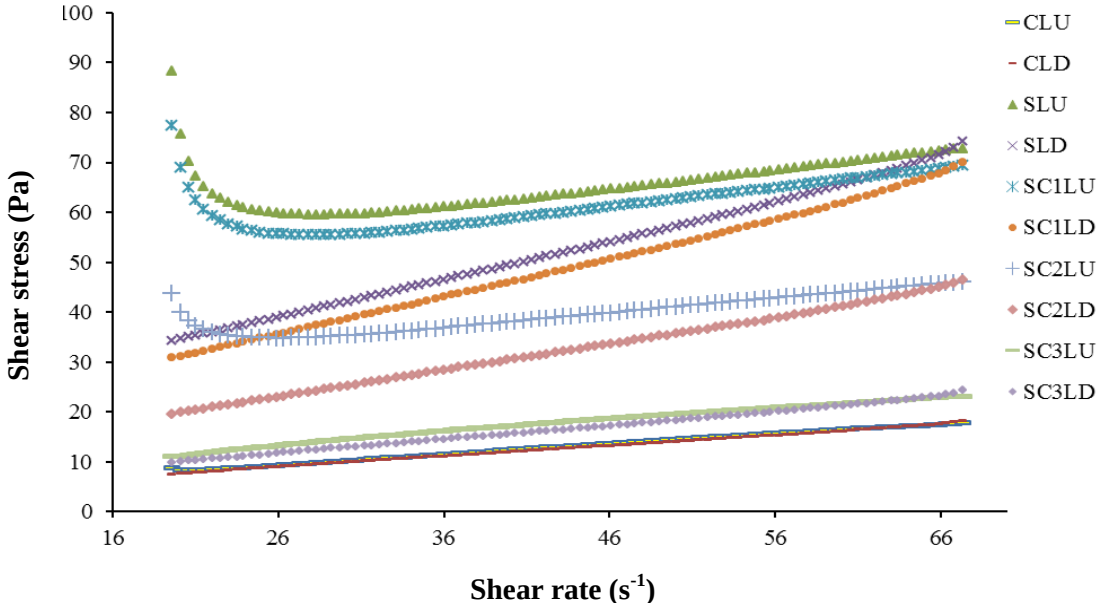


Figure 4.7 Shear stress & shear rate relationship upward (U) and downward (D) flow curves and hysteresis areas for fermented ice creams. Noted: ice cream inoculated with La-05 made using with 100% coconut milk: CLU & CLD; ice cream with 75% soybean extract+25% coconut milk: SC1LU & SC1LD; ice cream with 50% soybean extract+50% coconut milk: SC2LU & SC2LD; ice cream with 25% soybean extract+75% coconut milk: SC3LU & SC3LD.

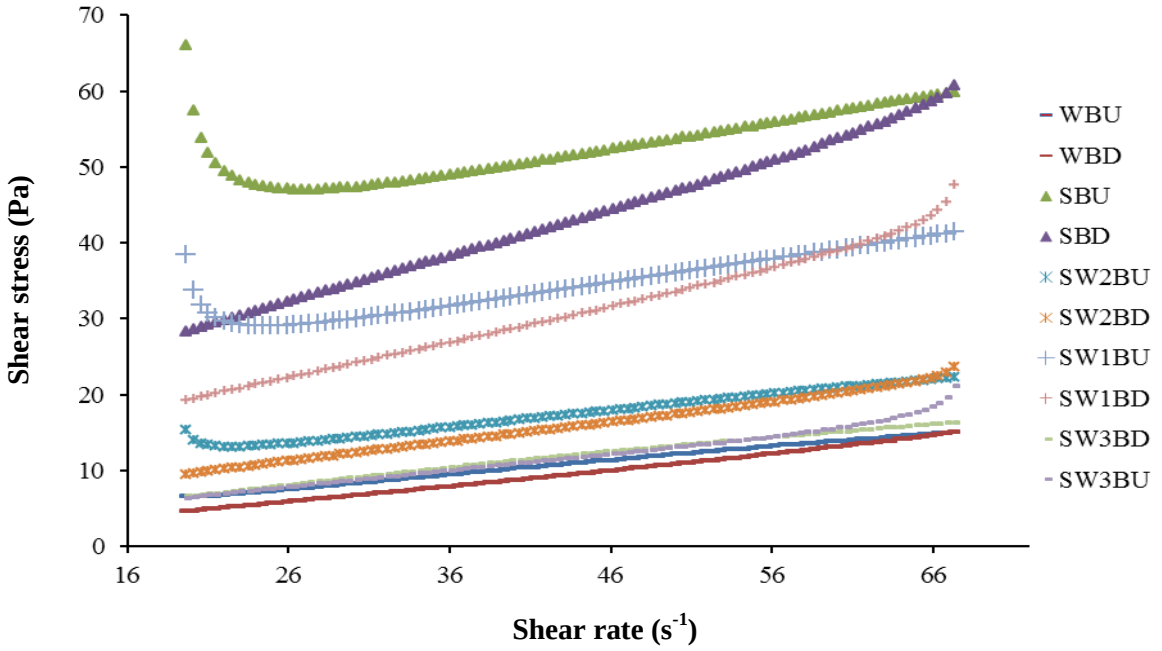


Figure 4.8 Shear stress & shear rate relationship upward (U) and downward (D) flow curves and hysteresis areas for fermented ice creams. Noted: ice cream inoculated with Bb-12 made using with 100% cow milk: WBU & WBD; ice cream with 100% soybean extract: SBU & SBD; ice cream with 75% soybean extract+25% cow milk: SW1BU & SW1BD; ice cream with 50% soybean extract+50% cow milk: SW2BU & SW2BD; ice cream with 25% soybean extract +75% cow milk: SW3BU & SW3BD.

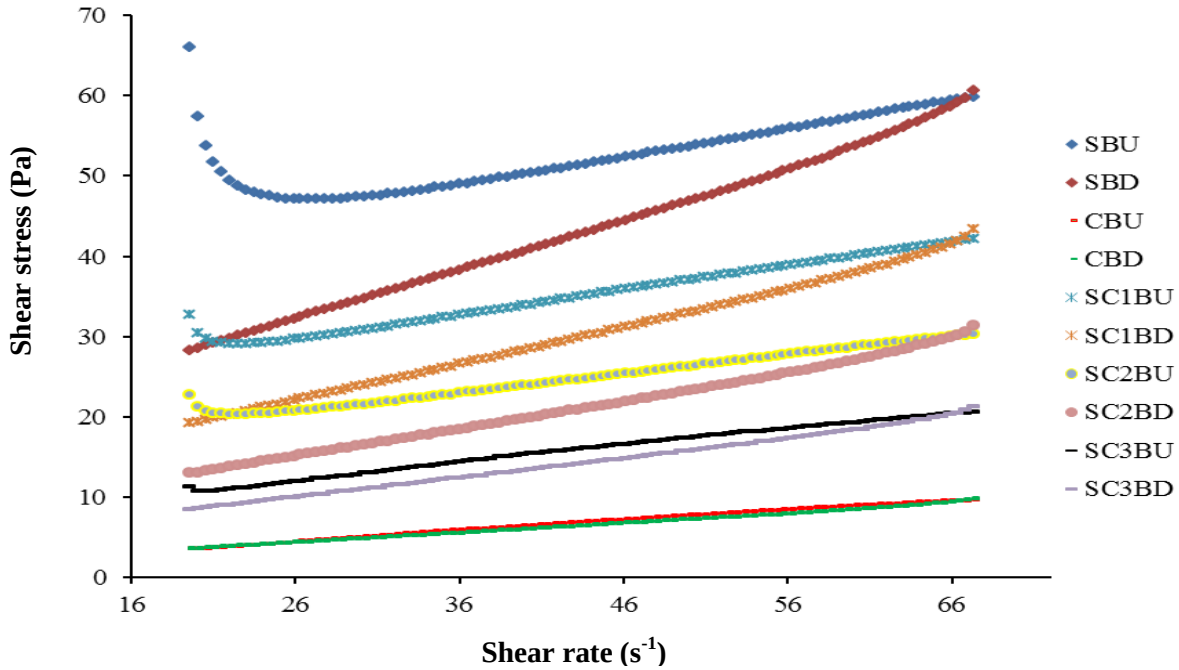


Figure 4.9 Shear stress & shear rate relationship upward (U) and downward (D) flow curves and hysteresis areas for fermented ice creams. Noted: ice cream inoculated with Bb-12 made using with 100% coconut milk: CBU & CBD; ice cream with 75% soybean extract+25% coconut milk: SC1BU & SC1BD; ice cream with 50% soybean extract+50% coconut milk: SC2BU & SC2BD; ice cream with 25% soybean extract+75% coconut milk: SC3BU & SC3BD.

Table 4.15 Hysteresis of integral area of shear rate sweep non fermented and fermented ice creams.

Samples [A]	Hysteresis (Pa)[a]		
	Non fermented ice cream	Fermented ice cream	
		Fermented ice cream by La-05	Fermented ice cream by Bb-12
W	23.93±0.96[f]	28.99±1.80[fg]	60.34±1.04[d]
C	36.19±1.14[e]	24.14±1.34[g]	15.30±1.10[e]
S	45.69±2.03[d]	605.17±0.93[a]	439.95± 1.32[a]
SW1	45.20±1.51[d]	242.02±1.05[d]	173.00± 1.02[c]
SW2	28.69±1.30[f]	218.71±1.72[e]	75.64± 1.86[d]
SW3	2.70±1.81[g]	44.42±1.56[f]	~0± 0.00[f]
SC1	100.41±1.42[a]	589.79±1.84[a]	231.83± 1.11[b]
SC2	60.00±1.61[b]	28.99±1.80[fg]	171.74± 0.92[c]
SC3	55.33±1.59[c]	24.14±1.34[g]	74.72± 1.02[d]

[A] Ice cream mixes with different milk. W: ice cream with 100% cow milk; C: ice cream with 100% coconut milk; S: ice cream with 100% soybean extract; SW1: ice cream with 75% soybean extract+25% cow milk; SW2: ice cream with 50% soybean extract+50% cow milk; SW3: ice cream with 25% soybean extract+75% cow milk; SC1: ice cream with 75% soybean extract+25% coconut milk; SC2: ice cream with 50% soybean extract+50% coconut milk; SC3: ice cream with 25% soybean extract+75% coconut milk.
[a] Mean values±standard deviation.
[a-f] Values with different letters in the same column are significantly different ($p<0.05$) (Tukey test).

4.2.3 Size and zeta potential

Table 4.16 shows the particle size and zeta potential of ice creams. In all non fermented and fermented ice creams, the particle size of ice creams with coconut milk were seen higher than ice creams containing cow milk ($p<0.05$; Table 4.16). The zeta potential of non fermented ice creams with cow milk were seen higher (more negative) than ice creams containing coconut milk ($p<0.05$; Table 4.16).

Table 4.16 Effect of milk replacement on zeta potential and particle diameter (Dm) of fat globules of non fermented and fermented ice cream

Samples [A]	Non fermented ice cream		Fermented ice cream			
			Fermented ice cream by La-05		Fermented ice cream by Bb-12	
	Particle size (μm)	Zeta potential (mV)	Particle size (μm)	Zeta potential (mV)	Particle size (μm)	Zeta potential (mV)
W	0.91±0.08^{e}	-36.56±0.80^{d}	4.86±0.11^{e}	-35.02±0.56^{b}	4.09± 0.09^{e}	-36.33±0.40^{d}
C	1.74±0.03^{b}	-30.70±0.60^{b}	5.29±0.06^{d}	-35.73±0.62^{b}	4.68±0.06^{d}	-37.40±0.70^{d}
S	1.60±0.10^{c}	-35.50±0.70^{cd}	7.05±0.08^{b}	-37.93±0.71^{c}	7.70±0.30^{b}	-37.67±0.90^{d}
SW1	0.81±0.03^{e}	-36.87±0.90^{d}	6.84±0.06^{c}	-37.57±0.62^{c}	6.19±0.50^{c}	-31.20±0.60^{a}
SW2	0.82±0.05^{e}	-37.60±1.08^{d}	6.56±0.10^{c}	-36.60±0.41^{bc}	6.09±0.21^{c}	-32.67±0.50^{b}
SW3	0.83±0.04^{e}	-26.40±0.78^{a}	4.20±0.08^{e}	-31.73±1.30^{a}	4.07±0.11^{e}	-30.80±0.48^{a}
SC1	1.57±0.06^{c}	-33.20±0.65^{bc}	8.13±0.04^{a}	-36.43±0.54^{bc}	8.09±0.30^{a}	-37.33±0.80^{d}
SC2	1.68±0.07^{c}	-34.30±0.08^{cd}	8.86±0.05^{a}	-38.13±1.10^{c}	8.42±0.30^{a}	-34.87±1.04^{c}
SC3	2.54± 0.11^{a}	-26.70±1.20^{a}	8.15±0.02^{a}	-37.77±0.72^{c}	8.07±0.20^{a}	-35.17±0. 80^{c}

[A] Ice cream mixes with different milk. W: ice cream with 100% cow milk; C: ice cream with 100% coconut milk; S: ice cream with 100% soybean extract; SW1: ice cream with 75% soybean extract+25% cow milk; SW2: ice cream with 50% soybean extract+50% cow milk; SW3: ice cream with 25% soybean extract+75% cow milk; SC1: ice cream with 75% soybean extract+25% coconut milk; SC2: ice cream with 50% soybean extract+50% coconut milk; SC3: ice cream with 25% soybean extract+75% coconut milk.

[a-j] Means in the same column followed by different letters were significantly different($p<0.05$).

4.2.4 Optical polarizing microscope imaging (OPM)

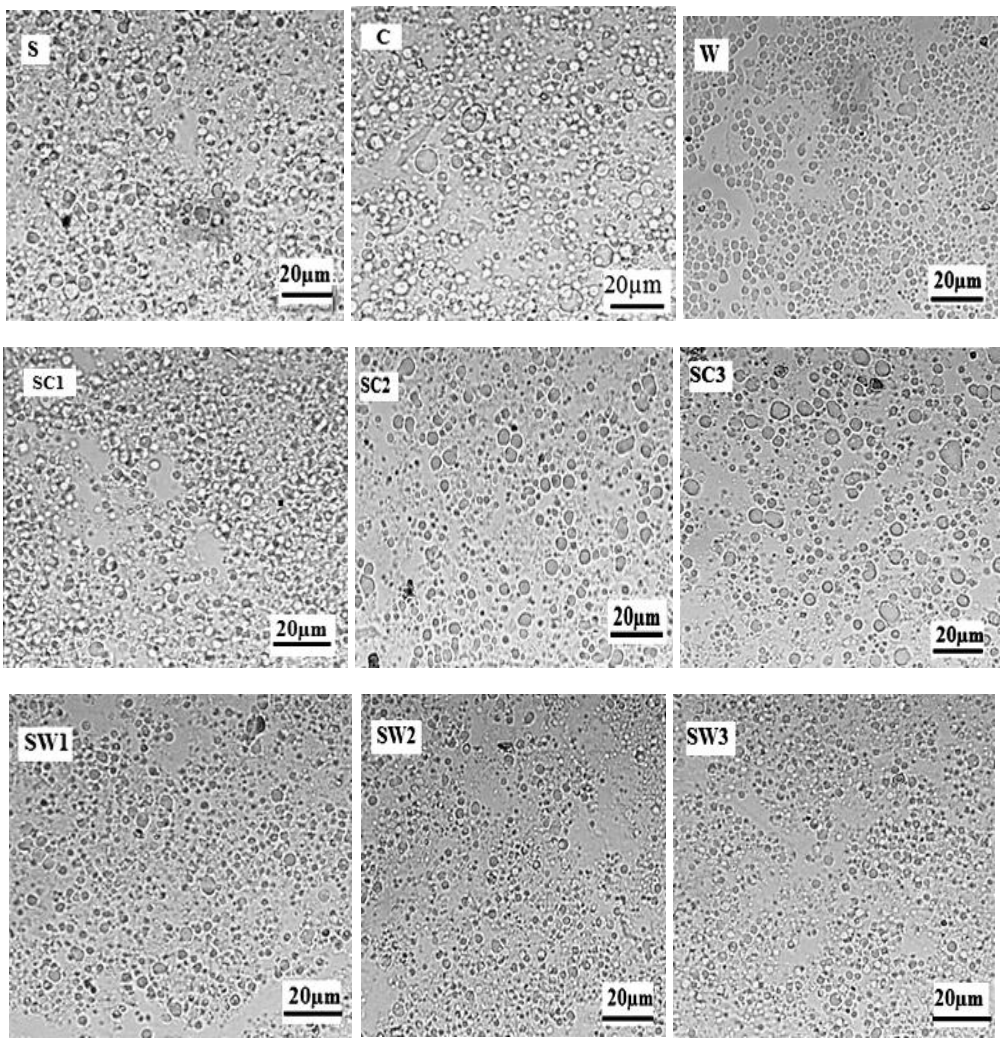


Figure 4.10 Micrographs (×50 magnification) of non fermented probiotic ice cream mixes with different milk. W: ice cream with 100% cow milk; C: ice cream with 100% coconut milk; S: ice cream with 100% soybean extract; SW1: ice cream with 75% soybean extract+25% cow milk; SW2: ice cream with 50% soybean extract+50% cow milk; SW3: ice cream with 25% soybean extract+75% cow milk; SC1: ice cream with 75% soybean extract+25% coconut milk; SC2: ice cream with 50% soybean extract+50% coconut milk; SC3: ice cream with 25% soybean extract+75% coconut milk.

