A study of product stability of commercial probiotic fermented milk and yoghurt

Produktstabilitet av kommersiell probiotisk syrnet melk og yoghurt under lagring

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PREFACE

This master thesis is a result of the study during my graduation at the Norwegian University of Life Science (UMB). Using this opportunity, I would like to thank several people who gave me full support during my dates at UMB and during my graduation period.

The writing of my thesis has gone through ups and downs. In the beginning I experienced some troubles finding a research topic and defining a suitable research question, which really interested me. During this period, numerous e-mails were sent back and forth with my supervisor professor Judith Narvhus. I would like to express my deepest gratitude to my major supervisor professor Judith Narvhus and Roger K. Abrahamsen, without whom my thesis would not be done. First, I would like to thank you for helping me defining my research topic which has been both interesting and challenging. Thank you for guiding my research in the right direction, letting me come by your office whenever a question needed an answer, and for taking the time to explain things thoroughly. Thank you for the patience and the extra time you have spent on correcting my language and grammatical mistakes.

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ABSTRACT

In this study, the stability of three probiotic fermented milk products (‘Cultura Naturell’, ‘Biola Syrnet Lettmelk Naturell’ and ‘Biola Pluss Yoghurt Mild Naturell’) produced by TINE BA was studied and samples from each production were studied at three different times within designated shelf-life. The study focused mainly on the viability of probiotic bacteria during storage, which is a key criterion for quality evaluation of probiotic dairy products. Other parameters (pH, viscosity, organic acids, carbohydrates, volatile compounds and sensory attributes) were also measured for a comprehensive quality evaluation of these products.

The three TINE probiotic fermented milk products studied are produced with different combinations of bacteria strains, raw materials and chemical ingredients and have shown different properties regarding to viable cell counts of bacteria, pH, viscosity, organic acids, carbohydrates, and volatile compounds. These variations determine their unique sensory characteristics and effect on customer’s decision upon purchase of the fermented milk products. In additional, variations between the three different productions of the same product were observed concerning pH, viscosity, organic acids, carbohydrates and volatile compounds, which however seem not to affect the sensory perception of these products in large extent .

The viable cell counts of probiotic bacteria in TINE probiotic fermented milk product were satisfactory, maintaining above a level of 7 log cfu/g during storage at 4 ℃ within designated shelf-life. This indicates that potential healthy beneficial could be obtained by regular consumption of TINE probiotic fermented milk products.

SAMMENDRAG

I denne oppgaven ble stabilitet av tre probiotisk fermenterte melkeproducter (Cultura Naturell, Biola Syrnet Lettmelk Naturell og Biola Pluss Yoghurt Mild Naturell) fra TINE studert. Produkter fra hver produksjonsenhet ble undersøkt ved tre forskjellige tidspunkter innen anbefalt holdbarhet. Oppgaven fokuserte hovedsakelig på overlevelse av protiotiske bakterier under lagringsforhold som er er et sentralt kriterium for kvalitet evaluering av probiotiske meieriprodukter. Andre parametere (pH, viskositet, organisk syre, karbohydrate, sensoriske parametere og flyktige forbindelser) ble også målt for en omfattende kavlitetsvurdeirng av disse produktene.

Det tre TINE probiotiske fermenterte melk produkter produseres med ulike kombinasjoner av bakteriestammer, råvarer og kjemisk ingredienser og viste ulike egenskaper med hensyn til overlevelse av bakteria, pH, viskositet, organisk syrer, karbohydrater og flyktige forbindelser. Disse variablene fastsetter deres unike sensoriske egenskaper og påvirker kundens avgjørelse ved kjøp av fermenterte melkeprodukter. I tillegg ble variasjoner mellom de tre ulike produksjoner av samme produkt observert når det gjelder pH, viskositet, organisk syrer, karbohydrater og flyktige forbindelser, som imidlertid synes ikke å påvirke den sensoriske oppfattelsen av disse produktene i stor grad.

Antall probiotisk bakterier som overlever under lagring ved 4 °C i TINE probiotisk fermenterte melkeprodukene var tilfredsstillende, ettersom de opprettholde et nivå på over 7 log cfu /g under lagring frem til fremstemplingsdatoen. Dette indikerer at potensielle helsegevinst kan oppnås ved regelmessig inntak av TINE probiotiske fermenterte melkeprodukter.

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1.0 INTRODUCTION

Since the term ‘functional food’ was first introduced in Japan in the mid-1980s, research on the connection between diet and health has evolved rapidly, and the functional food industry has grown substantially in the past two decades (Raghuveer & Tandon, 2009). Consumers, who have become more health conscious, are not satisfied with food products that only intend to satisfy hunger or provide necessary nutrients. Instead they have begun to focus on products that aim to prevent nutrition-related diseases and improve physical and mental well-being (Klaus, 2003).

In recent years, increased knowledge and understanding of gut micro-flora composition and activities has made the concept of functional food move markedly towards gastro-intestinal function (Gibson, 2007). Probiotics are one of the fastest growing categories within functional food ingredients and have been successfully used in many commercial food products. According to Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), probiotics can be defined as ‘live microorganisms, which when administered in adequate amounts confer a health benefit on the host (Moriya *et al.*, 2003). Probiotics are usually of intestinal origin. They beneficially affect gastrointestinal function by influencing compositions and activities of intestinal microbiota towards a more positive metabolism (O’Toole *et al.*, 2008). Probiotics are sold mainly as ingredients in fermented foods. Fermented dairy products, vegetable and meats are considered to be good carriers of probiotics. However, at present, probiotics are almost exclusively consumed as fermented dairy products such as yogurt, fermented milk or cheese (Soccol *et al.*, 2010).

As one of the most recognized and intensively studied functional products, various beneficial effects associated with consumption of dairy products with live probiotic bacteria have been demonstrated and sales of cultured dairy products containing probiotic bacteria strains have increased dramatically in recent years. However, despite the apparent success of probiotic fermented milk on the market, there are still problems that have to be studied and solved. A marketable probiotic product has to meet several requirements to guarantee its beneficial effect on human beings, among which good viability of probiotic bacteria in the product during storage is considered to be the most important (Heller, 2010). However, the results of some studies (Iwana *et al.*, 1993; Shah *et al.*, 1995; Coeuret *et al.*, 2004; Gueimonde *et al.*, 2004) carried out in different countries have shown low viabilities of probiotic bacteria cells in some commercial fermented milk products within their labeled shelf-life and this has created a negative image about these products. Recently, several studies (Dave & Shah, 1997; Shah, 1999; Güler-Akin & Akin, 2005; Christopher *et al.*, 2008) have concentrated on modifying and developing processes or supplementations, such as micronutrients, in order to improve the viability of probiotic bacteria in commercial products.

In Norway, TINE BA launched the Biola product range in 1997 as the first range of probiotic dairy products available on the market with a desire to develop a new range of products containing not only the ordinary nutrients contained in milk but also the probiotic bacteria in order to enhance the functional properties of their products. The probiotic bacteria strain *Lactobacillus*(*L.*) *rhamnosus* GG (LGG®) is used in addition to the traditional probiotic bacteria species *L. acidophilus* La-5and *Bifidobacterium* (B.) *lactis* Bb-12 in the new Biola products (Valio, 2009). This move has been proved to be a great success today ten years later. Biola range now has developed into several product branches including Biola fermented milk, Biola yoghurt, Biola juice (at present withdrawn) with a variety of taste and package.

This study is performed in order to obtain a better understanding of TINE probiotic fermented milk products. Three of the probiotic fermented milk products from TINE BA were chosen: 'Cultura Naturell', 'Biola Syrnet Lettmelk Naturell' and 'Biola Pluss Yoghurt Mild Naturell'. Since the presence of fruit flesh, flavor additives and preservatives could, for practical reasons, have an unpredictable influence on the analysis results, all three products were chosen as plain. Products from three production dates were studied to test the stability of probiotic products during their designated shelf-life. The viability of bacteria was studied by enumeration using selective agars. Other parameters (pH, viscosity, organic acids, carbohydrates, volatile compounds and sensory parameters) were also measured in order to give a comprehensive understanding of the properties and qualities of the probiotic fermented milk products. In addition, sensory analysis was used to see whether any changes in quality during storage at 4 ℃ within their designated shelf-life could be detected by a sensory panel (consumers).

The main objects of this study were:

1) To monitor viability and survival of probiotic bacteria in products storing at 4 ℃ up to the end of the recommended shelf-life.
2) To comprehensively evaluate the storage stability of probiotic fermented milk products based on measurements of different relevant parameters.

Chapter 1 and 2 contain a literature review of probiotic fermented milk and probiotic strains. Chapter 3 explains and describes materials and methods used in this study. Chapter 4 presents the results. Chapter 5 and Chapter 6 give discussions and conclusions. Chapter 7 and Chapter 8 are references and appendices respectively.

2.0 LITERATURE REVIEW

2.1 Probiotic fermented milk

2.1.1 History

The evidence shows that the exact origin of making fermented milk products could date from 10000 – 15000 years ago, the dawn of human civilization. It is said that fermented milk would have been discovered accidentally, with milk being left too long in the sun or a warm place (Tamime & Robinson, 2000). Another story is when the Bulgarians began migrating into Europe in the second century, they tried to store the fresh milk in goatskin bags. The milk became spontaneously fermented by wild bacteria present in the goatskin bags. The flavor of the fermented milk was appreciated by early man and they continued making it (Wikipedia, 2007).

It was not until the beneficial effects of yoghurt were proposed in the beginning of 20th century by a Russian scientist named Elie Metchnikoff, that fermented milk products became popular in the Western world. After researching on the people of Bulgaria who were reputed to have much longer life than the people of other countries, Metchnikoff claimed that the high consumption of cultured milk was responsible for their longevity. According to his opinion, the harmful bacteria are the culprits in many diseases and beneficial bacteria in fermented milk can suppress the diseases caused by bacteria and thus prolong the normal lifespan (Soccol, *et al.*, 2010; Lourens-Hattingh & Viljoen, 2001).

Since Isaac Carasso first industrialized the production of yogurt in 1919 in Barcelona (Tunick, 2009), production of yoghurt has spread over the world. Today, sophisticated techniques to produce a variety of fermented milk products in large scale production have been developed. Instead of using bacteria strains that exist naturally in the milk, microorganisms cultured and selected by microbiologists are commonly intensively used either solely or in combinations with other selected microorganisms (bacteria or yeasts) to obtain desired texture, flavor, consistence, shelf-life and nutritious value, which also made the process and quality of the products more controllable. In the meantime, marketing strategies have developed fermented milk products into a large product range with different properties such as: reduced/lower fat and calorific content, extended shelf-life and additive free. In addition, with the introduction of health promoting probiotic bacteria in the market, people can choose the products with more health-related properties.

2.1.2 Probiotics

The word probiotic comes originally from the Greek 'pro bios' which means 'for life' (Fuller, 1989) and a number of definitions have been used over the years. Today the definition of probiotics coming from Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) is considered to be the most authorized and they define probiotics as 'live microorganisms' which, when administered inadequate amounts, confer a health benefit on the host' (Moriya *et al.*, 2006).

The inventor of probiotics is considered to be Elie Metchnikoff, a Nobel Prize winner, who proposed a link between longevity and the consumption of lactic bacteria *Lactobacillus (L) delbrueckii* subsp. *bulgaricus* and *Streptococcus* (*S.*) *thermophilus)* present in yoghurt. He suggested that these bacteria suppress the putrefactive-type fermentations of the intestinal flora that contribute to aging and toxification (O' Toole & Cooney, 2008).

Scientists later found that *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cannot survive in the gastro-intestinal tract and therefore their beneficial effects are restricted. Instead the scientists turned their interest to the lactic bacteria of intestinal origin and several probiotic bacteria species (such as *L. acidophilus*) were then isolated from intestine of healthy people (Soccol *et al.*, 2010).

To exert to any probiotic effect, a microorganism has to meet strict criteria (Kailasapathy & Chin, 1999; Heller, 2010):

1) They have to be naturally resistant to gastric acid (such as stomach acid and bile acid pH ranging from 1 to 4).
2) They have to survive the action of bile salts and digestive enzymes (such as lysozymes) present in the intestines.
3) They have to be totally safe for the host and give beneficial effects on human health.
4) They have to arrive in the intestines in sufficient quantities and be able to adhere to the wall of the intestines in order to give an effect.