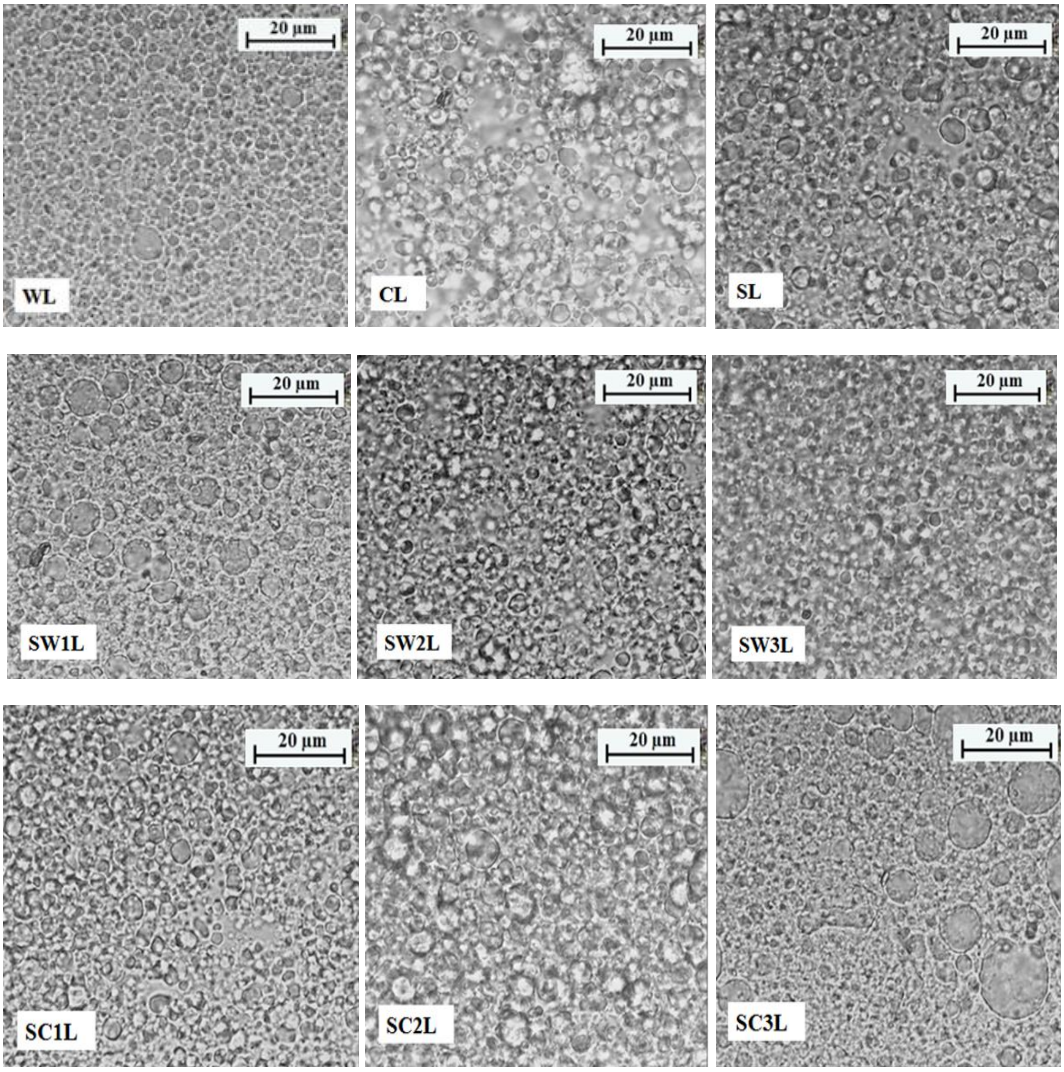


Figure 4.11 Micrographs (×50 magnification) of fermented ice cream samples incubated with La-05 and made with 100% cow milk: WL; 100% coconut milk: CL; 100% soybean extract: SL; 75% soybean extract+25% cow milk: SW1L; 50% soybean extract+50% cow milk: SW2L; 25% soybean extract+75% cow milk: SW3L; 75% soybean extract+25% coconut milk: SC1L; 50% soybean extract+50% coconut milk: SC2L; 25% soybean extract+75% coconut milk: SC3L

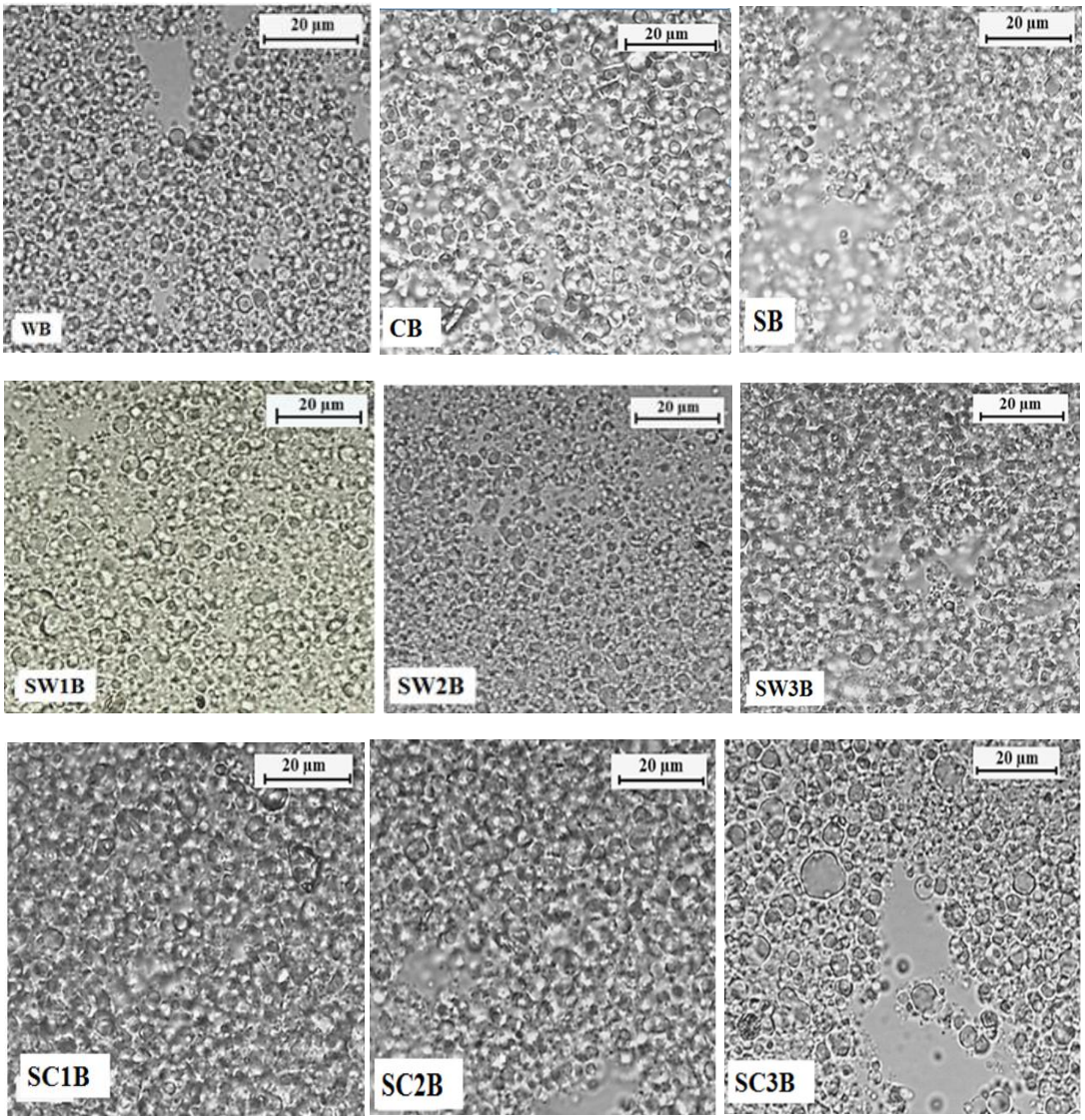


Figure 4.12 Micrographs (×50 magnification) of fermented ice cream samples incubated with Bb-12 and made with 100% cow milk: WB; 100% coconut milk: CB; 100% soybean extract: SB; 75% soybean extract+25% cow milk: SW1B; 50% soybean extract+50% cow milk: SW2B; 25% soybean extract+75% cow milk: SW3L; 75% soybean extract+25% coconut milk: SC1B; 50% soybean extract+50% coconut milk: SC2B; 25% soybean extract+75% coconut milk: SC3B.

4.2.5 The thermal properties of ice creams with different milks

The thermal properties associated with ice crystal-melting of non fermented and fermented ice creams with different milk (Figures 4.13, 4.14 and 4.15) were measured by differential scanning calorimetry (DSC).

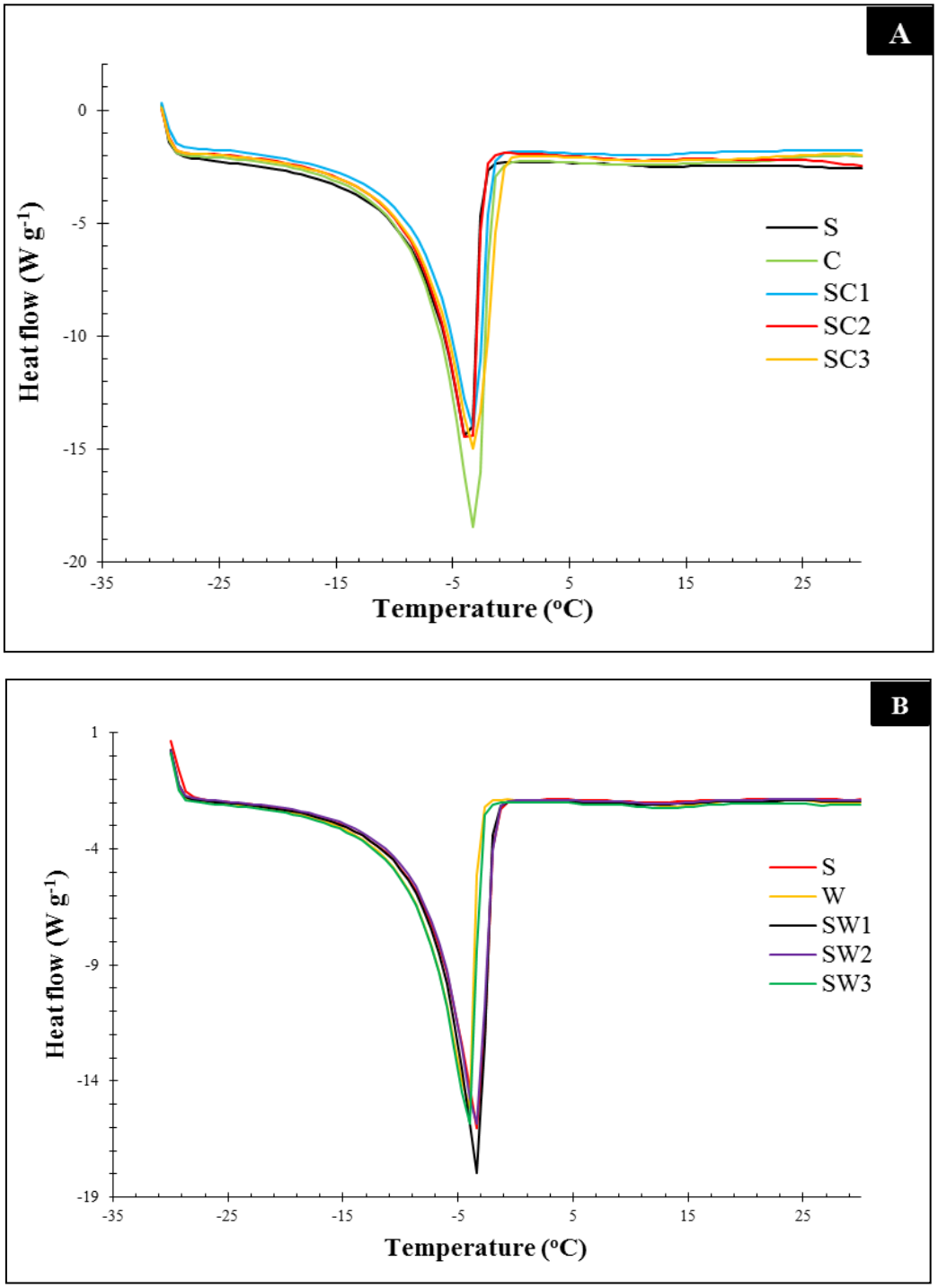


Figure 4.13 Effect of the replacement of cow milk with coconut milk and soybean extract on the ice crystal-melting of non fermented ice creams measured by differential scanning calorimetry: A) ice creams containing coconut milk, B) ice creams containing cow milk.

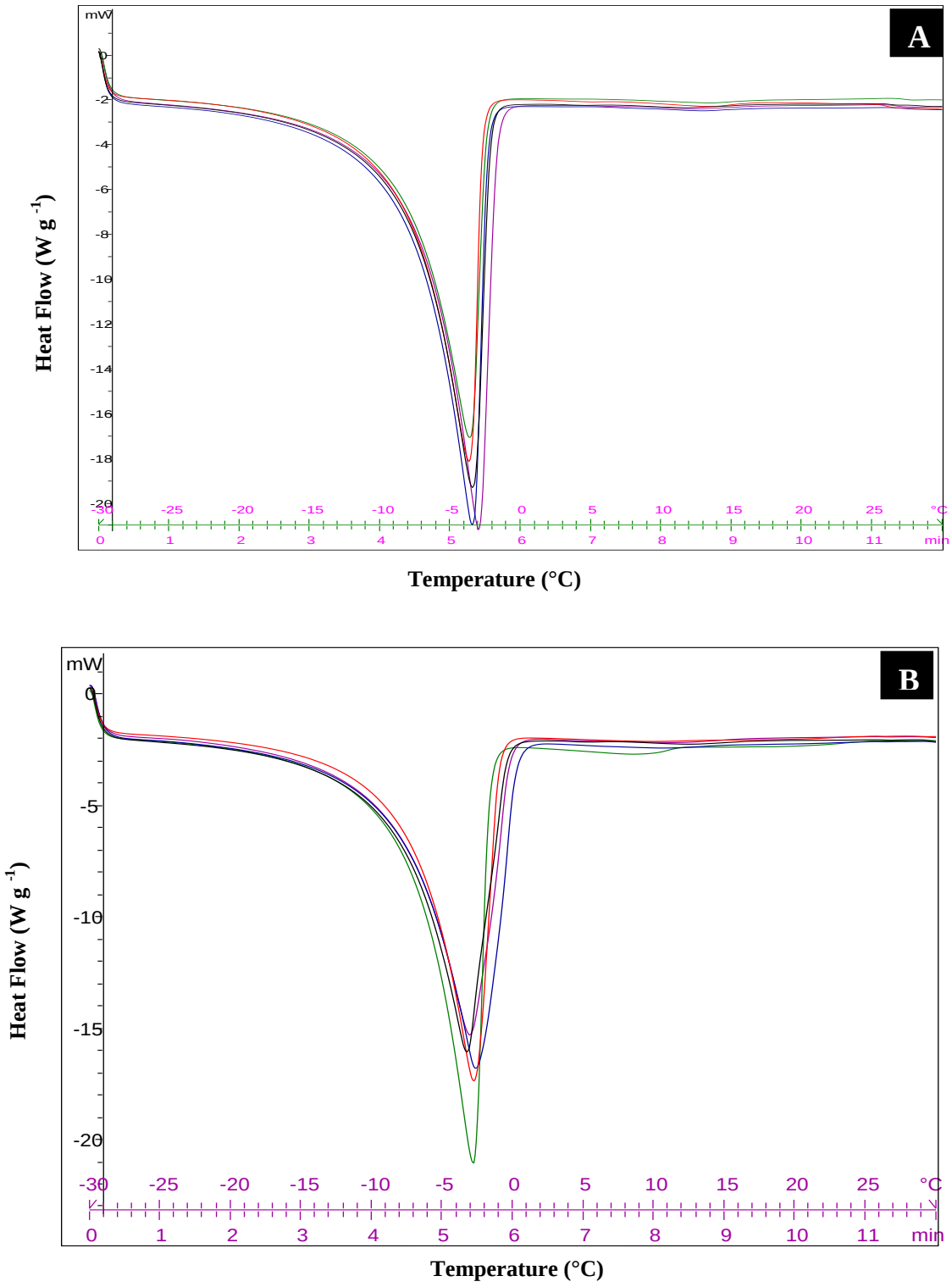


Figure 4.14 Effect of the replacement of cow milk with coconut milk and soybean extract on the ice crystal-melting of fermented ice creams incubated with La-05 measured by differential scanning calorimetry (DSC): A) ice creams containing cow milk (—**:** SL; —: WL; —: SW1L; —: SW2L; —: SW3L), B) ice creams containing coconut milk: (—: SL; —: CL; —: SC1L; —: SC2L; —: SC3L).

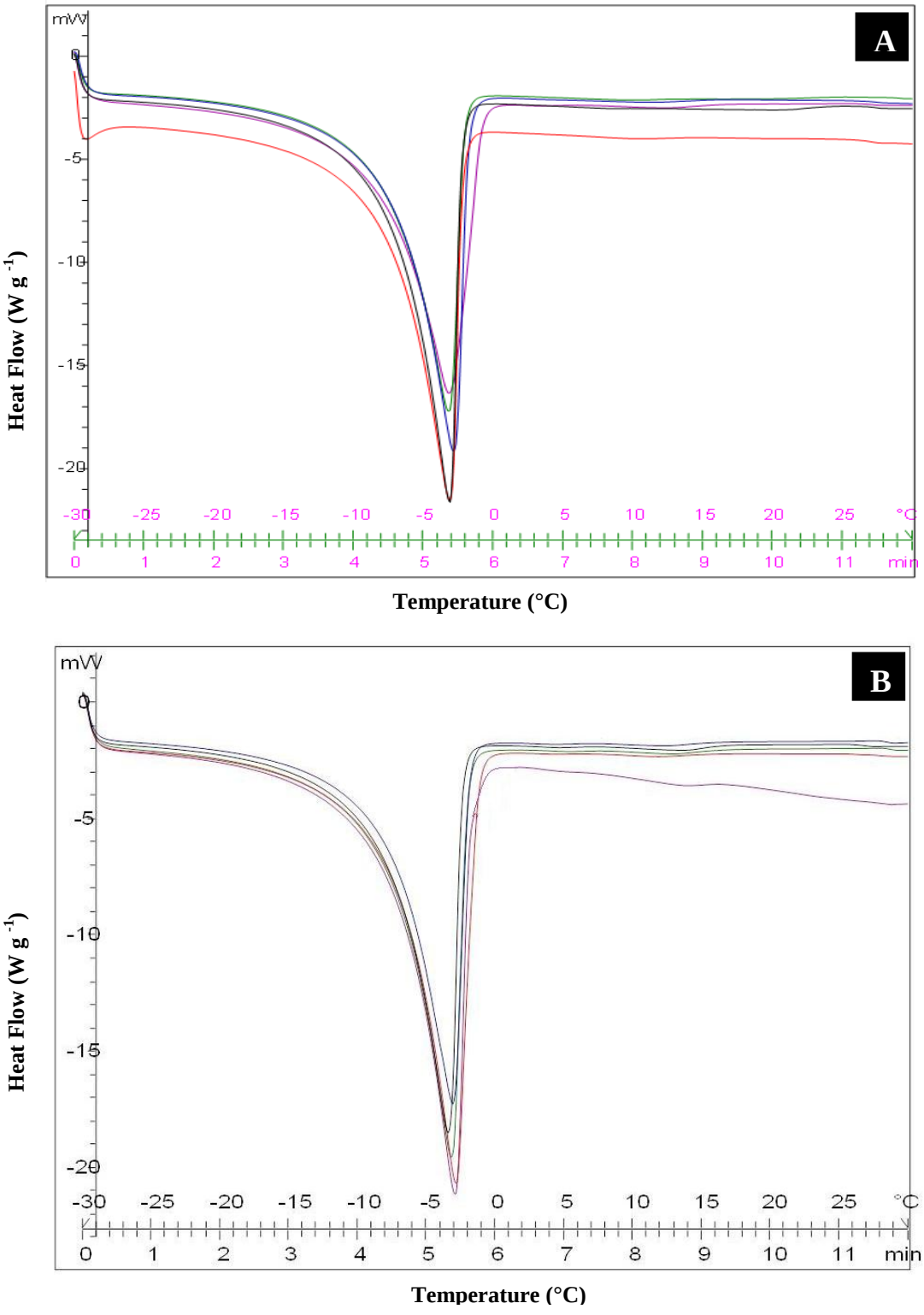


Figure 4.15 Effect of the replacement of cow milk with coconut milk and soybean extract on the ice crystal-melting of fermented ice creams inoculated with Bb-12 measured by differential scanning calorimetry (DSC): A) ice creams containing coconut milk (—: SB; —: CB; —: SC1B; —: SC2B; —: SC3B), B) ice creams containing cow milk: (—: SB; —: WB; —: SW1B; —: SW2B; —: SW3B).

In non fermented ice creams, there was no significant differences in peak temperature among ice creams (W = -3.82±0.15, C = -3.17±0.10, S = -3.90±0.13, SW1 = -3.44±0.21, SW2 = -3.75±0.14, SW3 = -3.68± 0.22, SC1 = -3.70±0.11, SC2 = -3.72±0.16 and SC3 = -3.70±0.12 ^{0}C; p>0.05) (Table 4.17). The highest onset temperature was in C ice cream sample (-6.93±0.12 ^{0}C) and the lowest was in W (-8.77±0.11 ^{0}C) and S (-8.50±0.11 ^{0}C) ice cream samples. The onset temperature was similar for all ice creams containing composite milk (SW1 = -7.77±0.19, SW2 = -7.91±0.13, SW3 = -7.86±0.21, SC1 = -7.86±0.10, SC2 = -7.91±0.18 and SC3 = -7.40±0.11 ^{0}C; p>0.05) (Table 4.17). The freezable water amounts of W, C and S were 32.50±1.18, 39.60±1.21 and 31.91±1.40%, respectively. The freezable water amounts of ice creams with coconut milk (SC1 = 34.07±1.07, SC2 = 34.64±1.04 and SC3 = 37.47±1.09%) were higher than ice creams containing cow milk (SW1 = 31.68±1.03, SW2 = 34.62±1.05, SW3 = 38.61±2.11%). The freezing point values were similar in all ice creams (p>0.05; Table 4.17). The enthalpy for the ice crystal melting of ice creams with coconut milk (SC1 = 113.79±5.60, SC2 = 115.72±6.20 and SC3 = 125.16±6.10 J/g) were higher than ice creams containing cow milk (SW1 = 105.81±6.00, SW2 = 115.65±4.80, SW3 = 128.96±5.30 J/g).

No significant effects (p>0.05) were observed between samples fermented with either with La-05 and Bb-12. In both types of fermented ice creams inoculated with La-05 and Bb-12, there is no significant difference in the onset temperature to among ice creams. The peak temperature was similar for all fermented ice creams containing composite milk (Tables 4.18 and 4.19). The freezable water amounts of fermented ice creams ice creams with coconut milk were seen higher than ice creams with cow milk (Tables 4.18 and 4.19). The enthalpy for the ice crystal melting of WL, CL, SL, SW1L, SW2L, SW3L, SC1L, SC2L and SC3L was 123.04±4.0, 131.83±3.18, 123.61±6.10, 131.76±5.00, 125.92±4.07,

122.24±3.98, 118.90±6.20, 133.16±5.50 and 138.54±5.12 J/g, respectively ($p>0.05$; 4.18). The enthalpy for the ice crystal melting of WB, CB, SB, SW1B, SW2B, SW3B, SC1B, SC2B and SC3B was 128.59±6.12, 130.33±5.37, 130.26±1.11, 130.86±5.23, 124.08±4.07, 120.24±2.18, 126.62±3.18, 129.02±5.03 and 129.42±4.27 J/g, respectively ($p>0.05$; Table 4.19).

Table 4.17 Differential scanning calorimetry analyses for non fermented ice cream mixes.

Samples	Peak temperature (^{0}C)	Onset temperature (^{0}C)	Freezing point (^{0}C)	Freezable water (%)	ΔH_f (J/g)
W	-3.82±0.15 ^{a}	-8.77±0.11 ^{c}	-5.52±0.09 ^{a}	32.50±1.18 ^{e}	108.57±4.10 ^{d}
C	-3.17±0.10 ^{a}	-6.93±0.12 ^{a}	-4.53±0.14 ^{a}	39.61±1.21 ^{a}	132.29±5.20 ^{a}
S	-3.90±0.13 ^{a}	-8.50±0.11 ^{c}	-5.21±0.12 ^{a}	31.91±1.40 ^{d}	106.57±3.10 ^{d}
SW1	-3.44±0.21 ^{a}	-7.77±0.19 ^{b}	-5.00±0.10 ^{a}	31.68±1.03 ^{d}	105.81±6.00 ^{d}
SW2	-3.75±0.14 ^{a}	-7.91±0.13 ^{b}	-5.31±0.17 ^{a}	34.62±1.05 ^{c}	115.65±4.80 ^{c}
SW3	-3.68±0.22 ^{a}	-7.86±0.21 ^{b}	-5.01±0.21 ^{a}	38.61±2.11 ^{ab}	128.96±5.30 ^{ab}
SC1	-3.70±0.11 ^{a}	-7.86±0.10 ^{b}	-5.06±0.16 ^{a}	34.07±1.07 ^{c}	113.79±5.60 ^{c}
SC2	-3.72±0.16 ^{a}	-7.91±0.18 ^{b}	-5.48±0.11 ^{a}	34.64±1.04 ^{c}	115.72±6.20 ^{c}
SC3	-3.70±0.12 ^{a}	-7.40±0.11 ^{b}	-4.94±0.19 ^{a}	37.47±1.09 ^{bc}	125.16±6.10 ^{bc}

ΔH_f = Enthalpy of fusion.
$^{a-e}$ Means in the same column followed by different letters were significantly different ($p<0.05$).

Table 4.18 Differential scanning calorimetry analyses for fermented ice cream mixes inoculated with La-05.

Samples	Onset temperature (^{0}C)	Peak temperature (^{0}C)	Freezing point (^{0}C)	Freezable water (%)	ΔH_f (J/g)
WL	-8.31±0.21 ^{a}	-4.05±0.15 ^{a}	-5.40±0.13 ^{a}	36.84±1.18 ^{a}	123.04±4.00 ^{a}
CL	-7.32±0.16 ^{a}	-3.20±0.11 ^{a}	-4.50±0.22 ^{a}	39.47±1.11 ^{a}	131.83±3.18 ^{a}
SL	-8.03±0.13 ^{a}	-3.82±0.16 ^{a}	-5.90±0.31 ^{a}	37.01±1.12 ^{a}	123.61±6.10 ^{a}
SW1L	-7.45±0.14 ^{a}	-3.45±0.20 ^{a}	-5.20±0.10 ^{a}	39.45±1.02 ^{a}	131.76±5.00 ^{a}
SW2L	-7.81±0.18 ^{a}	-3.86±0.17 ^{a}	-5.00±0.17 ^{a}	37.70±1.75 ^{a}	125.92±4.07 ^{a}
SW3L	-8.28±0.12 ^{a}	-3.94±0.20 ^{a}	-5.80±0.16 ^{a}	36.60±2.01 ^{a}	122.24±3.98 ^{a}
SC1L	-8.33±0.10 ^{a}	-3.41±0.10 ^{a}	-5.20±0.12 ^{a}	35.60±1.17 ^{a}	118.90±6.20 ^{a}
SC2L	-7.77±0.11 ^{a}	-3.02±0.14 ^{a}	-5.00±0.13 ^{a}	39.87±1.03 ^{a}	133.16±5.50 ^{a}
SC3L	-7.33±0.18 ^{a}	-3.12±0.11 ^{a}	-4.50±0.19 ^{a}	41.48±1.07 ^{a}	138.54±5.12 ^{a}

^{A}Calculated by subtracting freezing point and freezable water of fermented ice cream from freezing point and freezable water of fermented ice cream, dividing by freezing point and freezable water of fermented ice cream and multiplying by 100.
$^{a-g}$ Means in the same column followed by different letters were significantly different ($p<0.05$).
ΔH_f = Enthalpy of fusion.

Table 4.19 Differential scanning calorimetry analyses for fermented ice cream mixes inoculated with Bb-12.

Samples	Onset temperature (^{0}C)	Peak temperature (^{0}C)	Freezing point (^{0}C)	Freezable water (%)	ΔH_f (J/g)
WB	-7.86±0.10 [a]	-3.81±0.22 [a]	-4.90±0.19 [a]	38.50±2.01 [a]	128.59±6.12 [a]
CB	-7.41±0.21 [a]	-3.54±0.11 [a]	-4.94±0.11 [a]	39.02±1.21 [a]	130.33±5.37 [a]
SB	-7.35±0.11 [a]	-3.27±0.10 [a]	-4.50±0.13 [a]	39.00±1.20 [a]	130.26±1.11 [a]
SW1B	-8.10±0.23 [a]	-3.45±0.16 [a]	-5.80±0.18 [a]	39.18±1.00 [a]	130.86±5.23 [a]
SW2B	-7.74±0.15 [a]	-3.58±0.17 [a]	-5.00±0.08 [a]	37.15±1.05 [a]	124.08±4.07 [a]
SW3B	-7.38±0.23 [a]	-3.37±0.21 [a]	-4.98±0.14 [a]	36.00±2.11 [a]	120.24±2.18 [a]
SC1B	-7.35±0.18 [a]	-3.24±0.13 [a]	-4.78±0.07 [a]	37.91±1.09 [a]	126.62±3.18 [a]
SC2B	-7.71±0.29 [a]	-3.54±0.15 [a]	-4.90±0.09 [a]	38.63±1.01 [a]	129.02±5.03 [a]
SC3B	-7.85±0.10 [a]	-3.53±0.10 [a]	-5.01±0.09 [a]	38.75±1.07 [a]	129.42±4.27 [a]

[A]Calculated by subtracting freezing point and freezable water of fermented ice cream from freezing point and freezable water of fermented ice cream, dividing by freezing point and freezable water of fermented ice cream and multiplying by 100.
[a-g] Means in the same column followed by different letters were significantly different ($p<0.05$).
ΔH_f = Enthalpy of fusion.

4.3 Microbial assay

4.3.1 Colony forming unit of probiotics in non fermented ice cream during frozen storage

Colony forming unit of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* is as shown in Figure 4.16. In ice cream containing 100% soybean extract, the colony forming unit of *B. bifidum* and *L. acidophilus* in the mixture of ice cream was 7.86±0.03 and 7.86±0.15 Log10 cfu/mL, respectively before freezing (Table 4.20, Figure 4.1). *L. acidophilus* colony forming unit decreased to 7.85±0.01 Log10 cfu/mL and *B. bifidum* colony forming unit reached 7.80±0.04 Log10 cfu/mL after one day frozen storage (Figure 4.16). After 30 days of storage, *L. acidophilus* and *B. bifidum* colony forming units decreased to 7.85±0.01 and 7.77±0.04 Log10 cfu/mL, respectively. In ice cream containing 100% coconut milk, the *B. bifidum* and *L. acidophilus* colony forming units in the mixture of ice cream were 7.74±0.04 and 7.12±0.04 Log10 cfu/mL, respectively before freezing (Table 4.20, Figure 4.16). After one day of frozen storage, *L. acidophilus* and decreased to

6.95±0.01 Log10 cfu/mL and *B. bifidum* colony forming unit reached 7.52±0.04 Log10 cfu/mL. After 30 days of storage, *L. acidophilus* colony forming unit decreased to 6.87±0.06 Log10 cfu/mL whereas *B. bifidum* colony forming unit until 7.37±0.06 Log10 cfu/mL. In ice cream containing 100% cow milk, the *B. bifidum* and *L. acidophilus* colony forming units in the mixture of ice cream were 7.40±0.12 and 7.20±0.07Log10 cfu/mL, respectively before freezing (Table 4.20, Figure 4.16). After one day freezing, *L. acidophilus* and *B. bifidum* colony forming units reached 7.01±0.01 and 7.30±0.04 Log10 cfu/mL, respectively. After 30 days of frozen storage, *L. acidophilus* colony forming unit decreased to 6.85±0.02 Log10 cfu/mL and *B. bifidum* colony forming unit to 7.19±0.05 Log10 cfu/mL.

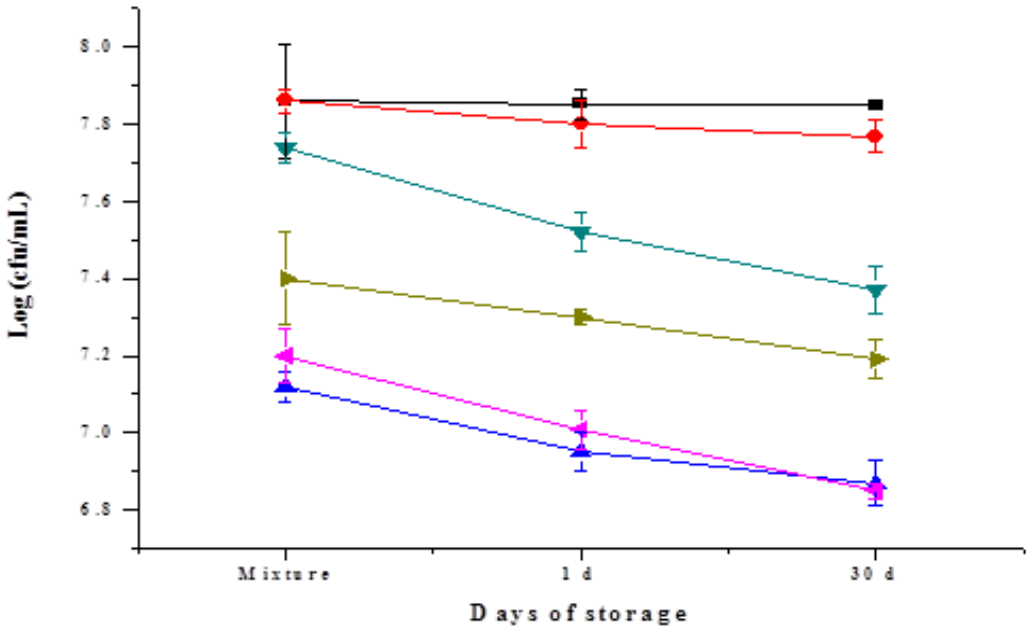


Figure 4.16 Viable colony forming unit of *L. acidophilus* and *B. bifidum* in ice-cream with different kind of milk during 30 days of frozen storage at -20 °C. Noted: ice creams inoculated with *L. acidophilus* made using cow milk (WL; ◄), soybean extract (SL; ■) or coconut milk (CL; ▲); ice creams inoculated with *B. bifidum* made using cow milk (WB; ►), soybean extract (SB; ●) or coconut milk (CB; ▼).

Table 4.20 Counts of probiotic bacteria in mixes and stored ice creams after 30 days.

Ice creams	Mixture (Log10 cfu/mL)	Ice cream 30 days (Log10 cfu/mL)	Survival (%)[A]
SL	7.86±0.15	7.85±0.01	99.87 [a]
SB	7.86±0.03	7.77±0.04	98.83 [a]
CL	7.12±0.04	6.87±0.06	96.48 [b]
CB	7.74±0.04	7.37±0.06	95.17 [b]
WL	7.20±0.07	6.85±0.02	95.08 [b]
WB	7.40±0.12	7.19±0.05	97.16 [b]

[A] Calculated by subtracting bacteria count in ice cream mixture before freezing from bacteria count in ice cream after 30 days of frozen storage, dividing by bacteria count in ice cream mixture before freezing and multiplying by 100.

[a-d] Values with different letters in the same column are significantly different ($p < 0.05$) (Tukey test).

4.3.2 The growth rate of probiotics in ice creams until pH reach to 5.50

Table 4.21 shows the time taken for reaching to pH = 5.50 (the time required for reaching to pH = 5.50 by probiotic fermentation activities) was longer for Bb-12 (SB = 11.13, WB = 18.10, CB = 10.50, SC1B = 10.50, SC2B = 10.50, SC3B = 10.50, SW1B = 11.30, SW2B = 13.56 and SW3B = 16.20 h) than those for La-05 (SL = 8.48, WL = 10.18, CL = 6.20, SC1L = 8.20, SC2L = 7.40, SC3L = 6.20, SW1L = 8.48, SW2L = 9.25 and SW3L = 9.25 h; $p<0.05$). The pH decreased faster in the ice creams containing vegetable extracts than those containing cows' milk. The acidification of ice creams containing coconut milk due to fermentation by La-05 was slower with increasing soybean extract amount ($p<0.05$). No significant difference were among composite milk ice creams containing coconut milk when the probiotic added was Bb-12 ($p>0.05$). The reduction in pH was faster in ice creams containing cow milk when higher soybean extract content was present during fermentation caused by both probiotics ($p<0.05$).

The growth rate of La-05 and Bb-12 due to fermentation until pH = 5.50 was increased with a higher soybean extract content in composite milk ice creams ($p<0.05$).