2.1.3 Health benefits

The human gastro-intestinal tract (GIT) possesses a dynamic bacterial ecosystem, which consists of up to10^{14} bacteria and more than 400 species, some of which play important roles in the digestive process. These microorganisms affect human health in harmful, favorable or neutral ways. The metabolism of harmful bacteria (such as, *Escherichia* (*E.) coli* and *Clostridium* spp.) is putrefactive and they produce a variety of harmful substances, such as amines, hydrogen sulfide or phenols, causing toxification and certain intestinal problems (Lourens-Hattingh & Viljoen, 2001).

The metabolism of beneficial probiotic lactic acid bacteria is non-putrefactive. In the course of their proliferation and survival in the GIT, probiotic lactic acid bacteria act in a favorable way at different levels. They change the metabolic properties of intestinal microbiota by competing for nutrients with other putrefactive microorganisms; they create unsuitable conditions for putrefactive microorganism by producing antagonistic substances, such as lactic acid and bacteriocins; they take part in the pre-digestion of food and produce different vitamins that improve the bioavailability of minerals (Sander, 2000; Fairclough, 2008).

To get obvious therapeutic benefits, it is recommended that minimum 10^6 cfu / g probiotic bacteria should be present in product. In order to compensate for the number of probiotic bacteria that lose in the transit, consumers are recommended to intake 100 g per day of probiotic milk products containing totally 10^9 cfu probiotic bacteria (Rybka & Kailasapathy, 1995).

Figure 2.1 shows schematically the beneficial effects of probiotic bacteria may have on humans.

The established health benefits of probiotics include:

1) Improved lactose digestion. Some probiotic bacteria (such as *L. acidophilus*) are a source for β-galactosidase (lactase) which is an enzyme needed for lactose digestion (Tamime, 2005). These bacteria begin to break down lactose early when they arrive in the intestine and they can exert their lactase activity in vivo in the gut lumen (McDonough, 1987). For example, a study shows that lactose intolerant people shows better tolerance to milk fermented with *B. longum*, *L. acidophilus* La-1or *L. acidophilus* NCFM compared to milk without probiotic bacteria (Jiang *et al.*, 1996; Jiang & Savaiano, 1997; Sanders & Klaenhammer, 2001).
2) Prevention of and shortening the duration of several types of diarrhea. A study shows that in the course of diarrhea, the composition of intestinal micro-flora changed remarkably. The number of *Bifidobacterum* spp. and *E. coli* decrease dramatically and *Candida* spp. increases. Probiotic bacteria such as *L. casei* and *B. bifidum* can effectively restore the micro-ecological balance and they are proved to have therapeutic effect on post-burn diarrhea in children (Chen *et al.*, 1999). Some other studies have showed that *L. rhamnosus GG*, *B. lactis* Bb-12, and *Enterococcus faecium* SF68 have potential effect on antibiotic associated diarrhea caused by *Clostridium difficile* (Gismondo *et al.*, 1999; D'Souza *et al.*, 2002).
3) Enhancement of immune system. Probiotic bacteria have been shown to increase: B-lymphocytes, IgA-, IgG- and IgM-secreting cells which help to defense foreign matter and increase antibody activity. For example, *B. lactis* N019 are shown to significantly enhance two different cellular immune response namely phagocytosis and tumour killing. (Chiang *et al.*,2001)

In addition, several potential health benefits of probiotics are pointed out by some studies. For example, *L. acidophilus* L1 and *L. plantarum* PH04 was shown to have

hypocholesterolemic effects (Anderson *et al.*, 1999; Nguyen *et al.*, 2007); *B. animalis* DN-173010 was shown to be able to shorten the gut transit time and therefore prevent occurrence of constipation (Bouvier, 2001; Meance *et al.*, 2001). *B. longum* ATCC15708 and *L. acidophilus* ATCC4356 are shown to have anti-oxidative effect and alleviate inflammation (Lin & Chang, 2000).

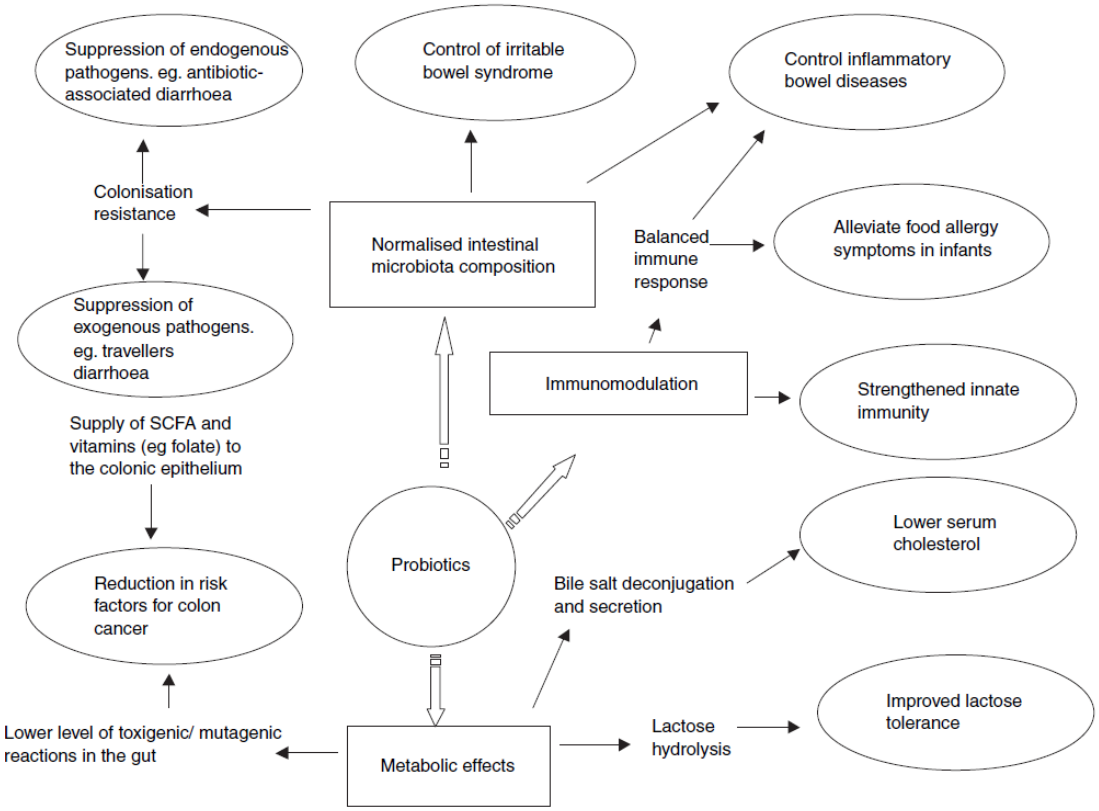


Figure 2. 1 Schematic representation of the functional activities and health benefits of Probiotics (Parvez *et al.*, 2006)

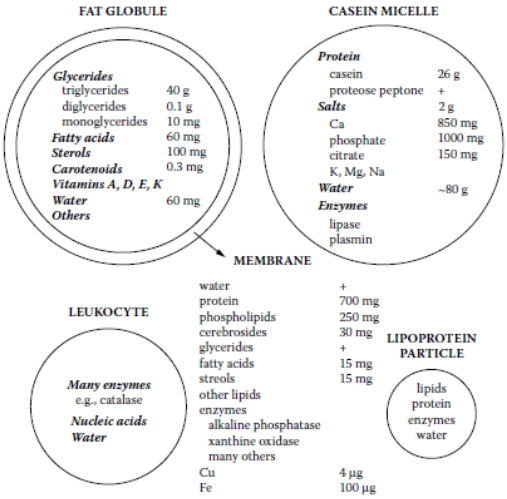
2.1.4 Milk as raw material

The milk of some animals, such as cows, sheep, goats and buffalos, has been used for human consumption for thousands of years. Liquid raw milk is composed of about 87% water and 13% solid (the content of dry mater) including fat, protein, sugar, vitamins, mineral substances and organic acids.

Table 2.1 gives a survey of the average composition and structure of milk. Chemical composition determines to a large extent, the nutritional value, flavor, or technological properties of milk and dairy products. Variation depending on season, stage of lactation, feeding, health status of cow and genetic factors could potentially offer problems and opportunities for dairy manufactures (Walstra *et al.*, 2006).

Table 2. 1 Composition and structure of milk (Walstra *et al.*, 2006)

Composition and Structure of Milk[a]



water	+
protein	700 mg
phospholipids	250 mg
cerebrosides	30 mg
glycerides	+
fatty acids	15 mg
streols	15 mg
other lipids	
enzymes	
alkaline phosphatase	
xanthine oxidase	
many others	
Cu	4 µg
Fe	100 µg

SERUM

Water	790 g	***Organic acids***		***Proteins***	
		citrate	1600 mg	casein	+
Carbohydrates		formate	40 mg	β-lactoglobulin	3.2 g
lactose	46 g	acetate	30 mg	α-lactalbumin	1.2 g
glucose	70 mg	lactate	20 mg	serum albumin	0.4 g
others		oxalate	20 mg	immunoglobulins	0.8 g
		others	10 mg	proteose peptone	+
Minerals				others	
Ca, bound	300 mg	***Gases***		***Nonprotein nitrogenous***	
Ca, ions	90 mg	oxygen	6 mg	***compounds***	
Mg	70 mg	nitrogen	16 mg	peptides	+
K	1500 mg	***Lipids***		amino acids	50 mg
Na	450 mg	glycerides	+	urea	250 mg
Cl	1100 mg	fatty acids	20 mg	ammonia	10 mg
phosphate	1100 mg	phospholipids	100 mg	others	300 mg
sulfate	100 mg	cerebrosides	10 mg	***Enzymes***	
bicarbonate	100 mg	sterols	15 mg	acid phosphatase	
		others		peroxidase	
Trace elements				many others	
Zn	3 mg	***Vitamins, e.g.***		***Phosphoric esters***	~300 mg
Fe	120 µg	riboflavin	2 mg	***Others***	
Cu	20 µg	ascorbic acid	20 mg		
many others					

[a] Approximate average quantities in 1 kg milk. *Note*: The water in the casein micelles contains some small-molecule solutes.

2.1.5 Probiotic fermented milk

Dairy products are considered to be the best vehicle for probiotic lactic acid bacteria. This is because fermented dairy products already have a high reputation as being healthful and customers have already accepted the fact that fermented products contain viable microorganisms.

Probiotics must meet several basic requirements in order to be incorporated in marketable probiotic products (Heller, 2010):

1) Probiotic bacteria should survive in the food matrix and their number and beneficial effects should be maintained in a sufficient level in the products during storage;
2) They should not have any changes on their physical and genetic properties during production, transportation and storage.
3) They should not have negative effects on the taste or aroma in the final products.
4) They should not accelerate post acidification during the shelf-life of the product.

In addition, the manufacturer of probiotic fermented products should have reliable methods for identification and evaluation of probiotic bacteria. They should also modify the process of traditional fermented milk production in order to guarantee the survival of probiotics microorganisms.

2.1.6 Acid gel formation

About 80% of protein consists of casein which is a mixture of four proteins (αs1, αs2-, β-, and κ-caseins) dispersed in water phase of milk. These protein units are held together by colloidal calcium phosphate (CCP) in the form of casein aggregates called casein micelles. During acidification of milk by a starter culture, casein micelles begin to undergo a physical-chemical change when pH is decreased to 5.5-5.0. In the course of pH reduction, CCP is solubilized and the charged 'hairs' of κ-caseins begin to shrink. Electrostatic repulsion between casein molecules is decreased when the pH of milk reaches the isoelectric point of casein (pH 4.6) and casein-casein attractions increase due to increased hydrophobic and plus-minus (electrostatic) charge interactions (Horne, 1998). This results in the formation of three-dimensional network consisting of clusters and chains of caseins (Walstra *et al.*, 2006; Lee & Lucey, 2010).

Figure 2.2 shows the casein micelle in the sub-micelles model showing the protruding C-terminal parts of κ-caseins as proposed by Holt & Horne, but the real structure of casein micelle is still under debate.