Their growth rate was better in ice creams containing coconut milk than those containing cow milk ($p<0.05$).

Table 4.21 The probiotic counts and the time required by probiotic bacteria in ice creams during to reduce the pH of ice cream mixes to 5.50.

Samples[A]	Probiotic counts before fermentation (A; Log10 cfu mL^{-1})[B]	Probiotic counts after fermentation (B; Log10 cfu mL^{-1}) [B]	Difference between probiotic counts before and after fermentation (A-B; Log10 cfu mL^{-1}) [C]	Time taken (h)[D]	Growth rate (Log10 cfu mL^{-1}/h) [E]
SL	7.11±0.08	8.40±0.05	1.29^{b}	8.48^{g}	0.15^{b}
WL	7.20±0.07	8.29±0.04	1.09^{d}	10.18^{e}	0.11^{e}
CL	7.16±0.04	8.30±0.06	1.14^{c}	6.20^{i}	0.18^{a}
SC1L	7.21±0.04	8.76±0.07	1.55^{a}	8.20^{g}	0.19^{a}
SC2L	7.19±0.06	8.18±0.09	0.99^{e}	7.40^{h}	0.13^{c}
SC3L	7.27±0.07	7.73±0.08	0.46^{i}	6.20^{k}	0.07^{g}
SW1L	7.07±0.05	8.33±0.09	1.26^{b}	8.48^{i}	0.15^{b}
SW2L	7.12±0.03	8.04±0.08	0.92^{e}	9.25^{f}	0.10^{e}
SW3L	7.26±0.08	8.13±0.07	0.87^{f}	9.25^{f}	0.09^{f}
SB	7.53±0.09	8.76±0.09	1.23^{b}	11.13^{d}	0.11^{e}
WB	7.21±0.08	8.05±0.10	0.84^{g}	18.10^{a}	0.05^{i}
CB	7.45±0.09	8.70±0.09	1.25^{b}	10.50^{e}	0.12^{d}
SC1B	7.50±0.05	8.57±0.06	1.07^{d}	10.50^{e}	0.10^{e}
SC2B	7.50±0.02	8.57±0.08	1.07^{d}	10.50^{e}	0.10^{e}
SC3B	7.47±0.04	8.59±0.05	1.12^{c}	10.50^{e}	0.11^{e}
SW1B	7.52±0.06	8.32±0.08	0.80^{g}	11.30^{d}	0.07^{g}
SW2B	7.40±0.09	8.19±0.09	0.79^{g}	13.56^{c}	0.06^{h}
SW3B	7.46±0.03	8.05±0.04	0.59^{h}	16.20^{b}	0.04^{j}

[A] Samples inoculated with La-05 and made with 100% cow milk: WL; 100% coconut milk: CL; 100% soybean extract: SL; 75% soybean extract+25% cow milk: SW1L; 50% soybean extract+50% cow milk: SW2L; 25% soybean extrac+75% cow milk: SW3L; 75% soybean extract+25% coconut milk: SC1L; 50% soybean extract+50% coconut milk: SC2L; 25% soybean extract+75% coconut milk: SC3L. Samples inoculated with Bb-12 made using 100% cow milk: WB; 100% coconut milk: CB; 100% soybean extract: SB; 75% soybean extract+25% cow milk: SW1B; 50% soybean extract+50% cow milk: SW2B; 25% soybean extract+75% cow milk: SW3B; 75% soybean extract+25% coconut milk: SC1B; 50% soybean extract+50% coconut milk: SC2B; 25% soybean extract+75% coconut milk: SC3B.

[B] means values±standard deviation.

[C] The differences between the numbers of probiotics before and after fermentation in ice creams.

[D] The time required for reaching pH 5.50.

[E] Growth rate = The differences between the numbers of probiotics before and after fermentation in ice creams divided by the time required for pH reduction to 5.50.

[a-j] Means in the same column followed by different letters were significantly different ($p< 0.05$).

4.3.3 Colony forming unit of probiotics in fermented ice cream during frozen storage

4.3.3.1 Colony forming unit of *Lactobacillus acidophilus*

The *L. acidophilus* colony forming unit in the mixture (before fermentation stage) of SL, SC1L, SC2L, SC3L, CL, SW1L, SW2L, SW3L and WL ice creams were 7.10±0.08, 7.21±0.09, 7.09±0.09, 7.27±0.07, 7.16±0.06, 7.07±0.07, 7.02±0.07, 6.96±0.05 and 6.99±0.08 Log10 cfu/g, respectively (Figure 4.17). The colony forming unit increased to 8.78±0.08, 8.61±0.07, 8.02±0.03, 8.42±0.04, 8.07±0.04, 8.55±0.03, 8.84±0.06, 8.20±0.07 and 8.08±0.04 Log10 cfu/g for SL, SC1L, SC2L, SC3L, CL, SW1L, SW2L, SW3L and WL ice creams, respectively after fermentation by La-05. However, the colony forming unit decreased to 6.89±0.06, 7.77±0.09, 6.88±0.07, 8.15±0.08, 7.40±0.08, 7.48±0.06, 7.21±0.07, 7.04±0.08 and 6.87±0.07 Log10 cfu/g for SL, SC1L, SC2L, SC3L, CL, SW1L, SW2L, SW3L and WL ice creams, respectively after one day freezing. After 30 days of freezing *L. acidophilus* colony forming unit of SL, SC1L, SC2L, SC3L, CL, SW1L, SW2L, SW3L and WL ice creams reduced further to 6.34±0.11, 6.77±0.04, 5.98±0.06, 7.25±0.07, 6.90±0.09, 6.44±0.09, 6.55±0.02, 6.31±0.05 and 6.49±0.03 Log10 cfu/g, respectively. *L. acidophilus* colony forming unit of SL, SC1L, SC2L, SC3L, CL, SW1L, SW2L, SW3L and WL ice creams were found to stabilized at 60 (6.26±0.04, 6.17±0.07, 5.54±0.10, 6.99±0.13, 6.89±0.06, 6.16±0.04, 6.09±0.07, 6.28±0.06 and 6.14±0.04 Log10 cfu/g, respectively) and 90 (6.16±0.04, 6.01±0.07, 5.42±0.05, 5.44±0.05, 5.18±0.08, 5.82±0.07, 5.79±0.07, 5.15±0.07 and 4.85±0.08 Log10 cfu/g, respectively) days of freezing at -20 ^{0}C (Figure 4.17). Table 4.22 shows the survival percentage of La-05 in all ice creams containing vegetable extracts was higher than ice cream containing 100% cow milk (control) after 90 days of storage at -20 °C.

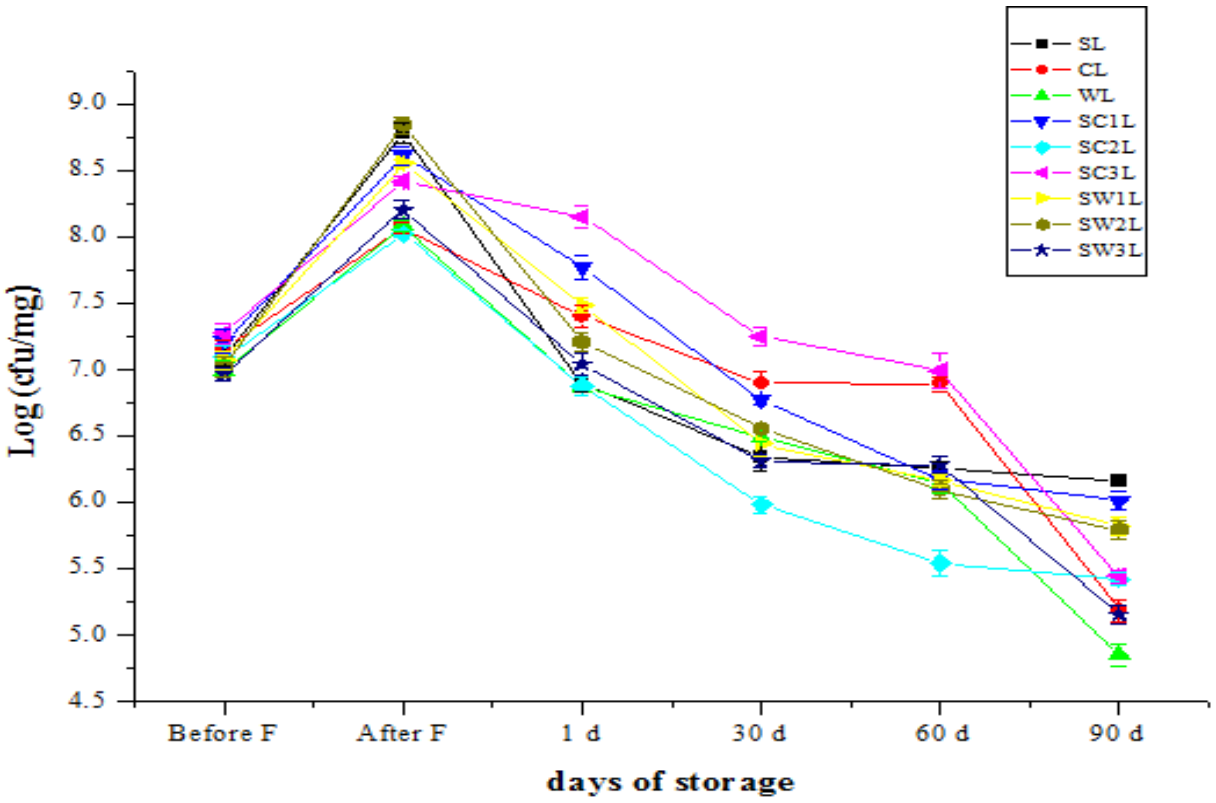


Figure 4.17 Viable colony forming unit of La-05 in ice creams with different kind of milk during 90 days of storage at -20 °C. Noted: Before F = the number of La-05 in ice cream mixture before fermentation stage; After F = the number of La-05 in ice cream mixture after fermentation stage.

Table 4.22 Survival of La-05 after 90 days of storage at -20 °C.

Samples	Mixture after fermented (Log10 cfu g^{-1})[A]	Ice cream after 90 days (Log10 cfu g^{-1})[A]	Survival (%)[B]
SL	8.78±0.08	6.16±0.04	70.20 [a]
CL	8.07±0.04	5.18±0.08	64.23[e]
WL	8.08±0.04	4.85±0.08	60.04[g]
SC1L	8.61±0.07	6.01±0.07	69.76[a]
SC2L	8.02±0.03	5.42±0.05	67.63[c]
SC3L	8.42±0.04	5.44±0.05	64.62[e]
SW1L	8.55±0.03	5.82±0.11	68.10[b]
SW2L	8.84±0.06	5.79±0.09	65.53[d]
SW3L	8.20±0.07	5.15±0.08	62.78[f]

[A] means values±standard deviation.
[B] Calculated by subtracting bacteria count in ice cream mixture after fermentation from bacteria count in fermented ice cream after 90 days of frozen storage, dividing by bacteria count in ice cream mixture after fermentation and multiplying by 100.
[a-c] Means in the same column followed by different letters were significantly different($p<0.05$).

4.3.3.2 Colony forming unit of *Bifidobacterium bifidum*

The *B. bifidum* colony forming unit in the mixture (before fermentation stage) of SB, CB, WB, SC1B, SC2B, SC3B, SW1B, SW2B and SW3B ice creams were 7.53±0.06, 7.45±0.32, 7.21±0.07, 7.50±0.09, 7.50±0.10, 7.47±0.04, 7.40±0.08, 7.52±0.07 and 7.46±0.09 Log10 cfu/g, respectively (Figure 4.18). The *B. bifidum* colony forming unit of SB, CB, WB, SC1B, SC2B, SC3B, SW1B, SW2B and SW3B ice creams increased to 8.21±0.05, 8.42±0.07, 8.06±0.09, 8.51±0.08, 8.56±0.10, 8.59±0.07, 8.19±0.05, 8.46±0.02 and 8.04±0.07 Log10 cfu/g after fermentation by Bb-12 (Figure 4.18). The *B. bifidum* colony forming unit of SB, CB, WB, SC1B, SC2B, SC3B, SW1B, SW2B and SW3B ice creams decreased to 7.93±0.07, 7.72±0.08, 6.65±0.06, 7.88±0.10, 7.73±0.09, 7.79±0.05, 6.51±0.04, 7.36±0.03 and 6.78±0.09 Log10 cfu/g, respectively after one day freezing. After 30 days of freezing *B. bifidum* colony forming unit of SB, CB, WB, SC1B, SC2B, SC3B, SW1B, SW2B and SW3B ice creams decreased to 7.42±0.09, 7.45±0.05, 5.87±0.03, 7.72±0.02, 7.33±0.04, 7.72±0.08, 6.43±0.07, 7.18±0.10 and 6.43±0.01 Log10 cfu/g, respectively. After 60 days of freezing *B. bifidum* colony forming unit of SB, CB, WB, SC1B, SC2B, SC3B, SW1B, SW2B and SW3B ice creams reduced further to 7.03±0.07, 7.35±0.04, 5.50±0.03, 7.60±0.10, 7.12±0.04, 7.65±0.02, 6.42±0.11, 6.56±0.08 and 5.41±0.07 Log10 cfu/g, respectively (Figure 4.18). *B. bifidum* colony forming unit of SB, CB, WB, SC1B, SC2B, SC3B, SW1B, SW2B and SW3B ice creams decreased to 7.00±0.07, 7.27±0.10, 5.18±0.07, 7.55±0.10, 7.05±0.07, 6.99±0.06, 6.35±0.04, 5.92±0.02 and 5.24±0.07 Log10 cfu/g after 90 days of freezing (Figure 4.18). Table 4.23 shows the survival percentage of Bb-12 in all ice creams containing vegetable extracts was higher than ice cream containing 100% cow milk (control) after 90 days of storage at -20 °C.

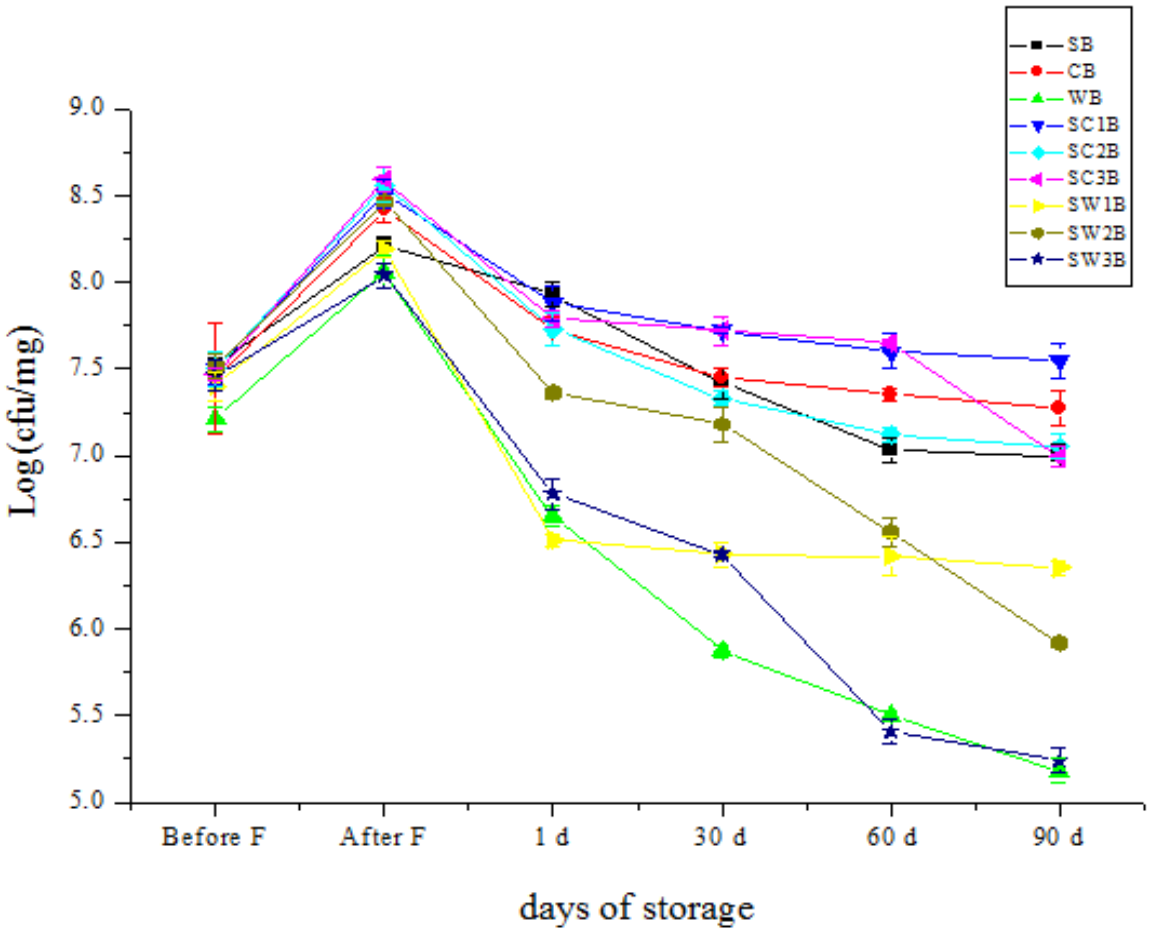


Figure 4.18 Viable colony forming unit of Bb-12 in ice creams with different kind of milk during 90 days of storage at -20 °C. Noted: Before F = the number of Bb-12 in ice cream mixture before fermentation stage; After F = the number of Bb-12 in ice cream mixture after fermentation stage.

Table 4.23 Survival of Bb-12 after 90 days of storage at -20 °C.

Samples	Mixture after fermented (Log10 cfu g^{-1})[A]	Ice cream after 90 days (Log10 cfu g^{-1})[A]	Survival (%)[B]
SB	8.21±0.05	7.00±0.07	85.30[b]
CB	8.42±0.07	7.27±0.10	86.36[b]
WB	8.06±0.09	5.18±0.07	64.24[f]
SC1B	8.51±0.08	7.55±0.10	88.73[a]
SC2B	8.56±0.10	7.05±0.07	82.40[c]
SC3B	8.59±0.07	6.99±0.06	81.47[c]
SW1B	8.19±0.05	6.35±0.04	77.58[d]
SW2B	8.46±0.02	5.92±0.02	70.00[e]
SW3B	8.04±0.07	5.24±0.07	65.15[g]

[A]means values±standard deviation.
[B] Calculated by subtracting bacteria count in ice cream mixture after fermentation from bacteria count in fermented ice cream after 90 days of frozen storage, dividing by bacteria count in ice cream mixture after fermentation and multiplying by 100.
[a-e] Means in the same column followed by different letters were significantly different($p<0.05$).