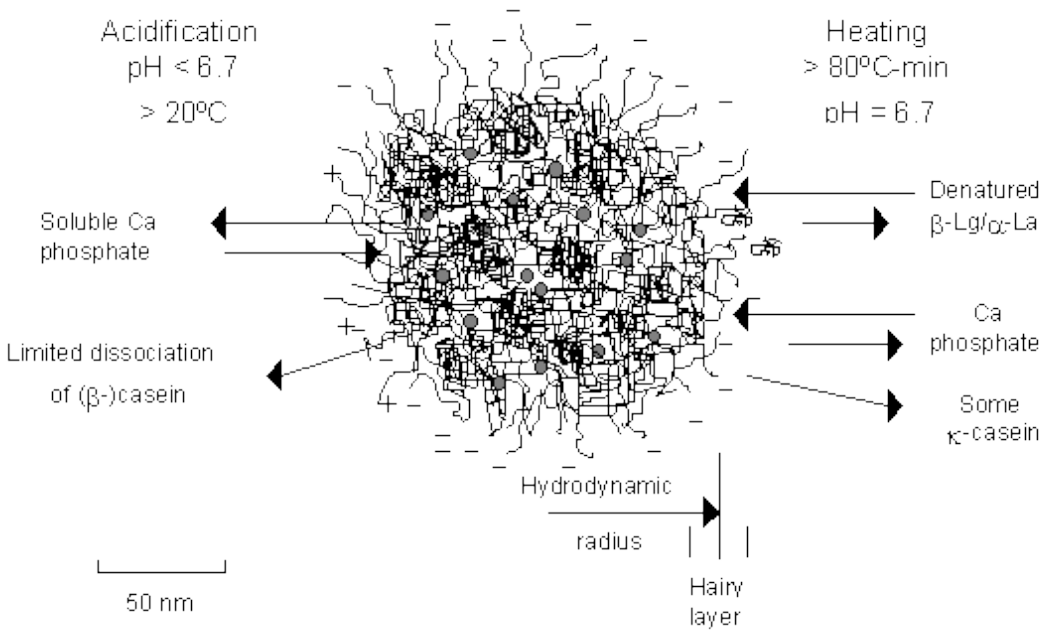


Figure 2. 2 Structure of casein micelle proposed by Holt and Horne (Holt & Horne, 2006)

In the production of stirred yoghurt or drinking type fermented milk product, fermentation is often followed by mixing, pumping, cooling and filling giving a smooth and viscous texture to product. The three dimensional network of protein will be partially destroyed under these processes and broken down into dispersion of gel pieces. Under storage these pieces of gel will try to form a new work under the force of intra-particle interaction and this phenomenon is called rebodying of yoghurt (Renan *et al.*, 2008).

2.1.7 Production of probiotic fermented milk products

Generally, fermented milk products dominating the market can be divided into two types: set-type and stirred-type. Set-type fermented milk products are characterized by a firm, gel-like structure, while stirred-type fermented milk products (as for instance, TINE 'Biola Pluss Yoghurt Mild Naturell') are characterized by a thick viscous consistency. In addition, a pourable stirred type called drinking type fermented milk (as for instance, 'Cultura Naturell' and 'Biola Syrnet Lettmelk Naturell') is becoming popular in the market. Despite their different properties, processes for production of these products are not radically different (Tamime & Robinson, 2000).

Figure 2.3 shows schematically different stages involved in production of probiotic yoghurt.

Generally, for production of probiotic yoghurt, fresh cow milk is first preliminary treated. It is preheated to 55 ℃ to 60 ℃ and goes through separation process in order to separate skim milk and cream. The fat content in milk is standardized to 4% by adding cream and milk solid-not-fat (SNF) is concentrated to 12%. Certain amount of milk solid including mainly lactose and milk protein can also be added to enhance the SNF level (Christopher *et al.*, 2008).

The standardized milk is then heated to 65 ℃ and homogenized at 20–25 MPa. Unlike traditional plain yoghurt, certain amount sugar is added after homogenization in the production of probiotic plain yoghurt and the milk is pasteurized at 95 ℃ for 5 min in order to remove any microbial infection under homogenization. The milk is then cooled down to 43 ℃ (Christopher *et al.*, 2008).

Probiotic set yoghurt is obtained by adding probiotic culture suspensions, packaging yoghurt milk in cups with lids and incubating at 41 ℃ for 4 to 4.5 hours in order to obtain of a pH round 4.7. Probiotic stirred yoghurt is obtained by incubating yoghurt milk under the same condition but in a bulk fermentation tank. Milk is added with yoghurt culture and probiotic culture suspension, stirred after coagulation and is packaged in cups. Both types of yoghurt are then cooled down to 4 ℃ for storage (Tamime & Robinson, 2000; Tamime, 2005; Christopher *et al.*, 2008).

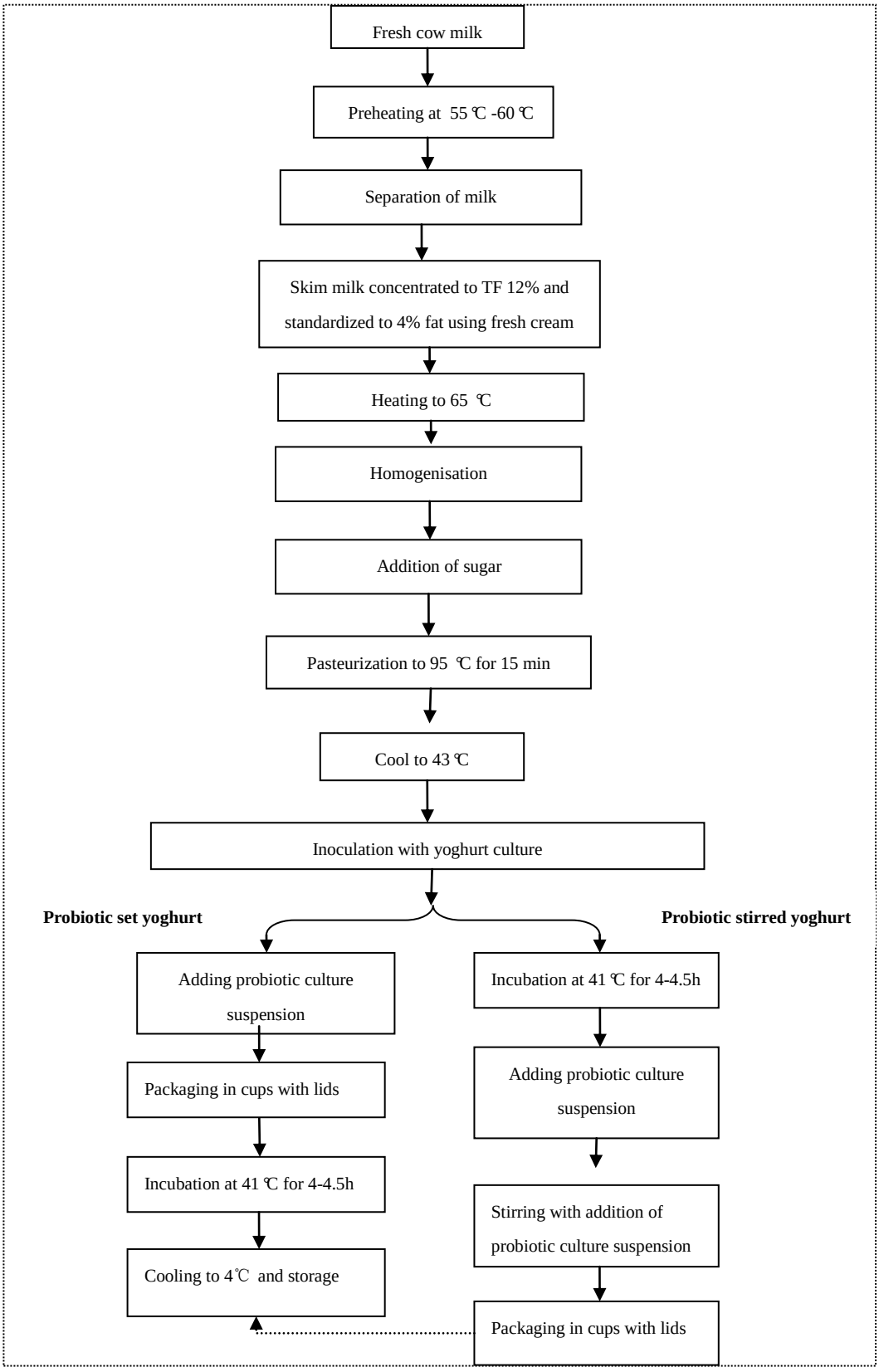


Figure 2.3An outline of various stages in the manufacture of probiotic yoghurt. (Adapted from Christopher *et al.*, 2008).

2.1.8 TINE probiotic fermented milk products.

TINE BA is Norway's largest producer, distributor and exporter of dairy products and a leader in probiotic development in Norway. In 1994, TINE BA obtained the license of Valio's *L. rhamnosus* GG strain (LGG ®) and launched the new range Biola in 1997. Today, the Biola range provides a big variety of yoghurt products, fermented low-fat milk and flavored fermented milk to which Valio's *L. rhamnosus* GG strain is added (Valio, 2009).

'Cultura Naturell' from TINE is produced by adding concentrated probiotic bacteria *L. acidophilus* La-5 and *B. lactic* Bb-12. 'Biola Syrnet Lettmelk Naturell' was previously called ABC milk and is produced by adding concentrated probiotic bacteria *L. acidophilus* La-5, *B. lactis* Bb-12 and *L. rhamnosus* GG. Skim milk (1.5% fat) is used as raw material and both of these products belong to the drinking milk which has lower milk solid content than ordinary yoghurt. The designated shelf-life of TINE 'Cultura Naturell' and TINE 'Biola Syrnet Milk Naturell is 3 weeks (Alnes, 2011).

TINE 'Biola Pluss Yoghurt Mild Naturell' is produced by adding probiotic bacteria *L. acidophilus* La-5, *B. lactis* Bb-12 and *L. rhamnosus* GG in addition to yoghurt culture. A level of extra milk solid in form of skimmed milk powder (2.5%) is added to full fat milk to make it more nutritious than many other dairy products. Sucrose (2%) is added to enhance the sweetness of TINE Biola Yoghurt. Inulin (2.9%), which has been shown to improve the viability of some probiotic bacteria strains (Donkor *et al*., 2007) is added claimed to increase the fiber content in yogurt. The designated shelf-life of 'Biola Pluss Yoghurt Mild Naturell' is 5 weeks (Alnes, 2011) (appendix 1 and 2).

Figure 2.4 shows products of TINE Cultura range and Biola range in the Norwegian market.

Figure 2. 3 TINE probiotic fermented milk products. Cultura range (left) (http://www.handelsbladetfk.no/id/21762). Biola range (right) (http://www.facebook.com/note.php?note_id=385259901891)

2.1.9 Quality and shelf life

The overall properties such as production of aroma compounds, texture characteristics, acid level, sensory properties and nutritional value are important for evaluating the quality of a food product. Determination of shelf-life is largely based on commercial experience or the use of predictive model. By monitoring the changing of the properties of a product under storage until they are unacceptable for consumption, a manufacturer predicts the shelf-life of a fermented milk product (Mataragas *et al.*, 2010).

However, quality deterioration of a fermented milk product can occur within very short time if it is produced using poor manufacturing practices or stored in insufficient conditions. Negative properties such as syneresis, appearance defects, atypical texture / mouth feel, loss of flavor, and post acidification are considered to be unacceptable for consumption and they are important indicators for quality deterioration. For probiotic fermented products, viability of probiotic bacteria is an additional important criterion for determination of shelf-life. This is because probiotic fermented milk products are not only produced to give enough nutrients or satisfy customer through their sensory properties. They also have to possess living probiotic bacteria in sufficient numbers in order to exert their health beneficial effects under consumption. It has been suggested that the probiotics should be present in a food to a minimum level of 10^6 cfu/g (Robinson, 1987).

Studies show that the viability of probiotic bacteria depends largely on the bacteria strains used, the interaction between different bacteria species, and the water activity of the food matrix. Availability of nutrients in the food matrix, presence of oxygen, acidity, redox potential, production of hydrogen peroxide, permeability of the package and storage temperature can also be factors that affect the viability of probiotic bacteria (Tamime, 2005).

2.2 Lactic acid bacteria

Lactic acid bacteria (LAB) are a large group of bacteria that produce mainly lactic acid as result of anaerobic carbohydrate fermentation. They have similar properties such as being gram-positive, non-motile, non-spore forming and in form of cocci, coccobacilli or rods. They are non-respiratory and cannot produce certain chemical compounds such as catalase and cytochromes. They grow well in anaerobic conditions, but unlike many other anaerobic bacteria, some LAB species can grow in the presence of oxygen (Walstra *et al.*, 2006).

The most important members of LAB are the genera *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus* and *Vagococcus* (Walstra *et al.*, 2006).

2.2.1 Sugar fermentation

All fermented milk products are produced utilizing the souring activity of lactic acid bacteria (Walstra *et al.*, 2006). Lactose is the main source of carbon and energy for the microorganisms in milk and it can be converted into lactic acid and other products by most of lactic acid bacteria under fermentation. Other kinds of sugar, such as sucrose can also be converted by certain lactic acid bacteria species depending on the enzymes available in the bacteria.

Figure 2.5 shows the molecular structures of carbohydrate lactose and sucrose. Both lactose and sucrose are disaccharides and their common empirical formula is $C_{12}H_{22}O_{11}$. Lactose is also called milk sugar and is the main source for lactic acid fermentation. One molecule of lactose consists of one molecule of glucose and one molecule of galactose. Sucrose is obtained from sugar cane or sugar beets commercially and is widely used as sweetening agent in food industry. One molecule of sucrose consists of one molecule of glucose and one molecule of fructose (Coultate, 2002).

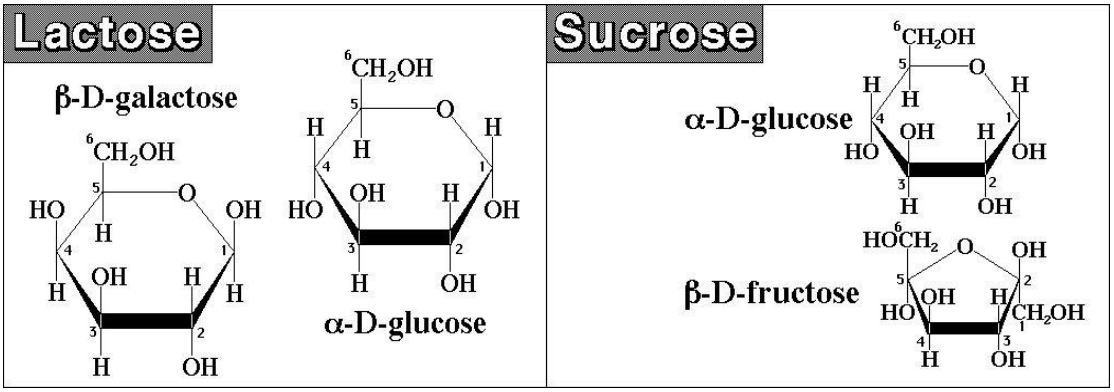


Figure 2. 4 Molecular structures of lactose (left) and sucrose (right) (http://www.hcc.mnscu.edu/chem/V.25/page_id_32265.html)

Unlike respiration, fermentation process does not require the presence of oxygen and therefore less energy is produced. A fermentation pathway always starts with sugar and finishes with various end products. In addition there are intermediate compounds produced along the metabolic pathway as one compound is converted into another. Pyruvate is considered to be the most important intermediate compound. In the metabolism of pyruvate, different chemical compounds, such as lactic acid, formic acid, acetic acid, ethanol and acetoin, are produced depending on the metabolic path way utilized by the bacteria and the kind of sugars available.

Depending on the metabolic pathway that is utilized in sugar fermentation, lactic acid bacteria can be divided into three different groups.

The first step in the metabolism of lactose is the transportation of lactose into the bacteria cell either via the phosphoenol pyruvate phosphotransferase system (PEP-PTS) (typical for lactococci) or lactose permease system.

During sucrose fermentation, it is transported into bacteria cell by a permease system and cleaved by sucrose hydrolase to glucose and fructose. In some lactococci, sucrose is transported by PTS forming sucrose-6-phosphate. Sucrose-6-phosphate is then cleaved by sucrose-6-phosphate hydrolase to glucose-6-phosphate and fructose. This enzyme will be induced when sucrose is present in the medium (Thompson & Chassy, 1981).

For further metabolism, Homo-fermentative LAB (such as lactococci) produce only lactic acid as the end product of carbohydrate fermentation. When there is excess glucose and limited oxygen in the environment, glucose is metabolized via the glycolytic or Embden-Meyerhof (EM) pathway and galactose-6-phosphate is metabolized via the tagatose pathway. Enzyme aldolase is characteristic in this process. Some thermophilic LAB cannot metabolize galactose and they excrete galactose out of cell as a metabolic shunt for uptake of lactose. The mechanism of homo-fermentative pathway can be simply explained as that a hexose is split into two identical 3-carbon molecules, which are transformed into lactic acid molecules in the following reaction sequences.

Hetero-fermentative lactic acid bacteria, such as *Leuconostoc* species and some of the *Lactobacillus* species also produce lactic acid as the major end product, but in addition they also produce ethanol, acetic acid and CO2 as end products. Glucose is metabolized via the phosphoketolase pathway. Galactose is first transformed into glucose-1-phosphate via the Leloir rote, which then enters the phosphoketolase pathway. One carbon is released in the form of CO2 from hexose leaving a pentose. Pentose is then split into one 3-carbon units which are converted into lactic acid and one 2-carbon units, and they will be then converted into ethanol or acetic acid. Important enzymes involved in the hetero-fermentative metabolism of *Leuconostoc* species are glucose-6-P dehydrogenase and phosphoketolase.

In addition to homo- and hetero- fermentative pathways, there are certain lactic acid bacteria (often lactobacilli) which perform a so-called facultative hetero-fermentative pathway. These bacteria are not restricted neither of the two pathways and which pathway is used depends on the sugar available and condition in the environment (Tamime & Robinson, 2000; Walstra *et al.*, 2006).

Figure 2.6 shows the different fates of pyruvate in energy metabolism of sugar under lactic fermentation.

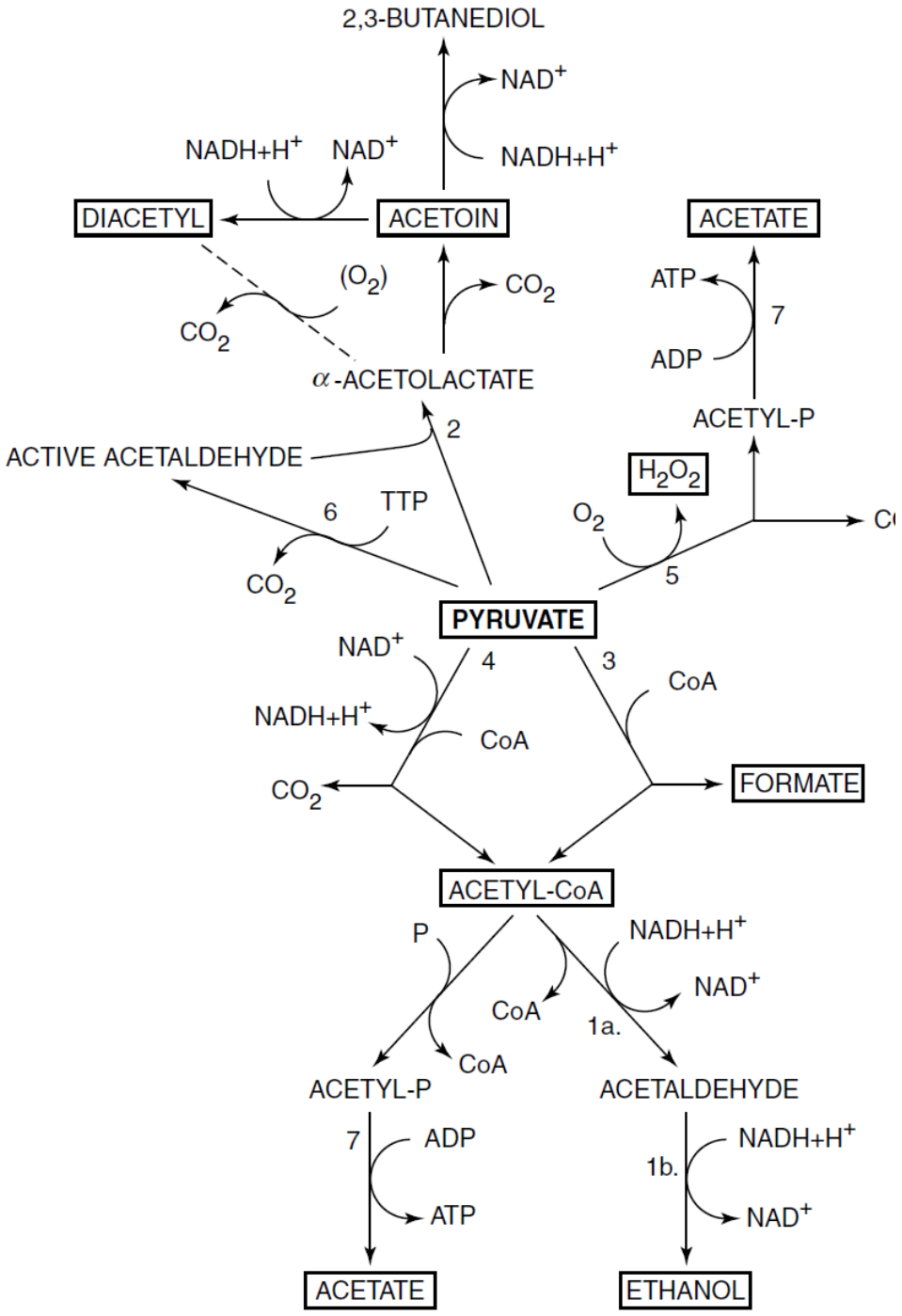


Figure 2. 5 Pathways for the alternative fates of pyruvate. Dashed arrow denotes a non-enzymatic reaction. Important metabolites and end products are framed. Selected enzymatic reactions are numbered: (1a) acetaldehyde dehydrogenase, (1b) alcohol dehydrogenase, (2) acetolactate synthase, (3) pyruvate formate lyase, (4) pyruvate dehydrogenase, (5) pyruvate oxidase, (6) pyruvate decarboxylase, and (7) acetate kinase. (Adapted from Axelsson, 1998)

2.2.2 Citrate metabolism

The major activity of LAB under fermentation is their catabolism of sugar, but some species also have the ability to metabolize citrate which originates naturally in milk.

The citrate is firstly transported into the cell membrane through a specific membrane protein and converted subsequently into acetate and oxaloacetate by enzyme citrate lyase. Oxaloacetate can be converted into pyruvate, which can be later metabolized into various compounds. The major end products of citrate metabolism are 4-carbon compounds, mainly diacetyl, acetoin and butanediol depending on bacteria strains and growth conditions (Quintans *et al.*, 2008).

Figure 2.7 shows the different stages involved in citrated utilization. Three stages are involved in citrate metabolism: 1) transportation of citrate by pemease. 2) its conversion into oxaloacetate by citrate lyase and 3) its further conversion to pyruvate and CO2. Metabolism of pyruvate will produce more different end products.