4.3.4 Colony forming unit of probiotics after *in vitro* gastrointestinal digestion

4.3.4.1 Colony forming unit of probiotics after SGD

The viability of La-05 and Bb-12 during 120 min of exposure to simulated gastric juice at pH 2.0 is as shown in Table 4.24. The colony forming unit of both probiotics after 1 min exposure to gastric juices in all ice creams decreased. Table 4.24 shows probaiotic tolerance to simulated gastric juice in ice creams increased with the addition of soybean extract.

Table 4.24 Effect of ice creams with different milks on the survival of probiotics during 120 min exposure to simulated gastric juice at pH = 2.0 (n = 3).

Probiotic	Sample	Viable colony forming unit (log cfu/g) during simulated gastric transit tolerance				Survival of bacteria after 120 min (%)[A]
		0 min	1min	30 min	120 min	
L. acidophilus (La-5)	SL	7.51±0.05^{d}	7.46±0.04^{d}	7.49±0.07^{c}	7.31±0.03^{*c}	97.34^{a}
	CL	7.77±0.04^{b}	7.71±0.02^{b}	7.64±0.05^{*b}	7.27±0.02^{*c}	93.56^{b}
	WL	7.97±0.04^{a}	7.88±0.02^{*a}	7.89±0.04^{*a}	6.70±0.07^{*d}	84.06^{c}
	SW1L	7.61±0.06^{c}	7.56±0.04^{c}	7.50±0.04^{*c}	7.49±0.05^{*b}	98.42^{a}
	SW2L	7.75±0.07^{b}	7.74±0.06^{b}	7.68±0.03^{b}	7.56±0.04^{*a}	97.55^{a}
	SW3L	7.33±0.03^{e}	7.16±0.07^{*e}	6.75±0.02^{*e}	5.44±0.02^{*h}	74.21^{d}
	SC1L	7.28±0.02^{e}	6.70±0.07^{*f}	6.66±0.09^{*e}	6.05±0.08^{*e}	83.10^{c}
	SC2L	7.27±0.02^{e}	6.26±0.02^{*g}	6.46±0.07^{*f}	5.75±0.09^{*f}	74.48^{d}
	SC3L	7.63±0.05^{c}	5.,59±0.01^{*h}	5.48±0.03^{*g}	5.56±0.03^{*g}	72.87^{d}
B. bifidum (Bb-12)	SB	7.40±0.08^{d}	7.30±0.07^{d}	7.27±0.04^{*c}	7.26±0.04^{*c}	98.11^{a}
	CB	7.82±0.08^{a}	7.51±0.06^{*c}	7.46±0.05^{*b}	7.44±0.02^{*b}	95.14^{c}
	WB	7.27±0.06^{d}	7.01±0.06^{*e}	6.95±0.05^{*a}	6.93±0.02^{*d}	95.32^{c}
	SW1B	7.07±0.03^{e}	7.01±0.04^{a}	7.00±0.07^{d}	6.95±0.09^{d}	98.30^{a}
	SW2B	7.38±0.04^{d}	7.30±0.03^{*e}	7.27±0.04^{*c}	7.25±0.06^{*c}	98.24^{a}
	SW3B	7.57±0.05^{c}	7.31±0.03^{*e}	7.19±0.09^{*c}	7.16±0.08^{*c}	94.58^{cd}
	SC1B	7.93±0.07^{a}	7.87±0.04^{a}	7.83±0.07^{a}	7.76±0.02^{*a}	97.86^{b}
	SC2B	7.70±0.04^{b}	7.57±0.07^{a}	7.47±0.03^{*b}	7.42±0.01^{*b}	96.36^{b}
	SC3B	7.96±0.04^{a}	7.64±0.04^{*b}	7.52±0.05^{*b}	7.39±0.05^{*b}	92.84^{d}

[A] Calculated by subtracting bacteria count at 0 min from bacteria count at 120 min, dividing by bacteria count at 0 min and multiplying by 100.

[*] In the same row indicates a significant difference of mean viable colony forming unit compared to that at 0 min ($p<0.05$).

[a-h] Values in the same column having different superscripts for mean viable colony forming unit for each probiotic differ significantly ($p<0.05$).

4.3.4.2 Colony forming unit of probiotics after SIJ

The viability of La-05 and Bb-12 during 120 min of exposure to simulated small intestinal juice at pH 8.0 is as shown in Table 4.25. The colony forming unit of both probiotics after 1 min exposure to gastric juices in all ice creams decreased. Table 4.25 shows probaiotic tolerance to simulated small intestinal juice in ice creams increased with the addition of soybean extract.

Table 4.25 Effect of ice creams with different milks on the survival of probiotics during 120 min exposure to simulated small intestinal juice pH = 8 (n = 3).

Probiotic	Sample [A]	Viable colony forming unit (log cfu/g)				Survival of bacteria after 120 min (%) [A]
		0 min	1min	60 min	120min	
L. acidophilus (La-5)	SL	7.45±0.02^{d}	5.97±0.05^{*b}	5.60±0.02^{*a}	5.23±0.04^{*a}	70.20^{a}
	CL	7.10±0.04^{f}	4.24±0.03^{*d}	3.93±0.02^{*e}	3.90±0.06^{*e}	54.93^{bc}
	WL	7.46±0.07^{d}	6.22±0.03^{*a}	5.64±0.04^{*a}	5.03±0.06^{*b}	67.43^{a}
	SW1L	7.21±0.04^{e}	6.14±0.08^{*a}	5.03±0.08^{*c}	4.18±0.07^{*d}	58.00^{b}
	SW2L	7.60±0.05^{c}	6.18±0.09^{*a}	4.93±0.07^{*c}	4.03±0.07^{*e}	53.03^{c}
	SW3L	7.93±0.02^{a}	5.92±0.09^{*b}	5.06±0.04^{*c}	4.13±0.09^{*d}	52.08^{cd}
	SC1L	7.70±0.01^{b}	6.01±0.06^{*b}	5.60±0.03^{*a}	4.40±0.08^{*c}	57.14^{b}
	SC2L	7.65±0.05^{b}	5.75±0.06^{*c}	4.39±0.03^{*d}	3.75±0.06^{*f}	49.02^{d}
	SC3L	7.68±0.03^{b}	5.43±0.03^{*c}	4.28±0.04^{*d}	3.15±0.06^{*g}	41.01^{e}
B. bifidum (Bb-12)	SB	7.61±0.08^{b}	7.16±0.04^{*a}	6.67±0.09^{*a}	6.35±0.03^{*a}	83.44^{ab}
	CB	7.83±0.08^{a}	6.30±0.04^{*d}	5.77±0.08^{*e}	5.54±0.02^{*d}	70.75^{f}
	WB	7.40±0.03^{d}	6.91±0.05^{*b}	6.67±0.03^{*a}	6.16±0.04^{*b}	83.24^{ab}
	SW1B	7.20±0.01^{f}	6.60±0.07^{*c}	6.32±0.01^{*b}	6.18±0.03^{*b}	85.83^{a}
	SW2B	7.80±0.07^{a}	6.36±0.06^{*d}	6.28±0.04^{*b}	6.18±0.02^{*b}	79.23^{cd}
	SW3B	7.03±0.05^{g}	6.53±0.03^{*c}	6.19±0.02^{*c}	5.36±0.04^{*f}	76.24^{de}
	SC1B	7.50±0.06^{c}	7.00±0.04^{*b}	6.65±0.02^{*a}	6.08±0.03^{*c}	81.06^{bc}
	SC2B	7.28±0.06^{e}	6.55±0.08^{*c}	5.95±0.03^{*d}	5.46±0.02^{*e}	75.00^{e}
	SC3B	7.68±0.03^{b}	6.02±0.08^{*e}	5.45±0.04^{*f}	4.80±0.05^{*g}	62.50^{g}

[A] Calculated by subtracting bacteria count at 0 min from bacteria count at 120 min, dividing by bacteria count at 0 min and multiplying by 100.
[*] In the same row indicates a significant difference of mean viable colony forming unit compared to that at 0 min ($p<0.05$).
[a-h] Values in the same column having different superscripts for mean viable colony forming unit for each probiotic differ significantly ($p<0.05$).
Means values±standard deviation.

4.4 Sensory analysis

In fermented ice creams, no significant effects ($p>0.05$) were observed between samples fermented with either La-05 or Bb-12 (Figure 4.19). In general in both non fermented and fermented ice creams, the colour score decreased with increasing soybean extract and close to dull colour (Tables 4.26, 4.27 and 4.28). The ice creams containing cow milk had a higher colour score than ice creams containing coconut milk. The texture score showed little differences among ice creams. There were no significant differences ($p>0.05$) in sweetness and cooked flavour. However, the flavour and taste score decreased with increasing soybean extract with the lowest flavour and taste score being seen in SB ice cream. Ice creams containing cow milk had a higher flavour and aroma than ice creams containing coconut milk. In general the highest of total acceptability was seen in ice creams containing 100% cow milk and lowest in ice creams containing soybean extract. The total acceptability was higher in ice creams containing cow than in those containing coconut milk and it decreased with increasing soybean extract amount in ice creams (Tables 4.26, 4.27 and 4.28).

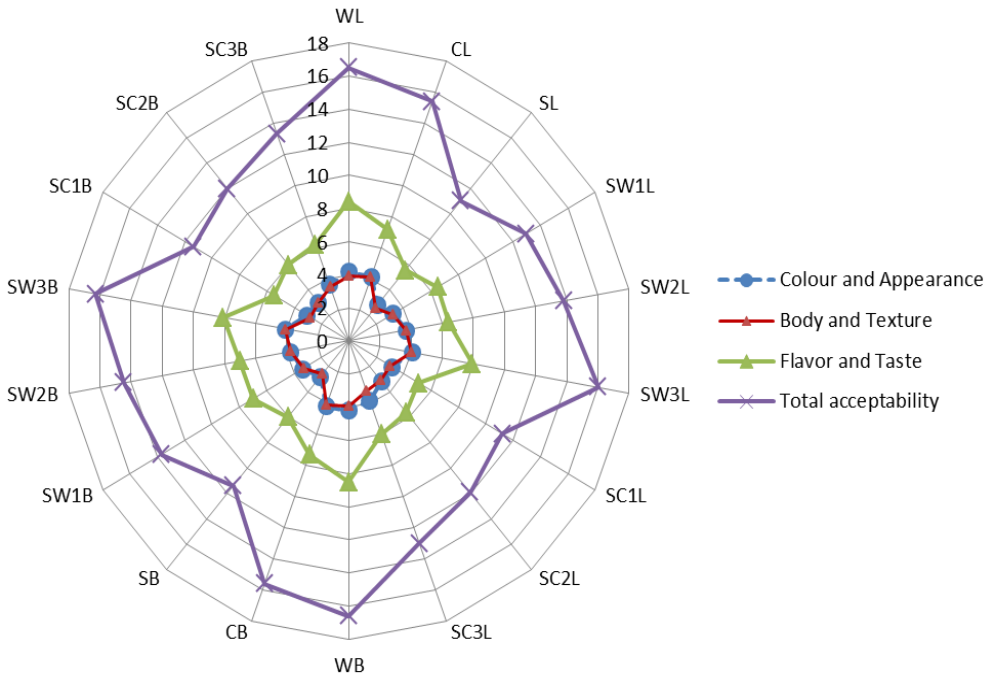


Figure 4.19 Changes in sensory evaluation of fermented ice cream by replacement of cow's milk with vegetable extracts ($p<0.05$).

Table 4.26 Organoleptic property scores of non fermented ice creams with different milks[A]

Samples	Colour and Appearance (1-5)	Body and Texture (1-5)	Flavour and Taste (1-10)	Total (1-20)
W	4.18 ± 0.05^{ab}	4.14 ± 0.04^{a}	7.94 ± 0.05^{ab}	16.26 ± 0.05^{a}
C	3.45 ± 0.05^{c}	3.61 ± 0.05^{b}	6.53 ± 0.06^{dc}	13.59 ± 0.03^{c}
S	3.22 ± 0.04^{dc}	3.00 ± 0.06^{bc}	5.10 ± 0.03^{e}	11.32 ± 0.02^{d}
SW1	3.82 ± 0.04^{b}	3.70 ± 0.04^{a}	6.72 ± 0.04^{a}	14.24 ± 0.05^{bc}
SW2	4.15 ± 0.06^{a}	3.91 ± 0.02^{a}	7.19 ± 0.02^{bc}	15.25 ± 0.06^{ab}
SW3	4.20 ± 0.07^{a}	4.07 ± 0.06^{a}	8.12 ± 0.05^{a}	16.39 ± 0.04^{a}
SC1	2.93 ± 0.07^{d}	2.62 ± 0.06^{c}	5.68 ± 0.04^{de}	11.23 ± 0.07^{d}
SC2	3.07 ± 0.06^{dc}	2.85 ± 0.04^{bc}	5.94 ± 0.05^{e}	11.86 ± 0.05^{d}
SC3	3.24 ± 0.05^{dc}	2.79 ± 0.04^{bc}	6.11 ± 0.04^{de}	12.14 ± 0.05^{dc}

[A] Mean values from 42 panelists.

[a-e] Means in the same column followed by different letters were significantly different ($p<0.05$).

Table 4.27 Organoleptic property scores of fermented ice creams with different milks and inoculated with La-05[A]

Samples[B]	Colour and Appearance (1-5)	Body and Texture (1-5)	Flavour and Taste (1-10)	Total (1-20)
WL	4.10±0.04^{ab}	4.03±0.05^{a}	5.30±0.05^{ab}	13.43±0.07^{a}
CL	3.30±0.05^{c}	3.16±0.04^{b}	4.79±0.08^{dc}	11.25±0.06^{c}
SL	3.18±0.05^{dc}	3.01±0.05^{bc}	3.37±0.04^{e}	9.56±0.07^{d}
SW1L	3.90±0.06^{b}	3.22±0.05^{a}	4.40±0.06^{a}	11.52±0.07^{bc}
SW2L	4.10±0.06^{a}	3.78±0.04^{a}	5.80±0.04^{bc}	13.68±0.07^{ab}
SW3L	4.14±0.07^{a}	4.17±0.06^{a}	6.41±0.05^{a}	14.72±0.09^{a}
SC1L	3.01±0.07^{d}	2.21±0.07^{c}	3.33±0.05^{de}	8.55±0.09^{d}
SC2L	3.10±0.06^{dc}	2.49±0.05^{bc}	4.10±0.07^{e}	9.69±0.07^{d}
SC3L	3.12±0.05^{dc}	2.61±0.06^{bc}	4.94±0.06^{de}	10.67±0.07^{dc}

[A] Mean values from 42 panelists.
[a-b] Values with different letters in the same column are significantly different ($p<0.05$) (Tukey test).

Table 4.28 Organoleptic property scores of fermented ice creams with different milks and inoculated with Bb-12[A]

Samples[B]	Colour and Appearance (1-5)	Body and Texture (1-5)	Flavour and Taste (1-10)	Total (1-20)
WB	4.12±0.05^{ab}	4.02±0.04^{a}	5.32±0.06^{ab}	13.46±0.04^{a}
CB	3.35±0.05^{c}	3.17±0.05^{b}	4.80±0.05^{dc}	11.32±0.04^{c}
SB	3.20±0.04^{dc}	3.00±0.05^{bc}	3.38±0.03^{e}	9.58±0.03^{d}
SW1B	3.91±0.05^{b}	3.20±0.04^{a}	4.41±0.04^{a}	11.52±0.06^{bc}
SW2B	4.12±0.07^{a}	3.80±0.03^{a}	5.82±0.03^{bc}	13.74±0.05^{ab}
SW3B	4.15±0.06^{a}	4.18±0.06^{a}	6.43±0.05^{a}	14.76±0.04^{a}
SC1B	3.00±0.07^{d}	2.20±0.06^{c}	3.34±0.04^{de}	8.54±0.06^{d}
SC2B	3.10±0.06^{dc}	2.50±0.05^{bc}	4.20±0.05^{e}	9.80±0.05^{d}
SC3B	3.11±0.04^{dc}	2.60±0.05^{bc}	4.95±0.04^{de}	10.66±0.05^{dc}

[A] Mean values from 42 panelists.
[a-d] Values with different letters in the same column are significantly different ($p<0.05$) (Tukey test).

CHAPTER 5 DISCUSSION

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5.1 Composition and chemical properties

The pH (just in fermented ice creams), total solid, fat and titratable acidity (TA) of non fermented and fermented ice creams were unchanged by replacement of cow milk with vegetables extracts. There is not any significant difference between non fermented ice creams containing 100% soybean extract, coconut and cow milk for protein (S = 2.47±0.03, C = 2.32±0.04 and W = 3.55±0.06 g $100g^{-1}$). However, pH in non fermented ice creams changed with milk replacement. In non fermented ice creams, the pH was found to be the highest in ice creams with C and SC3 ice creams and the lowest in W ice cream.

5.2 Physical properties

5.2.1 Melting rate of ice creams

All non fermented and fermented ice creams showed different melting behavior as a function of milk replacement. While the content of butter used to balance to fat (ice cream fat = 10.52% w/w) were less in coconut ice cream (butter used = 7.31 g) in contrast with ice creams containing cow and ice cream with 100% soybean extract (butter used = 10.37 g; Table 3.2), this is regarded to have minor effect on melting behavior. Hyvoen *et al.* (2003) reported that fat amount have effect on melting rate of ice creams and types of fat (dairy and vegetable fats) did not affect on their melting resistant. All vegetables and composite milk ice creams (16.27-33.36% w/w in non fermented ice creams; 0-30.78% w/w in fermented ice creams) showed a slower melting rate than W ice cream (35.88% w/w in non fermented ice cream; 35.51 and 30.51% w/w in fermented ice creams with Bb-12 and La-05, respectively) (Table 4.11). The melting resistance increased with increasing

soybean extract amount in ice creams containing composite milk in both non fermented and fermented ice creams. This presumably can be explained by the fact that soybean extract proteins is more hydrated and therefore prevent their free movement of water molecules associated with proteins (Akesowan, 2009) which lead to reduced syneresis and increased viscosity (Tables 4.12, 4.13 and 4.14). The relationship between the increase in viscosity and increase in the resistance of ice cream to melting rate was also reported by Kaya and Tekin (2001), Akesowan (2009) and Hermanto and Masdiana (2011). In addition soy lecithin protects the membrane proteins against damage because of freezing by its emulsifying properties (Aboulfazli *et al*., 2014) and assists good air distribution and fat structure in the ice cream can also affect the increase time to melt the ice cream (Hermanto and Masdiana, 2011).