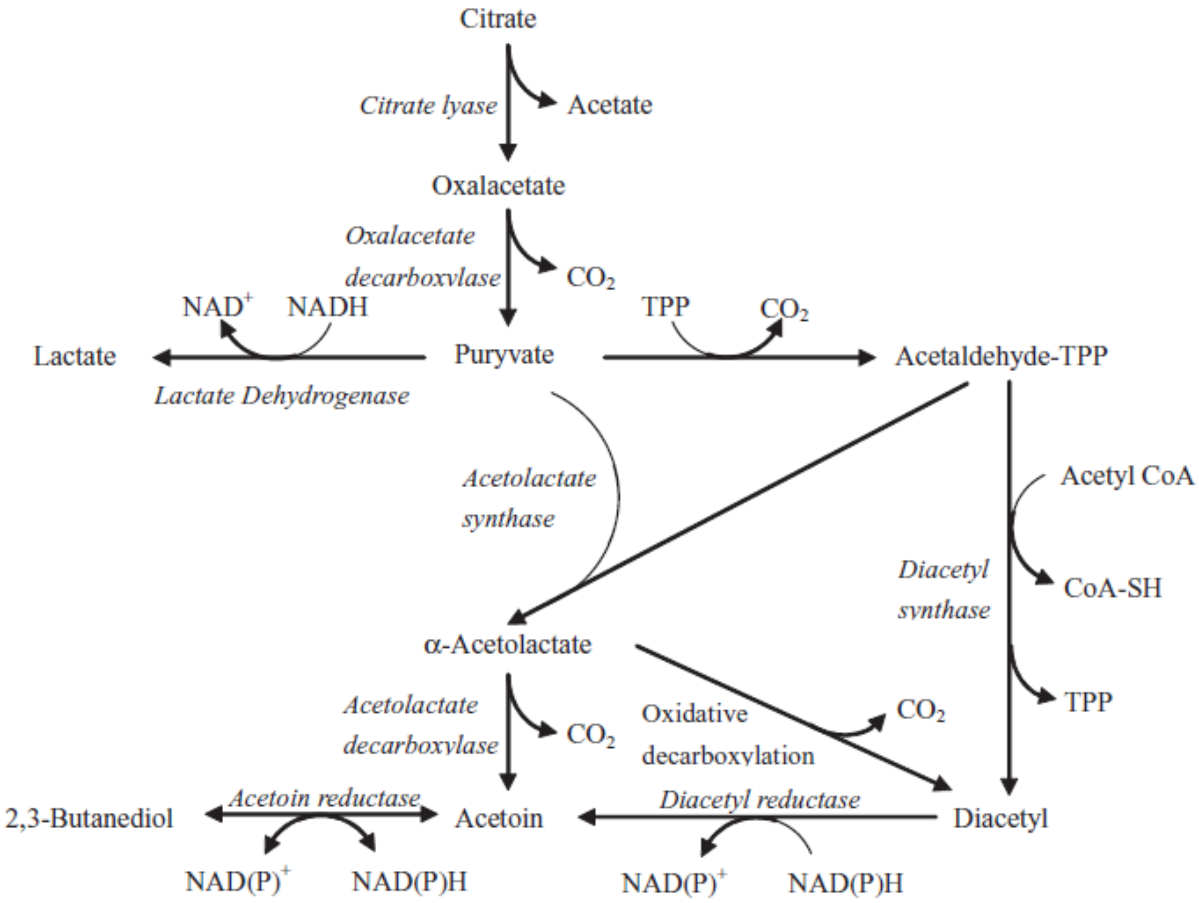


Figure 2. 6 Citrate utilization pathways in bacteria and its possible end products (Adapted from Fox & McSweeney, 1998 and McSweeney & Sousa, 2000).

2.2.3 Genus *Streptococcus*

The members of this genus are gram-positive spherical or ovoid cells, organized often in pairs and chains. They are facultatively anaerobic and ferment carbohydrates homofermentatively with major production of lactic acid. Streptococci can also produce small amounts of acetic and formic acids, ethanol and carbon dioxide (Hardie & Wiley, 1994).

*Streptococcus (S.) thermophilus i*s the only streptococcus that has been intensively used for commercial purposes. The most important characteristics of *S. thermophilus* for its commercial usage, is that it can survive pasteurization (72℃, 15s) and grows at temperatures up to 52 ℃. In addition, production of exopolysaccharides by certain *S. thermophilus* strains is important in the enhancement of yoghurt texture (Bridge & Sneath, 1999; Welman & Maddox, 2003).
Figure 2.8 shows the cell morphology of *S. thermophilus* observed by using scanning electron microscopy.

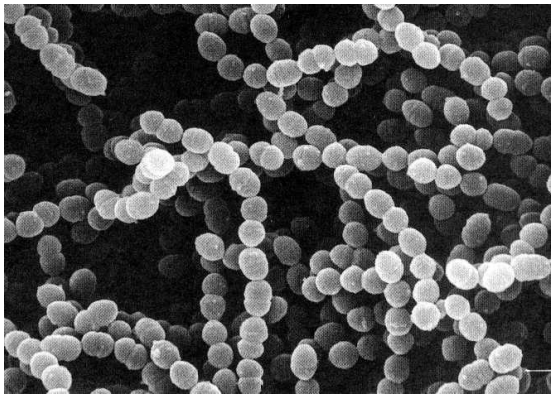


Figure 2. 7 Scanning electron micrograph of *S. thermophilus* (http://www2.unibas.it/parente/Starter/gruppi.html)

2.2.4 Genus *Lactobacillus*

The members of this genus are gram-positive, non-sporing rods or coccobacilli. They are micro-aerophilic and lack catalase. They are able to live in a highly acidic environment with pH 4-5 and are responsible for the final stages of fermentation in products. Lactobacilli have complex nutritional requirements and need rich media to grow. Lactobacilli comprise about 25% of all intestinal micro-flora and more than 100 species have been described (Felis & Dellaglio, 2007).

L. delbrueckii subsp. *bulgaricus* is gram-positive, and has very slender and long rods. It has an optimum growth rate at 42 ℃ and grows best under anaerobic and acidic (pH 4.6-5.4) conditions; *L. delbrueckii* subsp. *bulgaricus* is responsible for the production of acetaldehyde, which is a main contributor of the characteristic flavor in yoghurt. It

dominates the final stage of yoghurt fermentation and its metabolic activities under low pH are considered to be the reason for post acidification of yoghurt (Walstra *et al.*, 2006).

Figure 2.9 shows the cell morphology of *L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus* observed by using scanning electron microscopy.

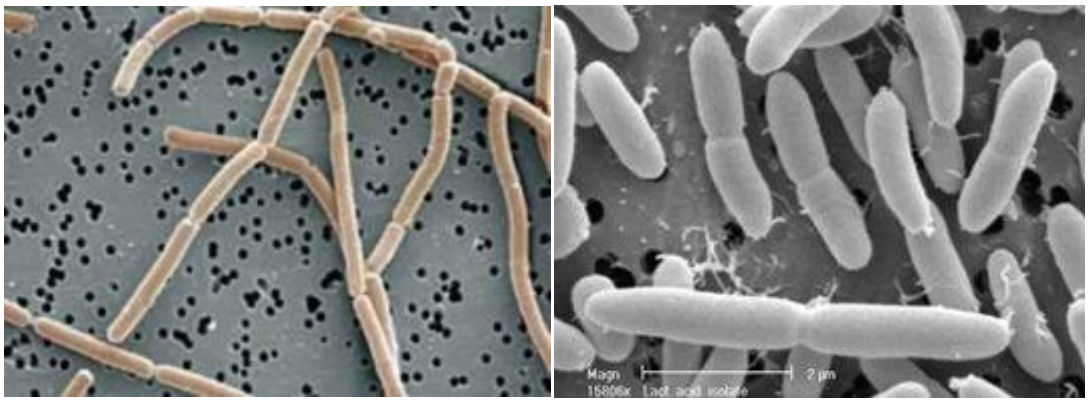


Figure 2. 8 Scanning electron micrograph of *L. delbrueckii* subsp. *bulgaricus* (left) and *L. acidophilus* (right).
(http://microbewiki.kenyon.edu/index.php/Lactobacillus;
http://www.musee-afrappier.qc.ca/fr/index.php?pageid=3114c&image=3114c_lactobacillus)

L. acidophilus is a component of the normal intestinal flora of healthy humans. They are Gram-positive rod-shaped, non-motile, non-spore forming bacteria with rounded ends. Typically, the cells are 0.6-0.9 μm in width and 1.5-6.0μm in length. It grows in or without presence of oxygen, but its growth is enhanced by anaerobic conditions. *L. acidophilus* exists either as single cell, in pairs or in short chains. Its optimum growth temperature is between 35- 40 ℃ and its optimum pH is between 5.5-6.0 (Shah, 2000). Henneberg from Kiel, Germany was the first who proposed the use of a combination of *L. acidophilus* and yoghurt culture to produce a so-called *Acidophilus – Milch* in the early 1980s'. This product finally became a big success in the German market under the name of 'yoghurt mild' (Heller, 2010).

L. rhamnosus GG was isolated from a healthy person by Gorbach and Goldin in 1987 and is the most clinically studied probiotic bacterium. The strain grows best under anaerobic conditions, but it grows also in the presence of CO_2. It does not ferment lactose or sucrose (Goldin *et al.*, 1992; Ouwehand *et al.*, 2002). *L. rhamnosus* GG survives but does not grow in fermented milk stored at 4 ℃ (Tamime, 2005).

Figure 2.10 shows the cell morphology of *L. rhamnosus* GG observed by using

scanning electron microscopy.

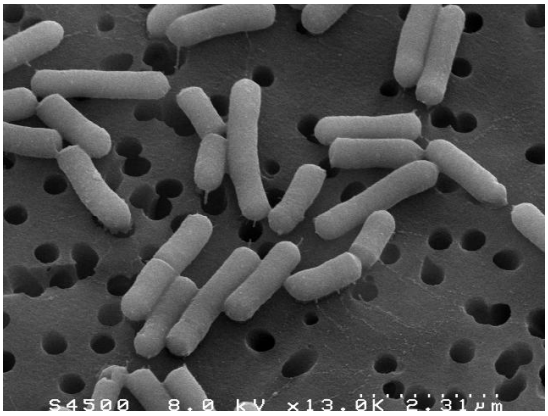


Figure 2. 9 Scanning electron micrograph of *L. rhamnosus* GG (http://www.geneferm.com/b5/Lactic_Acid_Bacteria.htm)

2.2.5 Genus *Bifidobacterium*

Bifidobacteria were first found in intestinal tract of new infants by Tissier in 1900. They are Gram-positive, non-spore forming, non-motile rods, often Y-shape or clubbed at the end. They are strictly anaerobic and lack catalase. *Bifidobacterium* is pleomorphic fermentative and it produces acetic acid in addition to lactic acid in the molar ratio of 3:2. The optimum growth temperature for bifidobacteria is 37 to 41 ℃ and the optimum pH is 6.5 to 7.0 (Scardovi, 1986; Shah, 2000).

Figure 2.11 shows the cell morphology of Bifidobacteria observed by using scanning electron microscopy

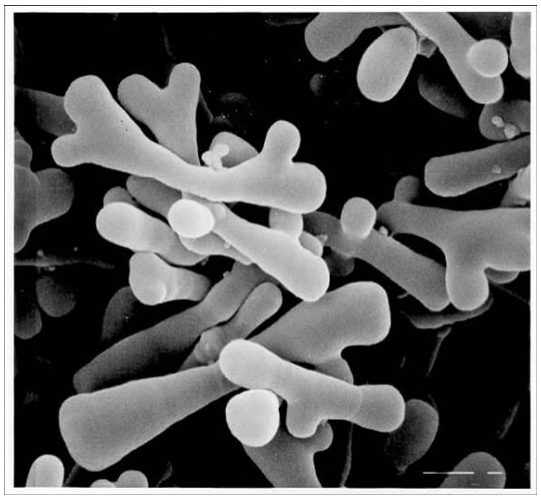


Figure 2. 10 Scanning electron micrograph of Bifidobacteria http://oncologiaesalute.wordpress.com/2008/02/17/i-probiotici-sono-letali-in-caso-di-pancreatite-acuta/

In some studies, a dramatic decline in the number of bifidobacteria was observed in some studied concentrated on commercial probiotic fermented products (Shah *et al.*,

1995; Iwana, 1993; Moriya *et al.*, 2006), this is because *Bifidobacterium* spp. requires a very rigorous growth conditions.

2.2.6 Yoghurt culture

Using a starter culture containing equal number of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* is generally accepted in the production of yoghurt. (Walstra *et al.*, 2006).

Figure 2.12 shows how *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* stimulate each other in growth and acid production.

L. delbrueckii subsp. *bulgaricus* provides *S. thermophilus* with amino acids through its proteolytic activity. The growth of lactobacilli in turn is promoted by formic acid and CO2 produced by *S. thermophilus*.