Ice creams containing coconut milk had a lower melting rate than those containing cow milk in both non fermented and fermented ice creams and also the melting rate in ice creams made with Bb-12 was higher than ice cream made with La-05 (Table 4.11). The differences in viscosity and freezing points of ice creams can influence on melting resistance (Aboulfazli *et al*., 2014). However, in the present study no differences ($p>0.05$) were observed in the freezing points amongst non fermented and fermented composite milk ice creams (Tables 4.17, 4.18 and 4.19) and also in freezable water amount amongst fermented ice creams (Tables 4.18 and 4.19). Noticeable differences in freezable water and the enthalpy of fusion of non fermented ice creams (Table 4.17) may be attributed to proteins and their differential hydration tendency (Alvarez *et al.,* 2005) which affect serum concentration and freezable water in the ice creams, hence their fusion enthalpies. Ice crystallisation is strongly dependent on the extent of freezing point and the percentage of bound water (unfrozen water) (Soukoulis *et al.,* 2009).

Whey protein and casein isolates have a higher amount of aspartic and glutamic acids (negative charge) than coconut protein, as well as a higher proportion of lysine and arginine (positive charge). The value of zeta potential is higher in whey protein than in coconut protein whereas the surface activity was shown to be higher in whey protein than in coconut protein (Onsaard *et al*., 2006). The coconut proteins are generally known for having poor solubility in water (Tangsuphoom, 2008), which may explain its contribution to the increase in the percentage of unbound water (freezable water) in ice creams. Therefore, the freezable water amount in the present studies may not be the main factor responsible for the reduction in melting rate of ice creams containing composite milk, because melting rate increased with increasing freezable water (Hwang *et al*., 2009).

Another effective factor on the variation in the melting rate of ice creams is their differences in apparent viscosity. Fermented ice creams incubated with La-05 and also non fermented and fermented ice creams containing coconut milk had higher melting rate because they had higher apparent viscosity than those ice creams and as a result a lower melting rate (Kaya and Tekin, 2001). On the other hand, the present studies showed the major contribution to the difference in melting rate can be attributed to the differences in ice cream apparent viscosity. For instance, ice creams containing higher amount of soybean extract despite having the lowest melting rate, had the highest apparent viscosity (see Tables 4.12, 4.13 and 4.14). During the fermentation, the production of lactic acid by bacteria resulted in a drop in pH and subsequently coagulation of proteins to form gel. The gelation processes retain all water present in the milk as a result of a peculiar microstructure of the protein network resembling a sponge with very small pores. This increases the viscosity and subsequently decreaced the ice cream melting rate (Table 4.11; Farnworth *et al*., 2007).

5.2.2 Rheological measurement

The data on the apparent viscosity, consistency index and flow behaviour index of the non fermented and fermented ice creams (Tables 4.12, 4.13 and 4.14) decreased with an increase in the shear rate, as illustrated by the non-Newtonian fluid behavior (Figures 4.1, 4.2 and 4.3). This decrease in viscosity of ice creams is partly because of the aggregation of fat globules, which decrease in size during shearing. Pinto *et al*. (2012) also noted the increase in shear rate decrease the apparent viscosity of frozen yogurt which is a common factor of milk products.

In non fermented ice creams, W and C (289 and 363 mPa s, respectively which are ice creams without soybean extract) melted ice creams had lower apparent viscosity than those containing soybean extract (Table 4.12). The highest apparent viscosity was in S ice cream (1120 mPa s), followed by SC1 and SW1 (982 and 818mPa s, respectively; Table 4.12). This could be explained by soy protein properties which are able to provide several functionalities such as water holding and emulsifying properties (Akesowan, 2009). Hence soy proteins form a stable network like a gel structure which create greater resistance to flow (Batista *et al*., 2005). This is in agreement to previous studies which showed grape wine less (Hwang *et al*., 2009) and inulin (Pinto *et al*., 2012) water retention effects and subsequent increase apparent viscosity of ice cream. Melted ice creams containing cow milk had a lower apparent viscosity than ice creams containing coconut milk. This could be due to the higher particle size of ice creams containing coconut milk because coconut proteins have poor emulsifying properties (Tangsuphoom and Coupland, 2009). Alvarez *et al*. (2005) found the addition of milk protein concentrates in ice cream increase their viscosity according to the Eilers equation because of the increased voluminosity of the dispersed particles.

In fermented ice creams, apparent viscosity was lower in ice cream made with Bb-12 than those made with La-05. This can be explained by the higher particle size in ice creams fermented by La-05 (Tables 4.13 and 4.14; Alvarez *et al*., 2005). According to Mathias *et al* (2011), the type of starter culture (capsular or ropy exopolysaccharide-producing or not) also affects the rheological behavior of fermented products such as yogurt and cheese. The texture of fermented products is strongly dependent on milk supplementations. The firmness of yogurt is highly dependent on total solids content, on the protein content of the product, and on the type of protein (Oliveira *et al.*, 2001). WL, WB, CL and CB samples had a lower apparent viscosity than ice creams containing soybean extract, and also the apparent viscosity increased with increasing soybean extract content. Thus this is possible that the mechanism of gel formation in these milks under fermentation is responsible to the viscosity changes. The pH plays a major role in the gelation of vegetable extract and cow proteins due to their isoelectric point of proteins. For example, the mechanism of the formation of the gel network in soybean extract and soy protein solutions is similar (Aboulfazli *et al*., 2014). The around pH 6, soy protein particles can approach each other and induce soy protein aggregation and form gel networks because the overall charge of soy proteins is lowest in this pH. Gels at pH below 6 are stiffer than gels above pH 6 due to the increased incorporation of proteins in the gel network. On the other hand, cow milk proteins generate maximum gel strength at pH 4.6 because the isoelectric point of caseins is below pH 4.6 (Grygorczyk, 2012). It is possible that the mechanism of the gel network formation in coconut milk is similar to that in cow milk because the isoelectric point (pI) of the coconut protein is around pH 4.3 (Aboulfazli *et al*., 2014). Hence, soybean extract can form stiffer gel networks than cow and coconut milk at pH 5.50, which creates greater resistance to the flow in soybean extract gels than in other milk gels. In addition, the apparent viscosity increases with the increasing soybean extract

content in ice creams (Tables 4.13 and 4.14). In a system containing a combination of proteins (from cow and soybean extract) aggregation was reported to occur earlier in the mixture (~pH 5.8) than in skim milk alone (~pH 5.3) due to the instability of soy proteins around pH 6.0 (Grygorczyk, 2012). Hence at pH 5.50, cow and coconut milks can form a stable gel when they combine with soybean extract. Confocal microscopy studies revealed that gelled soybean extract mixes have less branching and a more particulate structure than pure milk samples (Grygorczyk, 2012). Apparent viscosity in fermented ice creams containing coconut milk was higher than those containing cow milk, which it was similar to nonfermented ice creams.

The *K* (consistency index) varied from 0.87 to 4.81 Pa s^{-1} for non fermented ice creams and from 0.25 to 43.12 Pa s^{-1} for fermented ice creams (Table 4.12). SC1, S and SW1 ice creams amongst non fermented ice creams and SL, SB, SC1L and SC1B ice creams amongst fermented ice creams had the highest consistency indexes (Tables 4.13 and 4.14; $p<0.05$). The highest *K* values were in ice creams containing soybean extract and also increased with increasing soybean extract content due to the formation of gel by the aggregation of soy proteins which was caused to increase in water retention and the resistance to structural breakdown (Tables 4.12, 4.13 and 4.14; Aboulfazli *et al*., 2014).

The flow behavior index (*n*; the degree of pseudoplasticity of a fluid) ranged from 0.47 to 0.68 for non fermented ice creams and from 0.11 to 0.96 ($n = 1$) for fermented ice creams, the highest *n* values were higher in ice creams containing Bb-12 and decreased in ice creams containing higher soybean extract amount, but the differences were not significant (Tables 4.12, 4.13 and 4.14; $p>0.05$). Tables 4.12, 4.13 and 4.14 show the *n* values were higher in the downward curve than in the upward curve, which indicated a decrease in the pseudoplastic properties as the shear rate decreased. The increase in *n* and

decrease in *K* can be ascribed to the structural rupture of the protein network of the ice cream due to shearing (Rossa *et al*., 2012; Aboulfazli *et al*., 2014).

The formation of hysteresis (Table 4.15; Figures 4.4, 4.5, 4.6, 4.7, 4.8 and 4.9) is an important feature of the shear stress versus shear rate results. The fluid viscosity (regarding area formed between the curves of upward and downward) is time dependent (Rossa *et al*., 2012; Aboulfazli *et al*., 2014). It is a measure of energy, which is needed for the gel structural breakdown of the sample (Vega and Goff, 2005). González-Thomás *et al*. (2008) and Karaca *et al*. (2009) noted the presence of hysteresis in studies on ice cream. In fermented ice creams, ice creams made with La-05 tended to have a bigger hysteresis areas than ice creams made with Bb-12. In both non fermented and fermented ice creams, the addition of soybean extract increased ice cream hysteresis areas in samples containing cow milk lower than those containing coconut milk. It is probably due to poor emulsifying properties of coconut proteins (Tangsuphoom and Coupland, 2009) and thus a higher particle size of ice creams containing of coconut milk which lead to a higher apparent viscosity ice creams containing coconut milk. Tárrega *et al*. (2004) suggested that a high-viscosity thixotropic fluid may indicate a larger hysteresis area than a lower viscose, even if the latter undergoes a more accentuated destruction of the structure. An increase in hysteresis as an outcome of higher viscosity was also reported by Debon *et al.* (2010) for a dairy product with inulin and Pinto *et al.* (2012) for frozen yogurt containing microencapsulated *Bifidobacterium* Bb-12. In non fermented ice creams, the SW3 ice cream showed the lowest hysteresis area, and SC1 ice cream also showed the largest hysteresis area. In fermented ice creams, the ice cream without soybean extract showed the lower hysteresis area (CL = 28.99±1.80, WL = 24.14±1.34, CB = 60.34±1.04 and WB = 15.30±1.10 Pa) than others (except for SW3B ice cream = 0±0.00 Pa). The overall charge

at pH 5.50 for soy proteins (pI = 6.00) is lowest in contrast to cow milk proteins (pI = 4.6) and coconut proteins (pI = 4.3). Hence soybean extract ice creams had less surface active than others and thus the soy protein particles can form gel networks (Grygorczyk, 2012) which may increase the structural damage during processing (Mathias *et al*., 2011). SL, SB and SC1B ice creams showed the largest hysteresis area (605.17±0.93, 439.95±1.32 and 589.79±1.84 Pa, respectively) ($p>0.05$). Hence, SC1, SL, SB and SC1B ice creams provided a firmer product which need more energy for breaking the ice cream structure because of their protein networks (Rossa *et al*., 2012).

5.2.3 Effect of milk replacement on droplets suspension

Measurements of zeta potential (the electrical charge of the droplets) along with particle size can be used to predict the stability of ice cream emulsions. Theoretically, a high negative zeta potential prevents aggregation of the emulsion droplets and increases stability through electrostatic repulsion (Achouri *et al*., 2012). In non fermented ice creams, the zeta potential of fat globules was higher (more negative) ($p<0.05$) in ice creams containing cow milk (-26.40 to -37.60 mV*)* compared to ones containing coconut milk (-26.7 to -34.30 mV) and fat globule size of ice cream containing cow milk (0.81-0.91 μm) was lower than those containing coconut milk (1.57-2.54 μm) (Table 4.16 and Figure 4.10). The bigger fat globule size for coconut milk ice cream can be attributed to the less surface activity of the coconut proteins than whey proteins (Tangsuphoom and Coupland, 2009) and thus coconut proteins are not particularly effective in preventing droplet aggregation and also creating small droplets during or after homogenization (Onsaard *et al.*, 2006).

Particle size of fermented ice creams containing cow milk was lower than those containing coconut milk (Table 4.16 and Figures 4.11 and 4.12). In fermented ice creams,

samples with higher particle size made a gel structure with larger aggregates instead of compact structures (Puvanenthiran *et al*., 2002) and this results in a higher firmness and subsequently increase in the apparent viscosity of fermented ice creams (Amatayakul *et al*., 2006). In fermented and non fermented ice creams, the samples containing coconut milk were less stable than those containing cow milk because they had the bigger hysteresis areas which indicates the lower ability of coconut milk ice creams to recover their structure and viscosity (Table 4.15; Lopez and Sepulveda, 2012).

Data from rheological studies showed increased ice creams viscosity with increasing amount of soybean extract in ice cream made with composite milk. This can be attributed to the change in microstructure whereas the reduction in the fat particle diameters (Figures 4.10, 4.11 and 4.12) related in an increase in consistency index (*K* value; Tables 4.12, 4.13 and 4.14) and thus the increased product stability (Chiewchan *et al*., 2006). Also, the microscopic structure of ice cream (Figures 4.11 and 4.12) which showed the aggregation of droplets after fermentation, are visual evidence of indicates the enhancement of the gel network formation. These micrographs provide strong evidence to support the findings from the rheological studies that fermentation increases the viscosity of ice creams, and is caused by the change in the microstructure. As a whole, samples containing coconut milk showed larger droplet sizes than others because of poor emulsifying properties of coconut proteins adsorbed at the oil–water interface (Onsaard *et al*., 2006).

5.2.4 The thermal properties of ice creams with different milks

In non fermented ice creams, there are no significant differences in the peak temperature (T_p) and in the freezing point (T_f) between the ice creams (Table 4.17). However, there is significant variation in the onset temperature (T_0) containing purely

(100%) individual milk with the highest of T_0 value in C ice cream (-6.93±0.12 °C) and the lowest in S and W ice creams (-8.50±0.11 and -8.77±0.11 °C, respectively, $p<0.05$). The enthalpy values decreased with the addition of soybean extract in ice creams containing composite milks. The content of freezable water and the final moisture amount are effective factors on enthalpy value (Hwang *et al*., 2009). The moisture content was highly likely not the factor for the reduction in the enthalpy because the moisture content was same in all ice creams (ice cream total solid content~43-44% w/w). Hence the most probable reason is the freezable water amount (Table 4.17). Increasing soybean extract proportion and hence soy protein in ice creams made with composite milks could have increased water retention (Akesowan, 2009) and subsequently a decrease in the amount of freezable water and thus the melting rate. A similar positive relationship between the enthalpy of ice-melting transition and the amount of freezable water have been previously reported in wheat- and soy-containing breads (Vittadini and Vodovotz, 2003) and ice cream containing grape wine lees (Hwang *et al*., 2009).

In fermented ice creams, the thermal properties associated with ice crystal-melting of fermented ice creams with different milks were measured by DSC. Figures 4.14 and 4.15 show the typical DSC curves for the ice crystal-melting curves. No significant effects ($p>0.05$) were observed between ice creams incubated with La-05 and those incubated with Bb-12. The enthalpy values, the freezable water amount, onset temperature, peak temperature and freezing point were similar in all fermented ice creams (Tables 4.18 and 4.19). In general, the freezable water amount was increased after fermentation in ice creams due to the change in the electrical charge of the droplets of ice creams after fermentation which leads to a decrease in the stability of ice cream emulsion droplets and an increase in the freezable water (Tables 4.17, 4.18 and 4.19; Grygorczyk, 2012).

5.3 Microbiological analyses of probiotic ice creams

5.3.1 Viability of probiotics in non fermented ice cream during frozen storage

Figure 4.16 shows the changes in bacterial counts in non fermented ice creams made using cow or vegetable extracts. Colony forming units were similar among ice creams containing Bb-12 and La-05 (Table 4.20). The decrease of probiotic viability due to freezing may be related to mechanical stresses and freeze injury associated with the freezing and mixing process which incorporates oxygen into the mixes (Haynes and Playne, 2002). The decrease of viable probiotics was higher in after 1 day than after 30 days storage (Figure 4.16) because the mechanical and freezing processes which convert the ice cream mixture in the form of ice cream have a greater effect on survivability loss than throughout frozen storage (Hagen and Narvhus, 1999; Haynes and Playne, 2002; Alamprese *et al.*, 2002; Akalin and Erisir, 2008). The survival of both probiotics in ice cream increased ($p<0.05$) in the presence of vegetable extracts, although the colony forming unit among ice creams containing cow and coconut milk were not different ($p>0.05$). This could be explained by the higher pH of soybean extract and coconut milk ice creams than cow milk ice cream (see Table 4.20) which is known to be conducive to probiotic survival since these microorganisms are susceptible to inactivation when stored in acidic conditions (Hagen and Narvhus, 1999). Heenan *et al*. (2004) also demonstrated that the survival of probiotics increased in the frozen soy dessert due to the prevailing neutral pH. The survival of both probiotics was the highest in soybean extract ice cream. The reason for this high survivability is that soybean extract may provide physical protection against freezing damage by encapsulating probiotics with their proteins by forming a stable network looks like a gel structure (Akesowan, 2009). Keerati-u-rai and Corredig (2011) also demonstrated that soy proteins may cause adsorption at the interface of oil droplets, and form a layer

thickness. It is highly likely that the soy proteins may from a layer with subsequent increase in physical protection against freezing. Nousia *et al.* (2011) noted that the high survival of *L. acidophilus* cells during the frozen storage in ice cream was attributed to the protection provided to the cells by the solid ingredients and the high fat content of the ice cream in the form of emulsion. Wattanachai (2009) found that fat substitutes supplied in the industries were able to encapsulate probiotics in the yogurt ice cream and increased the survival probiotics during 4 weeks storage at -20 ^{o}C.