Once they are added into products, ratio between them begins to change. The number of *S. thermophilus*, which initiates the fermentation process, will increase in the beginning until redox potential of the milk medium is reduced to a low level and the pH is lowered to around 5.5. Growth of *L. delbrueckii* subsp. *bulgaricus* is then enhanced. When pH is lower than 5.0, its number will exceed *S. thermophilus* and dominantly acidify the yoghurt to a pH near 4.6 (Walstra *et al.*, 2006).

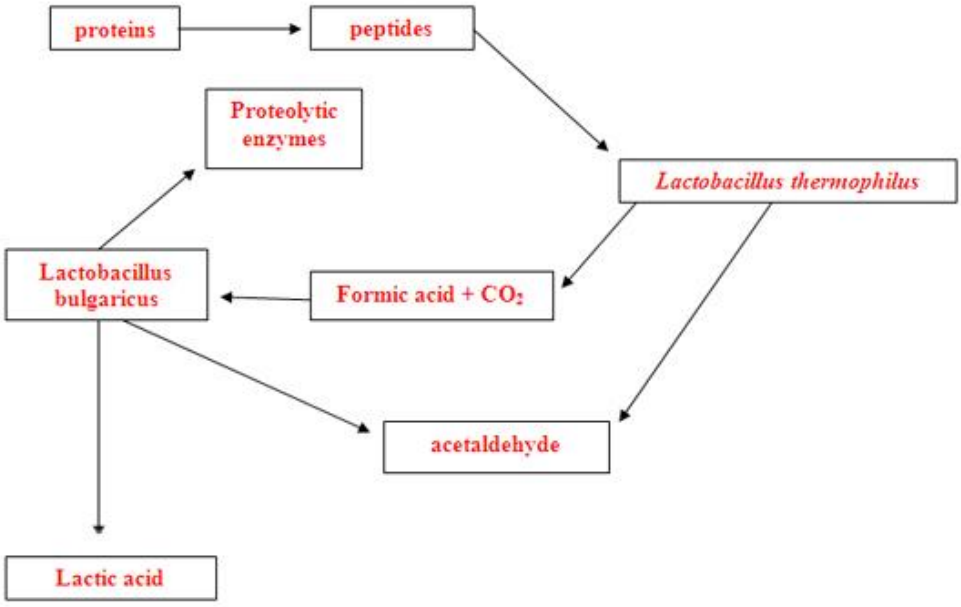


Figure 2. 11 Protocooperation interactions between yoghurt bacteria *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (http://www.biotechnology4u.com/industrial_microbia_food_beverage.html).

2.2.7 Commercial probiotic bacteria strains and cultures

There are already more than 90 probiotic products containing one or several probiotic organisms available worldwide (Shah & Tharmaraj, 2003). Table 2.1 shows the different probiotic fermented milk products dominating in Europe market. As we can see from table 2.1, probiotic bacteria strains used in these products vary from country to country. In Norway the major commercial probiotic bacteria strains is *L. acidophilus* La-5 and *B. lactis* Bb-12.

Table 2. 2 Examples of probiotic fermented milk products in the European market and probiotic bacteria strains used in these products (Tamime, 2005).

Type of product and trade name	Probiotic microorganisms present in the products as stated by the manufacturer
1. Non-drinkable fermented milks Bifosoft, Bifidus, Bioghurt, Biofit, BiofardePlus, Biola ,Biologic Bifidus, Cultura Dofilus, Dujat Bio Aktiv, Ekollogis Jordgubbs Youghure, Fit & Aktiv, Fjallyoughurt, fysiq, Gaio dofilus, Gefilac, Gefilus, L1, Probiotisches joghurt, proViva, RELA, Verum,Vifit Vitamel, Vitality, Weight Watchers, yogosan Milbona.	*L. acidophilus*, *L. acidophilus* La5, *L. rhamnosus* GG, LB21 and 271, *L. casei* (also strain F19), *L. johnsonii*, *L. plantarum* 299v, *L. reuteri* *Lactococcus. lactis* subsp. *lactis* L1A *B. bifidum*, *B. aminalis* subsp. *lactis* BB-12, *B. animalis* subsp. *animalis*
2. Drinkable fermented milks (including cultured buttermilk, yoghurt drink, dairy drink) A-fil, Actimel, Aktifit, AB-piim, Bella Vita, Bifidus, Biofit, Biola, Casilus, Cultura, Emmifit, Everybody, Fit & Aktiv,Fundo, Gaio, Gefilac, Gefilus, Kaiku Actif, LC1 go, LGG+, Onaka, Oresundsfil, Philura, Probiotic drink, Proviva, Pro.x, Verum, Vikt Vaktarna, Vitality, Vive+, Yakult, Yoco acti-vit	*L. acidophilus*, *L. acidophilus* La5, *L. casei* (F19, 431, *Imunitass*, *Shirota*). *L. rhamnosus* GG (271 and LB21), *L. johnsonii*, *L. plantarum* 299v, *L. reuteri*, *L. fortis* *Lac. lactis* subsp. *lactis* L1A *B. aminalis* subsp. *lactis* BB-12, *B. bifidum*, *B. animalis* subsp. *animalis*, *B. longum* BB536
3. Non-fermented dairy products (milk, ice-cream) Gefilus, god Hals, RELA, Vivi Vivo	*L. rhamnosus* GG, *L. johnsonii*, *L. plantarum* 299v, *L. reuteri*

The possible combinations of bacteria strains are very many. In order to obtain unique and specific aroma and flavor in fermented milk product, it is important to choose appropriated bacteria strains according to their technologic and sensory properties during fermentation and after fermentation. Several factors have to be considered with selection of bacteria strains for use in a starter culture (Stenby, 1998):

1) Acidity: Bacteria strains determine the acidity of the end product depending on their ability to produce acid. Certain bacteria strains, such as *L. delbrueckii* subsp. *bulgaricus*, are able to produce acid at low temperature resulting in post-acidification during storage, which was not desirable.
2) Flavor: their ability to produce flavor compounds determines the flavor of the end products. For example, *L. delbrueckii* subsp. *bulgaricus* produces acetaldehyde which is indispensable for formation of yoghurt taste. *L. acidophilus* and *Bifidobacterium* spp. produce acetic acid, which in high concentration could result in flavor defect.
3) Viscosity: their proteolytic activity and ability to produce EPS contribute to gel formation and gel stability during storage.
4) Fermentation: the time required by for the fermentation affects the production process of products.

Fermented milk with single LAB strain is not common in market. Practically, in order to achieve desired properties of product, bacteria strains are mixed in different combinations. A culture with *L. acidophilus* and *Bifidobacterium* spp. are known as AB culture. A culture with *L. acidophilu*s and *Bifidobacterium* spp. and *L. casei*/ *L. rhamnosus* GG is known as ABC culture. Since time for fermenting milk with only AB and ABC culture is relatively long, yoghurt culture is often added to shorten the time of fermentation (Schlichtherle-Cerny *et al.*, 2008; Tamime, 2005).

2.2.8 Selective enumeration of probiotic bacteria

One of the technical restrictions for probiotic milk products is that methods for evaluation and enumeration of probiotic bacteria are not fully satisfactory. In practice, the plate count method is preferred by manufacturers in routine quality control of probiotic microorganism. The difficulty with this method is that multiple microorganism cultures are commonly used in the product to achieve desired product and presence of other bacteria micro flora often have negative influence on the discrimination among the different bacteria groups present in the same product. The developing of reliable selective media with high applicability and simplicity is always a big topic that many researchers are working on.

Recent studies concentrating on developing, evaluating and comparing selective agar for probiotic bacteria in commercial products have shown that the performance of a culture medium for selective enumeration of commercial probiotic strains depends strongly on the product matrix, the target group of microorganism and diversity of the bacterial background flora in the product (Van de Casteele *et al.*, 2005). When monitoring the viability of probiotic bacteria it is important to enumerate them differentially and this has been proved to be very difficult, especially for lactobacilli (Tharmaraj & Shah, 2003).

Several studies concentrating on the selective enumeration of probiotic bacteria in

commercial products, especially in the presence of yoghurt bacteria have been performed in Australia and Europe. In these studies, MRS-Mupirocin and MRS-NPNL medium with addition of cysteine are recommended in many of these studies for selective enumeration of *B. lactis* Bb-12 (Vinderola, 1999; Shah, 1999; Van de Casteele *et al.*, 2005; Moriya, 2006); M17 and ST medium is recommended for selective enumeration of *S. thermophilus*; pH modified MRS (pH 4.58) is recommended for selective enumeration of *L. delbrueckii* subsp. *bulgaricus* (Tharmaraj, 2003). MRS-Vancomycin medium is recommended for selective enumeration of *L. rhamnosus* GG (Tharmaraj, 2003); MRS-Clindamycin medium is recommended for selective enumeration of *L .acidophilus* (Van de Casteele *et al.*, 2005; Moriya, 2006).

2.2.9 Sensory profile of probiotic fermented milk products

Customers get the most important image of a commercial product from its sensory properties including color, consistency (texture and mouth feel), flavor, viscosity and so on.

Flavor is considered to be the most important sensory property for a food product. The concept flavor often involves a perception of taste (detection of nonvolatile compounds,) and odor (detection of volatile compounds) such as sweetness, sourness and aroma. In a sensory profile of fermented milk products, sweetness and sourness corresponded respectively to sugar and organic acid. Aroma was affected by flavoring compounds divided in four groups including non-volatile acids, volatile acids, carbonyl compounds and miscellaneous compounds (Tamime & Robinson, 2000).

Fructose, glucose and sucrose are the main sources for sweetness. However, in a plain fermented milk product, fructose and glucose are largely consumed by LAB. Hence sweetness can be hardly detected in such products. Probiotic natural yoghurt is sweeter than traditional natural yoghurt because extra sugar, such as sucrose in TINE probiotic yoghurt, is added under processing (Tamime, 2005).

The sourness of fermented milk products is affected by organic acids on different levels. Different acids give sourness in different degrees. For example, acetic acid is intensely sourer than lactic acid (Hartwig *et al.*, 1995). The sourness of a fermented milk product comes mainly from lactic acid because of its high level in the product. Production of acetic acid by *L. acidophilus* and *Bifidobacterium* spp. is the main reason for the sharp sour taste of AB milk (‘Cultura’) stored under suboptimal conditions (Tamime, 2005; Walstra *et al*., 2006). Organic acids can be instrumentally detected by High pressure liquid chromatography (HPLC).

Volatile compounds contribute mainly to the odor of a fermented milk product and hundreds of volatile compounds have been identified in plain yoghurt. A large number of them exist natively in cow’s milk and their level is affected by processing,

fermentation, storage and raw material; others are produced by starter cultures during fermentation and are affected by type of bacteria strains and their enzymatic and chemical transformation ability of sugar, lipid and protein. Despite the large number of volatile compounds in yoghurt, some of them exist only in trace amount and not all of them are of sensory importance.

Several studies shows that flavor compounds that contribute to the desired flavor of a fermented milk product are only few, such as acetaldehyde, ethanol, acetone, diacetyl and 2-butanol (Badings & Neeter, 1980; Tamime & Deeth, 1980; Marshall, 1984; Ulberth, 1991; Kneifel *et al.*, 1992; Ulberth & Kneifel, 1992; Marshall,1993; Tamime& Robinson, 2000).

Table 2. 3 Descriptor of important flavor compounds found in fermented milk products (Adapted from Cheng, 2010).