5.3.2 Rate of probiotic growth during pH drop from initial to 5.50

The replacement of cow's milk with vegetable extracts decreased the time required for fermentation of ice cream mixes by both probiotics until pH = 5.50. La-05 showed higher growth rate than Bb-12 (Table 4.21). Some studies reported that *Bifidobacterium* strains were not as proteolytic as other LAB (*L. acidophilus*). This may explain why *Bifidobacterium* spp. grows slowly in milk and may require supplementation of peptides and amino acids from external sources (Klaver *et al*., 1993, Dave and Shah, 1998; Donkor, 2007). Regardless of the type of probiotics, the growth rate increased with increasing amount of soybean extract. Ice creams containing cow milk showed lower growth rate than ice creams containing coconut milk (Table 4.21). Lactic acid bacteria (LAB) including probiotic organisms are fastidious in nature, requiring numerous essential growth factors such as peptides and amino acids. Hence, *B. bifidum* and *L. acidophilus* tend to grow slowly in milk (Donkor, 2007). Although milk is rich in nutrients, it contains low concentration of free amino acids and peptides (ca. 0.1 g/L) to efficiently support growth of LAB (Shihata and Shah, 2000; Vasiljevic *et al*., 2005). *L. acidophilus* claimed for its probiotic properties require the presence of proline, arginine and glutamic acid for growth (Morishita *et al*., 1981) but is greatly stimulated by almost all 18 amino acids. Since, La-05

does not possess a fully functional pentose phosphate pathway, it requires the presence of aromatic amino acids (phenylalanine, tryptophan, histidine and tyrosine) too (Hebert *et al*., 2000). However, threonine, alanine, aspartic acid and asparagine are not considered essential for growth of La-05, which indicates that the amino acid precursor oxaloacetate is available in sufficient quantities for *de novo* synthesis from sugars or citrate or other supplied amino acids. Even though it is not considered essential, arginine seems to stimulate growth of tested CH-strain *L. acidophilus* (Ummadi and Curic-Bawden, 2010). *Bifidobacterium* need several amino acids which are either stimulatory or essential for their growth (e.g., arginine, glutamic acid, isoleucine, leucine, tryptophan, tyrosine, cysteine, and valine) (Prasanna *et al*., 2014). In response to this limitation, LAB have developed a complex system of proteinases and peptidases, which enable them to utilize casein as an additional source of organic nitrogen (Smid *et al*., 1991). On the other hand, Klaver *et al*. (1993) reported that *Bifidobacterium* strains were not as proteolytic as other LAB. However La-05 possesses a complex system of proteinases and peptidases which increase the availability of peptides and amino acids required for bacterial growth (Donkor *et al*., 2006), which explained the small increase in newly released amino acids groups and peptides during fermentation of milk by *L. acidophilus* and *B. bifidum* from 0 to 12 h (Donkor *et al*., 2007). Since probiotics need to use some amino acids and peptides for their cell growth and survival and hence the total amino acid content in fermented milk reflects the balance between assimilation and proteolysis by bacteria (Donkor *et al*., 2007). In some yogurt production, supplements such as whey powder, whey protein concentrates or acid casein hydrolysates are added to reduce the time required for fermentation with probiotics such as La-05, because they provide amino acids and/or carbohydrates to support the growth of microorganism (Farnworth *et al*., 2007). In addition, lactic acid bacteria are able to degrade different carbohydrates and related compounds (Salminen and Wright, 1998). Some of

researchers mentioned soybean extract is a good medium for growing *B. bifidum* due to presence of sucrose, raffinose and stachyose and also for growing *L. acidophilus* due to presence of sucrose, which are fermented by them (Kamaly 1997; Liu 1997; Scalabrini *et al*., 1998; Donkor, 2007). In the present study, apart from the types of milk used, the milk powder and sugar content and other ingredients (fat content was just corrected in all ice creams by using butter and their final fat were same (~10.52%)) are same (Table 3.2). Thus, the type of milk used could be seen as the determining factor on the growth rate of probiotic and changes in amino acids and carbohydrates contents in all ice creams. Table 4.6 shows all types of amino acids were higher in ice creams containing vegetable extracts (0.28–10 mg/mL) than ice cream containing 100% cow milk (control; 0.02-5 mg/mL). Coconut milk (100%) ice cream (C) showed higher amino acid concentration for glutamic acid, aspartic acid, alanine, serine, proline, isoleucine, leucine, valine, lysine and methionine than S and W ice creams (Table 4.6). Hence, the amounts for these amino acids increased with increasing coconut milk content in ice creams containing coconut milk (Table 4.6). The globulins and albumins are 80% of the proteins in the coconut milk which contain high levels of aspartic acid, glutamic acid and arginine (Yuliana *et al*., 2010). For other amino acids, ice cream containing 100 % soybean extract showed higher amino acid content for arginine, histidine, threonine, tyrosine and phenylalanine than C and W ice cream. Hence, the amounts for these amino acids increased with increasing soybean extract content in ice creams ($p<0.05$; Table 4.6). Soybean extract contains high levels of alanine and arginine (Saidu, 2005). Hence, all amino acids required for growth of both probiotics were higher in ice creams containing vegetable extracts than ice cream containing 100% cow milk (especially phenylalanine, tyrosine, threonine and arginine for La-05 and arginine, isoleucine, leucine, tyrosine and valine for Bb-12; Morishita *et al.*, 1981; Hebert *et al*., 2000; Pasupuleti and Demain, 2010; Prasanna *et al*., 2014). Tables 4.9 and 4.10 show

the amino acid change rate in ice creams in resulted of fermentation by La-05 and Bb-12. The data indicate higher utilization of amino acids during fermentation by both probiotics in ice creams containing vegetable extracts than in ice cream containing 100% cow milk ($p<0.05$; Tables 4.9 and 4.10). Threonine, tyrosine, valine and phenylalanine were utilized more than other amino acids in all ice creams by both probiotics. Table 4.9 shows amino acid change rate until pH of ice creams inoculated with La-05 reached to 5.50 and it reflects the balance between proteolysis and assimilation by bacteria. It is likely that because of higher essential amino acid requirement of La-05 growth, more tyrosine and phenylalanine are utilized in ice cream containing coconut milk more than those containing cow milk. The appearance of arginine, glutamic and proline was also higher in those containing coconut milk than in those containing cow milk. In ice creams inoculated Bb-12, isoleucine, lysine, tyrosine and valine (amino acids essential of its growth) was used in ice creams containing coconut milk more than others by Bb-12 (Table 4.10). Other reason of growth rate differences is related to the type of carbohydrates (sugars) and their amount in non fermented ice creams. Table 4.3 shows the stachyose and sucrose amounts increased in non fermented ice creams with higher soybean extract amount ($p<0.05$). Ice creams containing higher cow milk amount had higher lactose amount (Table 4.3). The replacement of cow milk with vegetable extract increased the sucrose amount in non fermented ice creams and the amount of sucrose was higher than lactose in non fermented ice creams and in general, they were the main sugar of non fermented ice creams (Table 4.3). The main sugar for soybean extract and coconut milk is sucrose (41–67% of total sugars), and for cow milk, it is lactose (Yulian *et al*., 2010; Bozanic *et al.,* 2011; Zare, 2011). Hence, La-05 and Bb-12 can utilize sucrose as well as lactose as a source of energy, which enhanced better cell growth during fermentation (Donkor, 2007). Table 4.5 shows lactose and sucrose were the primary sugars being consumed by the bacteria during the fermentations of ice creams.

Regardless of the starter culture used, the fermented concentrations of stachyose and sucrose (their change rate) increased with higher soybean extract amount by fermentation ($p<0.05$). Bb-12 was found to cause the disappearance of stachyose content more than can La-05 ($p>0.05$; Table 4.5). In general, total sugar amount increased in non fermented ice creams with the replacement cow milk with vegetable extracts (larger energy sources). In general, according to Tables 4.3 until 4.10 high growth rate of La-05 and Bb-12 in ice creams containing higher amount of vegetable extracts may be explained by the vegetable extract ability to provide amino acids and energy sources required for their growth. Considerably, total sugar and TFAA contents increased with replacement of cow milk (7.38 and 14.71 mg/mL, respectively) with vegetable extract (5.19-8.34 and 24.15-47.85 mg/mL, respectively) (Tables 4.3 and 4.6, respectively). So, soybean extract and coconut milk provide a richer growth medium than cow's milk in ice creams for both La-05 and Bb-12, thus the capacity to support faster microbial growth and metabolism resulting in a faster rate of pH decline in ice creams containing vegetable extract (Table 4.21). These findings in the present study are in line with other studies which reported *L. acidophilus and B. bifidum* are capable of growing in soybean extract and also coconut milk is a very rich medium which can support the growth of both probiotics (Shah, 2000a; Farnworth *et al.*, 2007; Donkor, 2007; Yuliana *et al.*, 2010).

5.3.3 Viability of probiotic bacteria in ice cream during frozen storage

Figures 4.17 and 4.18 show the changes in bacterial counts in ice creams made using cow or vegetable extracts under frozen storage for 90 days. After 90 days, the probiotic viability in samples tends to be lower in La-05 than in Bb-12 (Tables 4.22 and 4.23). Haynes and Playne (2002) noted that the viable bacterial counts reduce during freezing due to injury of cells associated with freezing, the mechanical stresses associated

with the mixing and freezing process which incorporates oxygen into the mixture also contribute to further decline in bacterial count. Bb-12 tend to have a better viability than La-05 in all ice creams which is in agreement to previous studies (Haynes and Playne, 2002). The presence of soybean extract and coconut milk increased both probiotics survivability in ice creams ($p<0.05$). The survival of Bb-12 was 85.30, 88.73, 82.40, 81.47, 86.36, 77.58, 70.00, 65.15 and 64.24% in SB, SC1B, SC2B, SC3B, CB, SW1B, SW2B, SW3B and WB samples, respectively, after 90 days (Table 4.23). The survival percentage of La-05 was 70.20, 69.76, 67.63, 64.62, 64.23, 68.10, 65.53, 62.78 and 60.04 in SL, SC1L, SC2L, SC3L, CL, SW1L, SW2L, SW3L and WL samples, respectively, throughout 90 days storage at -20 ^{0}C (Table 4.22). These results supported with numerous studies which have shown that probiotic cultures were capable of maintaining their stability in ice creams with minimum loss of viability throughout frozen storage. Hekmat and McMahon (1992) found that both *L. acidophilus* and *B. bifidum* were able to survive in fermented ice cream during 119 days of storage at -29 °C. Also, Hagen and Narvhus (1999) observed that the viable count of *L. reuteri*, *L. acidophilus*, *B. bifidum* and *L. rhamnosus* in ice cream did not change significantly during 52 weeks of frozen storage and remained above of 10^6 cfu/g. Others have also demonstrated that *L. johnsonii* La1 and *L. acidophilus* are capable of surviving in ice cream (Alamprese *et al.*, 2002; Andrighetto and Gomes, 2003). Hamed *et al*. (2004) observed no evidence of freeze injury to *B. bifidum* in frozen yoghurt over 10 weeks of frozen storage. Salem *et al.* (2005) noted the colony forming unit of *B. bifidum*, *L. gasseri*, *L. acidophilus*, *L. rhamnosus* and *L. reuteri* decreased by 1.68, 1.23, 2.23, 1.77 and 1.54 log cfu/g, respectively, in ice cream by storage at -26 °C for 84 days. Magarinos *et al.* (2007) observed 86-90% of the La-05 and Bb-12 were survived in ice creams containing 4% fat during 60 days of storage at -25 °C. Mohammadi *et al*. (2011) noted the probiotics can be survived for 180-360 days in ice cream (the shelf life of ice cream). Recently, Silva

et al. (2015) noted their ice cream with *B. animalis* received satisfactory probiotic viability was maintained throughout the 120 days of frozen storage. Others studies also showed the ability of probiotic for surviving in ice cream during storage at −18 to −28 °C for up to 180 days and remain above of 10^6 cfu/g (Christiansen *et al*.,1996; Haynes and Playn, 2002; Kailasapathy and Sultana, 2003; Fávaro-Trindade *et al*., 2006).

The highest survival of La-05 and Bb-12 was in SC1B and SL samples, respectively and the lowest in WB and WL samples, respectively (Tables 4.22 and 4.23). Among ice cream samples with composite milk, both probiotics studied had a higher survival in ice creams containing coconut milk than those containing cow milk, is due to lack of free amino acids in cow's milk (10 mg. 100 mL^{-1}; Magarinos *et al.*, 2007; Zare 2011). These are generally present in insufficient amounts, and are either unbound or compose low molecular mass peptides in milk (Rasic and Kurmann, 1983; Kurmann, 1998; Gomes *et al.*,1998; Gomes and malacata, 1999; Shihata and Shah, 2000; Vasiljevic *et al*., 2005; Zare, 2011; Mohammadi *et al*., 2011; Prasanna *et al*., 2014). Mohammadi *et al*. (2011) mentioned that milk supplemented with different growth promoters and/or growth factors (such as amino acids and carbohydrates) can increase probiotic viability in ice creams. Amino acids derivatives promote probiotic due to their nutritional value for the cells and their ability to reduce the redox potential of the medium (Dave and Shah, 1998; Mortazavian *et al*., 2011). Sugar (carbohydrate) can increase the survival of probiotics by its cryoprotectant activity (Champagne and Rastall, 2009) and also act as growth factors (Mortazavian and Sohrabvandi, 2006), and improve the retention of probiotic survivability in ice creams (Gibson *et al*., 2004; Mizota, 1996; Rycroft *et al*., 2001). The appearance of prebiotics and growth promoting factors can improve probiotic viability in ice cream (Crittenden *et al*., 2001; Akin *et al*., 2007; Palframan *et al*., 2003). Hence, the survival of

both probiotic was lower in ice cream containing 100% cow milk (control) than all ice creams containing vegetable extracts and it also increased with increasing soybean extract content in them. In fermented ice creams inoculated Bb-12, the arginine, leucine, isoleucine, valine, and tyrosine amount (some of free amino acids essential to the growth of bifidobacteria; Gomes *et al*., 1998; Donkor *et al*., 2006; Prasanna *et al*., 2014) were higher in ice creams containing coconut milk than those containing cow milk and also increased with increasing soybean extract amount (Table 4.8). Moreover Bb-12 can grow more extensively in soybean extract than in cow's milk under comparable conditions (Farnworth *et al.,* 2007). Because soybean extract contains oligosaccharides (stachyose and raffinose) and sucrose which may be utilized by *Bifidobacterum* (Donkor, 2007). Table 4.4 shows stachyose and sucrose amounts of fermented ice creams inoculated with Bb-12 increased with increasing soybean extract amount. The composition of nutrients with varying soybean extract, coconut and cow milks content (Tables 4.5, 4.9 and 4.10) suggest that the change of soybean extract proportion in the composite milk affect the utilization of sugars and amino acids. In fermented composite milk ice creams, the TAA and sugar content were also higher in ice creams with higher soybean extract amount. In fermented ice creams inoculated with La-05, sucrose amount increased with increasing soybean extract amount and it was higher in ice creams containing coconut milk that those containing cow milk (Table 4.5). The main sugar in soy and coconut milk is sucrose which La-05 can utilize it as well as lactose (Božanić *et al*., 2011; Yuliana *et al*., 2010; Donkor, 2006). In addition, sucrose and prebiotic compounds' have cryoprotective effect on probiotics in frozen products (Champagne and Rastall, 2009). Table 4.7 also shows tyrosine, phenylalanine, arginine, glutamic and proline (some of amino acids required for growth of La-05; Morishita *et al*, 1981; Hebert *et al*., 2000; Pasupuleti and Demain, 2010) increased in concentration in fermented ice creams inoculated with La-05 with increasing soybean extract content.

5.3.4 Viability of probiotic bacteria in ice cream to the exposure to simulated gastric and intestinal conditions

Each probiotic showed a progressive reduction in viability during a 120 min exposure to gastric juice. Bb-12 showed much greater tolerance to the exposure to gastric juice than La-05 (Table 4.24), this characteristic of Bb-12 is due in part to the low pH induction of H+-ATPase activity, an enzyme complex involved in maintaining intracellular pH homeostasis in bacteria (Matsumoto *et al*., 2004). This is in agreement with the results of Grimoud *et al.* (2010), which found that La-05 was more sensitive to high acid conditions, compared to Bb-12. For ice creams made with composite milk, the viability of both La-05 and Bb-12 was higher in samples containing cow's milk, than those containing coconut milk after 120 min. The bacteria survival after 120 min exposure to *in vitro* gastric conditions also increased with a higher soybean extract content in the ice cream. The highest tolerance of Bb-12 to gastric juice was found in SW1B, SW2B, and SB ice creams, whereas the lowest tolerance was in SC3B ice creams after 120 min. The highest survival of La-05 during *in vitro* gastric conditions was in SW1L, SW2L, and SL ice creams, whereas the lowest was found in SC3L, SW3L, and SC2L ice creams after 120 min.

The simulated intestinal juice which content 0.3% bile salt reduced significantly probiotic viability (Table 4.25). This occurred as early as one minute after exposure to bile for both bacteria, whereas Bb-12 showed a higher survival than La-05. Bb-12 contains the gene coding for bile salt hydrolase, an enzyme which is important for coping with the high bile salt concentrations in the small intestine. This enzyme is present and active in Bb-12 at all times, a fact which is documented by both microarray analyses and protein studies using 2-D gel electrophoresis (Garrigues *et al*., 2005). Having such an enzyme ready for action will provide an advantage for the cell as it allows a quick response to high bile salt

concentrations and thus facilitates the viable passage from the small intestine to the large intestine. These data suggest that Bb-12 is well-equipped to endure this critical passage in the gastrointestinal tract (Jungersen *et al*., 2014). Among the ice creams with composite milk, the survival of both probiotics was higher in those containing cow's milk, and their survival increased in ice creams made with composite milk, where the soybean extract content was higher after 120 min. The highest survival of Bb-12 in the presence of simulated small intestine juice comprising 0.3% bile was noted in SW1B ice cream, whereas the lowest occurred in SC3B ice cream. For La-05, the highest survival was in SL and WL ice cream and the lowest was in SC3L ice cream after 120 min.

In the present study, transit time had a significant influence on the bile salt and gastric tolerance of probiotics. When probiotics were exposed to gastric conditions for longer time periods, the loss of probiotic viability increased. In accordance with other research, the survival of both the probiotic strains was progressively reduced during an *in vitro* 120 min gastric and small intestine transit. In general, La-05 showed lower bile and acid tolerance than Bb-12 in ice creams after 120 min (Mishra and Prasad, 2005). These results and other (Chen *et al*., 2005) demonstrated that probiotics have a lower tolerance to bile than to gut acid.

The results of the present study provide support for a recent clinical study, which indicated that bacterial strains as well as the food matrix, profoundly affect probiotic viability in the presence of small intestine and simulated gastric juices (Ranadheera *et al*., 2012). Ranadheera *et al*. (2012) showed that the addition of carrier foods containing probiotics increased the pH of the gastric transit test mixture. The pH of the original mixes was 2.0, 3.0, and 4.0, and these increased to 2.8, 3.9, and 6.3, respectively, in the presence of ice cream, and 2.6, 3.6, and 4.2, respectively, in the presence of fruit and plain yogurts.

The survival of the probiotics was improved by an increase in the pH of the gastric content, as a result of the addition of the food matrix, because of the buffering capacity of the food carrier. However, in the present study, all the ice creams had a pH of around 5.50, so there were similar changes to the pH of the combined food and simulated juice mixes, shown in Table 4.1. Klingberg and Budde (2006) mentioned that the survival during gastrointestinal transit of *Lactobacillus plantarum* MF 1298 improved in human subjects when administered with fermented sausage, because the sausage could protect the bacteria, for example by a simple physical "encapsulation" within the matrix of sausage meat and fat, or by acting as a buffer. Ranadheera *et al*. (2012) found the survival of probiotics in ice cream was better than in yogurt during gastrointestinal transit in human subjects, because of the higher fat content in ice cream at 10%, rather than 5% in yogurt. In addition, the presence of ingredients in ice creams, such as cocoa powder and stabilisers, such as dextrose and guar gum, may also provide a protective barrier against small intestine and gastric juices. However, in the present study, apart from the types of milk used, the fat content and other ingredients (Table 3.2) are same. Thus, the type of milk used could be the determining factor on probiotic viability, during simulated gastric and gastro intestinal transit. In general, the addition of soybean extract significantly improved probiotic survival. This is because of the ability of soy proteins to form a stable protein network (Akesowan, 2009), soy proteins can adsorb at the interface of oil droplets, with surface loads varying between 2 and 4 mg m^{-2} and a layer thickness of between 30 and 40 nm (Keerati-u-rai and Corredig, 2011). Soy proteins may be able to form a stable layer with a thickness of between 30 and 40 nm and thus increase physical protection by coating probiotics with these proteins. In the present study both probiotics viability remained significantly higher in gastric and small intestinal juices when fortified with ice cream containing cow milk. Ice cream is an emulsion of oil in water, in which fat droplets in the ice cream mix is stabilized by milk

protein and emulsifiers (surfactant adhesion) to the oil/water interface (Ruger *et al*., 2002). Milk protein and emulsifiers covered the oil surface in ice cream (Goff, 2006). Probiotics may also be covered to considerable extent by a layer of protein and emulsifiers. This coating can protect probiotics from gastric conditions, the stability of which may depend on the emulsifying properties of milk proteins (their surface activity) at the outer oil water interface (or the outer probiotic) (Pimentel-González *et al*., 2009). Coconut proteins have lower emulsifying property than cow milk proteins and this can be attributed to the less surface active for coconut proteins than for cow milk proteins (Tangsuphoom and Coupland, 2009). This may imply a protein coverage around probiotics with a lower stable than can cow milk proteins and thus results in faster elimination of the coating surrounding probiotics and the release of probiotics under gastric conditions in ice creams made with coconut milk than in those made with cow milk. This could partially explain the lower survival of probiotics in coconut milk ice creams in contrast with cow milk ice creams under gastric conditions.

5.4 Sensory evaluation

Mean scores of flavour, body-texture and taste and colour of the samples are shown in Tables 4.26, 4.27 and 4.28. In all fermented and non fermented ice creams, the colour, taste and body-texture were decreased by the replacement of cow milk by vegetable extracts, whereas the creaminess, structure, aroma, colour and flavour of the products decreased with increasing soybean extract content ($p<0.01$). The total acceptability which decreased with increasing soybean extract content in the ice creams was most likely due to soybean extract woody or beany off flavours (Abdullah *et al*., 2003). Ice creams containing higher amount of soybean extract showed lower body-texture score, possibly caused by an increase in gummy texture of ice creams due to emulsifying properties of lecithin and

proteins of soybean extract (Salem *et al.*, 2005). Among ice cream samples with composite milk, those containing cow milk had higher total acceptability than those containing coconut milk (Tables 4.26, 4.27 and 4.28). In fermented ice creams, no significant effects ($p>0.05$) were observed between samples with respect to the kind of probiotic for all sensory scores (La-05 and Bb-12) (Figure 4.19).

In additional, the fermentation decreased slightly taste and body–texture scores of ice creams (Figure 5.1). The lower total acceptability was in fermented ice creams because of a decrease in their taste and flavour scores (Donkor, 2007). Increase freezable water amount after fermentation increase coarseness score which was also caused to the decrease of texture score of the ice creams (Salem *et al.*, 2005). There were no significant differences for colour scores between non fermented and fermented ice creams. The increase in the soybean extract content decreased the total acceptability in both non fermented and fermented probiotic ice creams due to the soybean extract beany or woody flavour (Donkor, 2006).

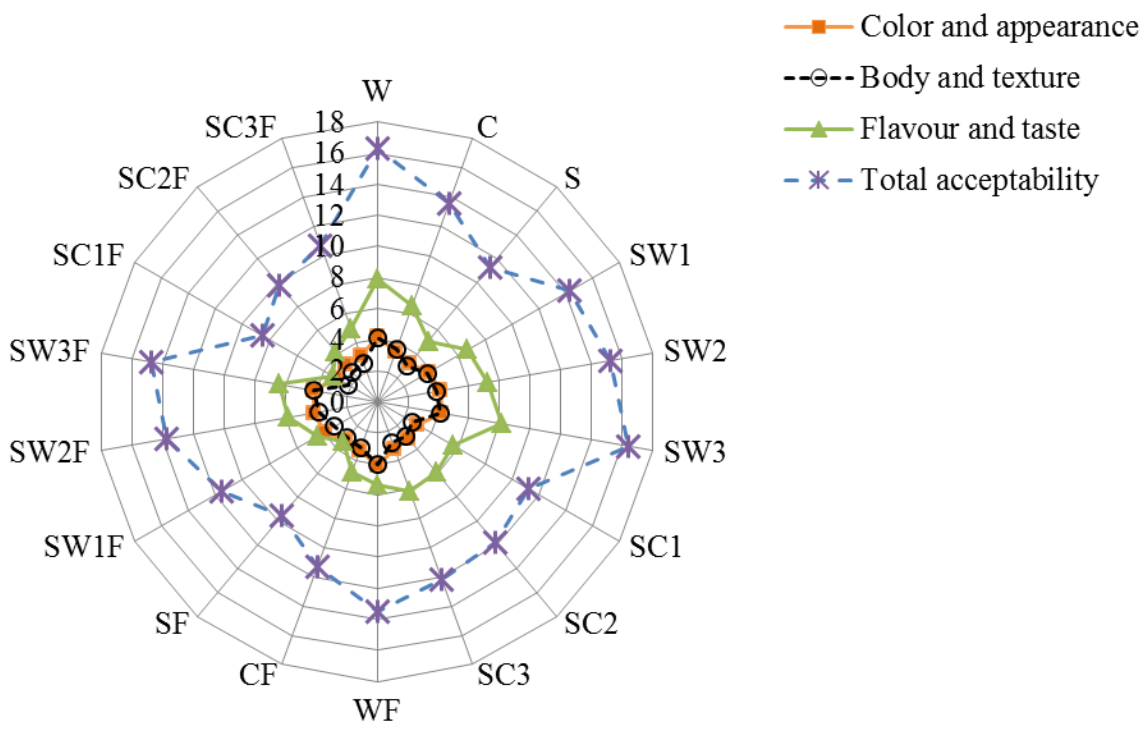

Figure 5.1 Changes in sensory evaluation of ice creams after fermentation (F) ($P < 0.05$). Note: F = fermented ice creams. In fermented ice creams, No significant effects (p>0.05) were observed between samples with respect to the kind of probiotic for all sensory scores (La-05 and Bb-12).

The PCA was carried out on two principal components (Figure 5.2). The first axis (PC1) explained 62.49% of the total variation in the data set and was dominated by coarse, dull colour, unnatural colour and lack of flavour attributes. The second axis (PC2) explained 33.51% of the total variation and was dominated by sourness attribute. Some of the descriptors could be correlated (Figure 5.2). For example, coarseness, lack of flavor, unnatural colour and dull colour were positively correlated with one another. Fermented ice cream had less sweetness and higher sourness flavour than non fermented ice creams. None of the ice creams were judged to be coarse, sandy, crumbly, fluffy, weak or have a cooked flavour. All fermented ice creams had a good total impression with medium sour taste.

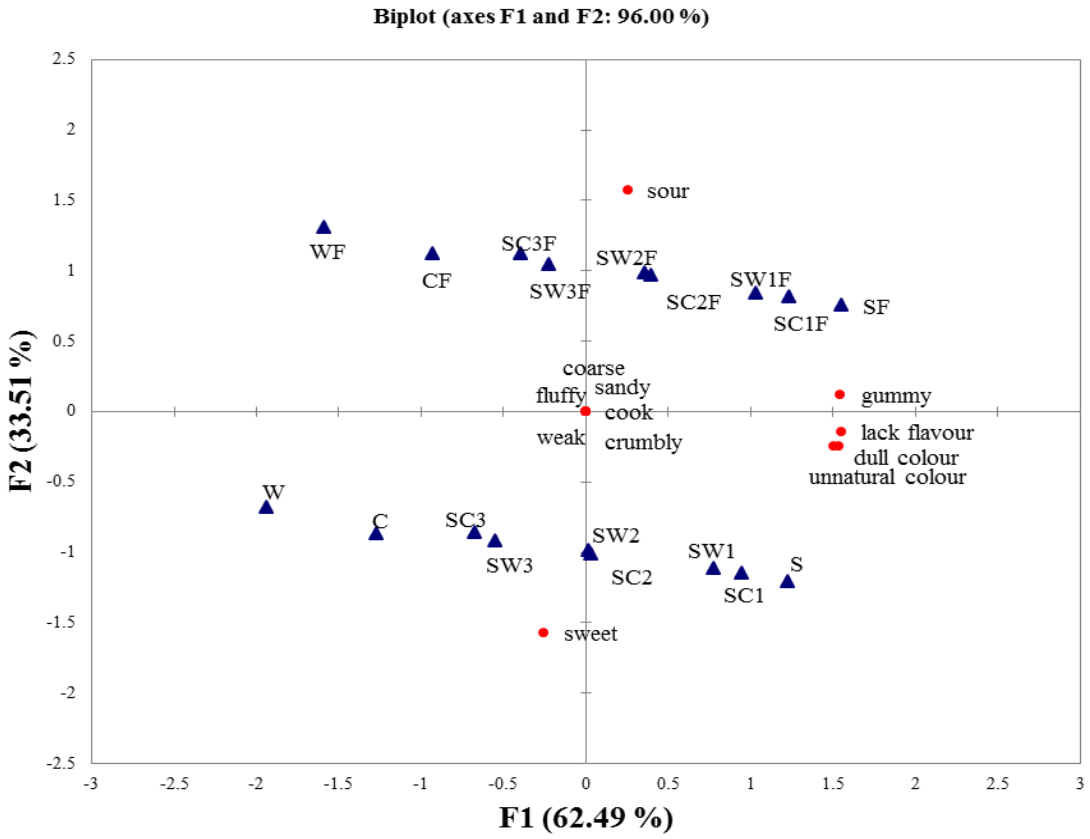

Figure 5.2 Principal component analysis (PCA) of probiotic ice creams with sensory attributes on PC1 and PC2 ($P < 0.05$). Note: F = fermented ice creams. In fermented ice creams, No significant effects ($p>0.05$) were observed between samples with respect to the kind of probiotic for all sensory scores (La-05 and Bb-12).

CHAPTER 6 CONCLUSION

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6.1 Conclusion

The examination of selected physical properties showed significant differences among non fermented and fermented ice creams containing vegetable extracts compared with the ice cream containing 100% cow milk (control). The addition of soybean extract in non fermented and fermented ice creams containing cow and coconut milk improved their physical (freezable water in non fermented ice cream, viscosity and melting rate of non fermented and fermented ice creams) properties. The fermentation increased the viscosity and melting resistance with slightly the decrease in the total acceptability of the ice creams. The replacement of cow's milk with vegetable extracts, decreased the time required for fermentation of ice cream mixes by both probiotics until pH = 5.50. The survival of Bb-12 and La-05 was also increased by replacing cow's milk with vegetable extracts in non fermented and fermented ice creams during storage at -20 °C. Soybean extract improved probiotic survival in simulated gastric and intestinal conditions. In general, this study has provided much valuable evidence on how vegetable extracts can alter ice cream physical properties and to what extent the survival of probiotics in ice cream can be enhance. Vegetable extracts, in addition to their capability to increase the nutritional and health benefits of ice cream, can improve the survival of probiotics during 90 days of frozen storage (-20 ^{o}C) and after being subjected to simulated gastric and intestinal digestions. Hence, fermented ice creams made with vegetable extracts have the potential to be used as new functional food in dairy industry because they provide customized technofunctionality such as the enhancement in viscosity, emulsification and melting resistance with minimal change to the taste and also improve health-related and nutritional aspects.

6.2 Future research

Soybean extract and coconut milk used in the present studies have tremendous promise in enhancing the growth and survival of probiotics during frozen storage and exposure to gastrointestinal conditions. The survival-enhancing effects of these vegetable extracts on probiotics are clearly of advantage to ice cream nutritional and functional properties. The exact mechanisms as to how these are achieved should be further studied in future studies. Several findings from the present studies may however be immediately applied after several studies are carried out to optimize the conditions whereby probiotics survival in these vegetable extracts are maximized. Donkor (2009) found *Lactobacillus* and *Bifidobacterium* release bioactive compounds (peptides and isoflavones) during fermentation in bovine milk and soybean extract. These can increase the functional properties of ice cream and thus future study are required to investigate these compounds in fermented ice cream made with different mixes of soybean extract and coconut milk. The stability of these bioactive peptides also need to be studied because minimum quantities of are known to exist (Elfahri, 2012) at the point of consumption in order to achieve the nutritional and health effect of these compounds. Large-scale fractionation of protein hydrolysates to obtain products enriched with biologically active peptides with specific functions may also be attempted because these peptides could be used as nutraceutical additives in functional foods. The understanding of droplet aggregation, gel network formation and the form of coat surrounding probiotics in fermented ice cream for example, are required to explain the changes in rheology of ice cream and survival probiotics during frozen storage and when exposed to gastrointestinal conditions. In this regard the use of cryo-electron microscopy analysis which can help to unravel the importance of unique

molecules from soybean extract and coconut milk on the physicochemical and rheological properties of composite cow-vegetable extracts ice cream.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

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Refereed Research Papers

1) Aboulfazli, F., Baba A. S., and Misran, M. (2014). Effect of vegetable milks on the physical and rheological properties of ice cream. *Food Science and Technology Research*, 20 (5), 987-996 (*ISI/SCOPUS Cited Publication*).

2) Aboulfazli, F., Baba A. S., and Misran, M. (2015). Effects of fermentation by *Bifidobacterium bifidum* on the rheology and physical properties of ice cream mixes made with cow and vegetable extracts. *International Journal of Food Science and Technology*, 50, 942–949 (*ISI/SCOPUS Cited Publication*).

3) Aboulfazli, F., Baba, A.S. and Misran, M. (2015). Replacement of bovine milk with vegetable milk: Effects on the survival of probiotics and rheological and physicochemical properties of frozen fermented dessert. *International Journal of Dairy Technology* (online published; DOI: 10.1111/1471-0307.12219) (*ISI/SCOPUS Cited Publication*).

4) Aboulfazli, F., and Baba, A.S. (2015). *In vitro* analysis of gastrointestinal tolerance of probiotics in fermented ice cream with vegetable and cow milks. *Food Science and Technology Research*, (In press) (*ISI/SCOPUS Cited Publication*).

5. Aboulfazli, F., Baba, A.S. and Misran, M. (2015). Survival of probiotics in ice creams made with dairy alternatives and physicochemical and rheology properties of ice cream. *Journal of Agricultural Science and Technology* (Submitted April 2015).

6. Aboulfazli, F., Baba, A.S., Misran, M. and Shori. A. B. (2015). Effects of the replacement of cow milk with vegetable extracts on the count of probiotics and changes in sugar and amino acid amounts in fermented probiotic ice creams. *Journal of Food Science* (Submitted March 2015).

7. Aboulfazli, F., Baba, A.S. and Misran, M. (2014). Effects of the replacement cow milk with vegetable extracts on rheology and physical properties of fermented probiotic ice creams. *International Journal of Food Engineering* (Submitted January 2015).

8. Low Hu Pin., Baba, A.S. and Aboulfazli, F. (2015). Effects of different levels of refined cane sugar and unrefined coconut palm sugar on the survivability of Lactobacillus acidophilus in probiotic ice cream and its sensory and antioxidant properties. *Food Science and Technology Research* (Submitted March 2015).

Book chapter

Shori, A.B., Aboulfazli, F., Baba, A.S. (2015). *Viability of probiotic in dairy products: A review focusing on yogurt, ice cream and cheese*. Microbes in Food book. (In press).

Conference Proceedings

1. Aboulfazli, F., Baba, A.S. & Misran, M. (2013). Effect of the addition of soy milk on the physical and rheological properties of ice cream. Challenges in Multidisciplinary Research and Practice (ICMRP, December 13-14, 2013) Kuala Lumpur, Malaysia. (Oral presentation).

2. Aboulfazli**, F**., Baba, A.S. & Misran, M. (2014). Effects of vegetable extract on survival of probiotics and rheological and physicochemical properties of bio-ice cream. International Conference on Biological and Medical Sciences (ICBMS' 15-16, 2014).Kuala Lumpur, Malaysia (Oral presentation).

Awards and Grants

1) The post graduate research grand (PPP), University Malaya, Kuala Lumpur, Malaysia. Account No: PV113-2012A.