Volatile compounds	Flavor description	Typical concentration in yoghurt (mg /kg)	References
acetaldehyde	ethereal, fresh, green, pungent	23 – 40 Min. 8-10	(Rasic & Kurmann, 1978; Kang *et al.*, 1988; Gaafar, 1992; Kneifel *et al.*, 1992)
diacetyl	buttery, creamy, vanilla	0.2 - 3	(Marshall, 1984; Rysstad & Abrahamsen, 1987; Kang *et al*., 1988; Ulberth, 1991; Hernandez *et al.*, 1995; Pourahmad & Assadi, 2005;)
ethanol	mild, ether	0.2-9.9	(Hild, 1979; Rysstad & Abrahamsen, 1987; Ott *et al.*, 1999; Pourahmad & Assadi, 2005),
acetone	sweet, fruity	0.3- 4	(Vescovo, 1970; Hild, 1979; Ulberth, 1991; Ott *et al*., 1999; Pourahmad & Assadi, 2005; Tamime & Robinson, 2000; Yu & Nakanishi, 1975)
2-butanone	varnish-like, sweet, fruity	0.1-7	(Ulberth, 1991; Ott *et al.*, 1999; Pourahmad & Assadi, 2005;Kaminarides *et al.*, 2007),
2,3-pentanedione	butter, vanilla, creamy, mild		(Schlichtherle-Cerny & Oberholzer, 2007)
acetoin		1.2 – 28.2	(Beshkova *et al.*, 1998; Alonso & Fraga, 2001; Pourahmad & Assadi, 2005).
acetic acid	vinegary	0.5 -18.8	(Beshkova *et al.*, 1998; Suomalainen & Mayra-Makinen, 1999; Alonso & Fraga, 2001).

Figure 2.13 shows the molecular structure of major aroma compound in fermented milk products.

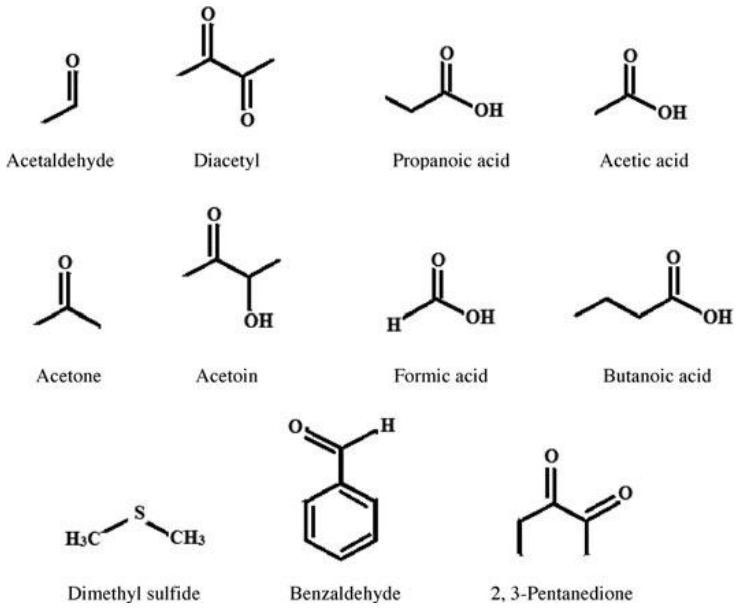


Figure 2. 12 Major aroma compounds present in fermented milk products (Routray *et al.*, 2011)

The texture of a fermented milk product is also an important parameter in sensory analysis. The concept of texture can be perceived in different ways and it can be expressed by different words in a sensory scale. For example, smoothness is often associated to fat content; thickness is often associated to SNF content. In more complex conditions, word creaminess is preferred in some sensory analysis. Creaminess of a fermented milk product is generally difficult to define and it is often remind people of relatively high viscosity and thickness, fat-related flavor, smooth and fatty mouth feel (Cayot *et al.*, 2008).

Sensory properties of fermented milk products are affected by several factors including raw materials, processes, food additives (such as thickening agent), choice of starter cultures and so on. For example, a thick product can be produced by concentrating (heating) or the addition of SNF or thickening agent. A pourable drinking product can be produced by using skimmed milk as raw material or by further homogenization. Studies show that products fermented with ABY culture (*L. acidophilus* + *Bifidobacterium* + yoghurt starter culture) have the highest acetaldehyde content and products fermented with ABT culture(*L. acidophilus* + *Bifidobacterium* + *S. thermophilus*) have relatively high firmness (Tamime, 2005)

3.0 MATERIALS AND METHODS

3.1 Experiment design

Three products (‘Cultura Naturell’ coded as C, ‘Biola Syrnet Milk Naturell’ Coded as S and ‘Biola Pluss Yogurt Mild Naturell’ Code as Y) produced by diary company TINE were selected as the three products to study. Three productions of each of the products were analyzed on three different days evenly distributed from the first day they were received to the expiry (best before) date. Products from three different production dates are coded as A, B and C.

Four cartons of each product were received from TINE. At each sampling time, a new carton was opened. Sample analyses performed on three different days were coded as 1, 2, and 3 (appendix 4). The fourth carton of product was kept for back up. All products were stored at 4 ℃ until analysis.

According to experiment design, there were in total 27 sampling stages in this study (3 products x 3 production dates x 3sampling times). Different analyses were performed at each sampling time including selective enumeration of lactic acid bacteria, pH measurement, volatile compounds measurement, organic acid measurement, viscosity measurement and sensory analysis.

Enumeration of lactic acid bacteria was always carried out first and other tests were performed the following day.

3.2 Enumeration of lactic acid bacteria

3.2.1 Preparation of base medium

Agar for the enumeration of *L. acidophilus* La-5, *L. delbrueckii* subsp. *bulgaricus*, and *B. lactis* Bb-12 was based on MRS-base medium. MRS-base medium was made by suspending 55 g of MRS powder (Difco 288210, USA) and 15 g agar (Saveen Werner B1000-1, Sweden) in 1000 ml of distilled water, and heating to boiling point with frequent agitation until complete solution was obtained. MRS medium was then distributed into 100 ml bottles and sterilized by autoclaving at 121 ℃ for 15 min.

The base medium for enumeration of *L. rhamnosus* GG was MRS-IM agar, which was made by suspending 10 g Tryptone (Oxoid LP-0042, UK), 5g Yeast extract (Oxoid LP-0021, UK), 1g Tween 80 (Sigma, P-4780, UK), 2.6 g di-Potassium hydrogen

phosphate (Merck No. 5104, Germany), 5 g Sodium acetate, 3 H2O (Merck No. 6267, Germany), 2 g di-Ammonium hydrogen citrate 2 g (Merck No. 1154, Germany), 0.2 g Magnesium sulphate , 7 H2O (Merck No. 5882, Germany), 0.05 g Manganese(II)- sulphate, H2O (Merck No.5960, Germany) and 13 g agar (Saveen Werner B1000-1, Sweden) in 1000 ml of distilled water, and heating to boiling point with frequent agitation until complete solution was obtained. MRS-IM medium was then distributed into 100 ml bottles and sterilized by autoclaving at 121 ℃ for 15 min..

3.2.2 Selective medium for *B. lactis* Bb-12

Method for enumeration of *B. lactis* Bb-12 was that used by and obtained from Christian Hansen. The method is standardized in International organization for standardization (ISO 29981:2010 /IDF 220: 2010) and is modified by Christian Hansen (Chr. Hansen, 2007b). Medium for enumeration *B. lactis* Bb-12 (MRS-M agar) was prepared by adding 5 ml L-cysteine hydrochloride (CyHCl) (Merck No 2838, Germany) stock solution and 2.5 ml mupirocin stock solution to 1 L MRS base medium. CyHCl stock solution was prepared by suspending 10 g CyHCl in 100 ml distilled water and mupirocin stock solution was made by suspending 100 mg Lithium mupirocin powder (Sigma, No. 69732, UK) in 10 ml distilled water. The final concentrations of CyHCl and mupirocin in MRS-M agar were 0.5g/L and 25 mg/L respectively. CyHCl and mupirocin solutions were filtered by using a 10 ml syringe (Becton Dickinson, UK) with a disposable needle (Leuven, Belgium) and a syringe filter with a pore size of 0.22 μm (Becton Dickinson, UK). Agar plates inoculated with sample dilutions were incubated anaerobically at 37 ℃ for 3 days.

3.2.3 Selective medium for *L. acidophilus* La-5

The method for enumeration of *L. acidophilus* La-5 was that used by and obtained from Christian Hansen. It is based on International standard (ISO 20128/IDF192) but is modified by (Chr. Hansen, 2007a). Medium for enumeration of *L. acidophilus* La-5 (MRS-C medium) was prepared by adding 0.5 ml clindamycine (Pfizer, 150 mg/ml, Norway) solution into 1 L MRS-base agar. Clindamycine stock solution was prepared by diluting clindamycine solution with concentration of 150 mg / ml to 0.2 mg/ml with distilled water. The clindamycine solution was filtered by using a 10 ml syringe (Becton Dickinson, UK) with a disposable needle (Leuven, Belgium) and a syringe filter with a pre size of 0.22 μm (Becton Dickinson, UK). The final concentration of clindamycine in MRS agar is 0.1 mg /l. Agar plates inoculated with sample dilutions were incubated anaerobically at 37℃for 3 days.

3.2.4 Selective medium for *L. delbrueckii* subsp. *bulgaricus*

Method for enumeration of *L. delbrueckii* subsp. *bulgaricus* is modified by (Shah &

Tarmaraj, 2003) and pH of MRS base agar was adjusted to 4.58 by using 1.0 M HCl (Merck No 0317, Germany). Agar plates inoculated with sample dilutions were incubated anaerobically at 43 ℃ for 3 days.

3.2.5 Selective medium for *L. rhamnosus* GG

The method for enumeration of *L. rhamnosus* GG was that used and described by (Christian Hansen, 2001). Selective medium for enumeration of *L. rhamnosus* GG was prepared by adding 5 ml vancomycin (Sigma, 75423, UK) stock solution and 10 ml glucose (Merck No. 108342, Germany) solution in to 1 L MRS-IM base medium. The vancomycin stock solution was made by suspending 100 mg vancomycin powder in 10 ml distilled water. Glucose solution was made by suspending 20g glucose in 100 ml distilled water. The final concentrations of vancomycin and glucose in the MRS-V medium were 50 mg /l and 20g/l respectively. Vancomycin and glucose solution were filtered by using a 10 ml syringe (Becton Dickinson, UK) with a disposable needle (Leuven, Belgium) and a syringe filter with a pore size of 0.22 μm (Becton Dickinson, UK). Agar plates inoculated with sample dilutions were incubated anaerobically at 37 ℃ for 3 days.

3.2.6 Selective medium for *S. thermophilus*

Method for enumeration of *S. thermophilus* is recommended by International dairy Federation (IDF, 1995). Medium for enumeration of *S. thermophilus* is M17 agar (Merck, 15029, Germany) which was prepared by suspending 55 gram of M17 powder and 15 gram agar (Saveen ,Werner B1000-1, Sweden) in 1000 ml of distilled water and heating to boiling point with frequent agitation until a complete solution is obtained. M17 medium was distributed into 100 ml bottles and sterilized by autoclaving at 121 ℃ (250 ℉) for 15 min. Agar plates inoculated with sample dilutions were incubated aerobically at 37 ℃ for 3 days.

3.2.7 Sample preparation

One gram mixed product was suspended in a tube with 9 ml peptone–saline water and mixed uniformly with a vortex mixer (0.9%, w/v saline; 0.1%, w/v peptone) to make the first dilution of bacteria and further diluted up to 10-9 in peptone–saline water. Proper dilutions of bacteria (10^{-5} to 10^{-9}) were used in plate counting. Pour plating technique was used for enumeration of *S. thermophilus, B. lactis* Bb-12, *L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus* La-5; Spread plating technique was used for enumeration of *L. rhamnosus* GG. Duplicate plates were made and the average count of the duplicate plates was used for result presentation.

Table 3.1 shows the selective media and incubation conditions for enumeration of probiotic bacteria.

Table 3. 1 Selective media and incubation conditions for the enumeration of probiotic bacteria in fermented dairy products.

Bacteria	Media	Supplement	Incubation conditions
L. acidophilus La-5	MRS	clindamycine	Anaerobic 37 ℃, 72h,
B. lactis BB-12	MRS	Li-mupirocin cysteine- HCl	Anaerobic 37 ℃, 72h,
L. rhamnosus GG	MRD-IM	vancomycin	Anaerobic 37 ℃, 72h,
S. thermophilus	M17	---	Anaerobic 37 ℃, 72h
L. delbrueckii subsp. *bulgaricus*	MRS	HCl (pH 4.58)	Anaerobic 43 ℃, 72h

3.3 pH measurement

The pH values of the probiotic fermented milk products were measured at 4 ℃ using a digital pH M 210 standard pH meter after calibrating at pH 4.0 and 7.0 (Merck, Germany) with standard buffer solutions (Merck, Germany). Products were stirred before measurement.

3.4 Head Space Gas Chromatography (HSGC)

The determination of volatile compounds in probiotic fermented milk products was performed by automatic static headspace gas chromatography (HSGC) in this study. With HSGC, liquid or solid samples are heated in a closed vessel until the volatile compounds present in the sample reach equilibrium between the sample and the gas above it, the so-called headspace. A 1ml aliquot of the headspace is automatically introduced into the gas chromatographic (GC) column for analysis.

Methods and procedures for volatile compounds (VOCs) analysis by HSGC is described by (Narvhus *et al.*, 1990). A total 6 important VOCs (acetaldehyde, diacetyl, ethanol, acetone, acetoin and 2, 3-petadione) compounds measured in this study.

10.00 g of each product was weighed and transferred to a N20-20 PE (Machery nagel, Düren, Germany) headspace tube. The tube was sealed with Chromacol 20-CT3 Teflon rubber and a Chromacol 20-ABC aluminum cap (Herts, UK) immediately after sample transferring to prevent loss of volatile compounds. The samples were extracted and analyzed using an HP 7694 Headspace sampler equipped with a 6890 GC system (Agilent, Wilmington, USA), A series 900 interface connector clips (Perkin Elmer, Sheltoon, Connecticut, USA) with a hydrogen generator (Model 75-32, Whatman,

Haverhill, MA, USA) holding a pressure of 1.6 bar (Merck, Germany) and total Chrom LC software (Perkin Elmer, Sheltoon, Connecticut, USA)

The nitrogen Ultra Plus 6 carrier gas (Eiva, Rjukan, Norway) was used as carrier gas at a constant flow of 0.5 ml/min. The GC temperature program was carried out as following: 53℃ for 1 minute, 2.70℃ for 2 minute, 3.130 ℃ for 3 minute. The increase of temperature between the first and second step is 10 ℃ / min and the increase of temperature between the second and third step is 12 ℃ /min. A CP-SIL 5CB GC column (Varian, Middelburg, the Netherlands), 25 m x 0.53 mm x 5.0 µm film thickness, was used for the HSGC analyses with flame ionization detection (FID) at 200℃.

The peaks were identified according to their retention times and their concentration in the sample is proportional to concentration of analyte in the headspace. The final result was calculated by external calibration using standard solutions. Standard solutions used include acetaldehyde, 2-butanone, ethyl acetate, 2-methyl-1-propanol, 2-methyl-butanal, 3-methyl-butanal, 3- methyl-1-butanol, 2-methyl-1-butanol, 2-methyl-1-propanal, diacetyl (Sigma- Aldrich); 2-butanol, acetoin, acetone, 2.3-pentadion (Merck, VWR), and ethanol (Arcus, Oslo, Norway).

3.5 Viscosity measurement

The viscosity of the probiotic fermented milk products was determined by the time in seconds of through-flow of sample in a SMR funnel at 4℃. The samples were stirred well before viscosity measurement.

3.6 High Performance Liquid Chromatography (HPLC)

The determination of organic acids and carbohydrates of the probiotic fermented milk products was done by High-performance liquid chromatography (HPLC) in this study.

The method and procedures of organic acids analysis is a modification of the method described by (Marsili *et al.*, 1981). The procedure used was as described by (Narvhus *et al.*, 1998).

1.00 g of each sample was weighed and transferred into a 10 ml Belco tube. 2.5 ml of ion exchanged water, 200 µl H_2SO_4 (Merck, Germany) and 8 ml acetonitrile (CH_3CN) (Merck, Germany) were added to the sample. The solution was then mixed by using a multi RS-60 Biosan Rotary mixing machine for 30 minutes and centrifuged at 3500 rpm for 15 minutes by using a Kubota 2010 centrifuge (Bunkyo-ku,Tokyo, Japan). The supernatant was then filtered into a HPLC tube (Agilent, Germany) with a Chromacol 8-ST101 septa and Chromacol 8-SV plastic cap by using a PTFE syringe filter with pore size of 0.2µm (Becton Dickinson, UK). 25µl of sample solution was then automatically injected into the HPLC column.

The HPLC used comprises a series 200 auto-injector with series 410 pumping system (Perkin Elmer), 200 series UV detector (Perkin Elmer), 200 series RI-detector (Perkin Elmer) and series 900 interface (Perkin Elmer), LC oven 101 column oven (Perkin Elmer).

For separation of organic acids, the sample solution were forced through an Aminex HPX-87H column (Bio Rad, CA, USA) with help of 5 mM H_2SO_4 as mobile phase (Merch, Germany) under a flow at 0.4 ml /min. The temperature of the column was 30 ℃. In order to protect the column, the sample ran through a Cation-H refill pre-column (Bio Rad, CA, USA). Different compounds were detected by a UV detector at wave length of 210 nm. The concentration of the different organic acids was then determined by comparing the retentions time and integrated peak areas with those of different organic acid and carbohydrate standard solutions. Standard solutions used included citric acid, orotic acid, pyruvic acid, succnic acid, DL-lactic acid, uric acid, pyroglutamic acid, α-ketoglutamic acid, propionic acid, acetic acid, formic acid (Sigma, USA), glucose, lactose, maltose, fructose and galactose (Merck, Germany).

3.7 Sensory analysis

Sensory properties of the probiotic fermented products were evaluated by a sensory panel (comprising 5 assessors) using a descriptive sensory method. The properties assessed include sweetness, sourness, vinegar taste, cultured milk taste, bitter taste, tart flavor, off-flavor, viscosity, creaminess, graininess and fresh taste. The samples were coded randomly and were evaluated by assessors using a sensory scale with 9 levels indicating intensity of sensory properties from low to high. The panel was calibrated by using one random sample before each sensory analysis. The average points of result were used in result presentation.

3.8 Data analysis

Data of viable cell counts of lactic acid bacteria, pH, viscosity, volatile compounds, organic acid and carbohydrates were subjected to Principal Component Analysis (PCA) by using Unscramble X software (CAMO, software AS, Oslo, Norway) and were represented in bi-plot. Variables were standardized by dividing by the standard deviation for each variable.

PCA is a popular multivariate technique which is used to reduce the dimensionality of multi-dimensional space to two or three dimensions by summarizing the variation in a correlated multi-attribute to a set of uncorrelated components. Bi-plot displays the inter-relationships between the samples and variables in multivariate data.

4.0 RESULTS

4.1 Microbiological results

In this study, it soon became clear that there were sufficient differences between the results from samples from the three productions of each product, that it would be misleading to present the results as mean values. The results from each production (A, B and C) are therefore presented as individual lines on the same figure.

4.1.1 Media performance

Results from direct observation of colonies on the different selective agars indicated that only one type of microorganism was able to grow on MRS-M, MRS- pH4.58, M17, and MRS-IM-V. In the case of MRS-C, 2 different bacteria strains (two types of bacterial colonies with different sizes were observed) were able to grow on MRS-C agar indicating that MRS-C medium was not totally selective for *L. acidophilus* La-5.

4.1.2 Colony morphology

The morphology of bacteria cells and colonies were observed under microscope.

Table 4.1 shows the colony morphology and bacteria morphology observed directly or microscopically.

Table 4. 1 Colony and cell morphology of bacteria grown in different medium.

Media	Media Selectiveity	Colony morphology	Bacteria morphology under microscope
MRS-C	*L. acidophilus* La-5	small (＜1mm), whitish circular colonies with smooth edge.	non-motile rods in forming of short chains
		large (about 2 mm), whitish circular colonies with smooth edge	non-motile rods in form of short chains.
MRS-M	*B. lactis Bb*-12	large (about 2 mm), whitish circular colonies with smooth edge.	non-motile rods with club ends
MRSIM-V	*L. rhamnosus* GG	large (about1-2 mm), creamy circular convex colonies with smooth edge.	non-motile rods in form of short chains.
M17	*S. thermophilus*	small (＜1mm), whitish circular colonies with smooth edge.	non-motile cocci, in form of pairs or short chains
MRS-pH 4.58	*L. delbrueckii* subsp. *bulgaricus*	small (about 1 mm), whitish-grey, irregular colonies with rough, undulate edge.	non-motile long rod shape, in form of short chains

4.1.3 Viable cell counts from product ‘Cultura Naturell’

The viable cell counts of *L. acidophilus* La-5 and *B. lactis* Bb-12 in the product ‘Cultura Naturell’ are shown in figures 4.1 and 4.2.

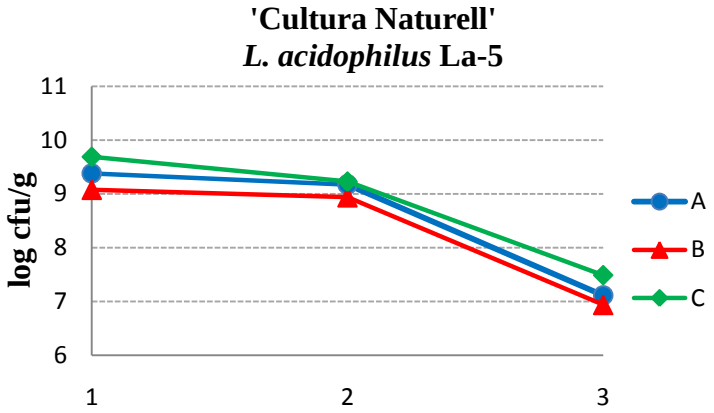


Figure 4. 1 Viable cell counts of *L. acidophilus* La-5 in the product 'Cultura Naturell' (A, B, C = production A, production B and production C. 1, 2, 3 = first sampling, second sampling and third sampling).

The initial viable cell counts of *L. acidophilus* La-5 in the product 'Cultura Naturell' from three productions were between 9.08 and 9.67 log cfu/g. Consistent declines in the number of *L. acidophilus* La-5 of about 2-log were observed from all three different productions. Variations between the three productions of 'Cultura Naturell' were small. *L. acidophilus* La-5 retained viable cell numbers above 7.11-7.49 log cfu/g after three weeks of storage. The decreasing rate of *L. acidophilus* La-5 between the first and second sampling was low (the number of *L. acidophilus* La-5 maintained above 9 log cfu /g). But its viable count decreased dramatically between the second and third sampling.

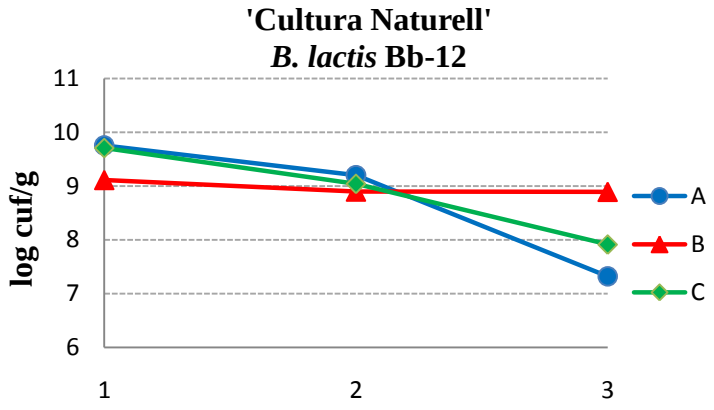


Figure 4. 2 Viable cell counts of *B .lactis* Bb-12 in the product 'Cultura Naturell' (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial viable cell counts of *B .lactis* Bb-12 in the product 'Cultura Naturell' from three productions were between 9.8 and 9.1 log cfu/g. Marked variations were observed between the three productions of 'Cultura Naturell'. Consistent declines in the number of *B. lactis* Bb-12 of about 2-log units were observed in 'Cultura Naturell' from

production A and C despite their highest initial viable cell counts of 9.8 and 9.7 log cfu/g respectively. Despite its lower initial viable cell counts, *B. lactis* Bb-12 in 'Cultura Naturell' from production B was stable, maintaining above 8.9 log cfu/g during three weeks of storage.

4.1.4 Plate counts of product 'Biola Syrnet Lettmelk Naturell'

The viable cell counts of *L. acidophilus* La-5, *B. lactis* Bb-12 and *L. rhamnosus* GG in the product 'Biola Syrnet Lettmelk Naturell' are shown in figures 4.3, 4.4 and 4.5.

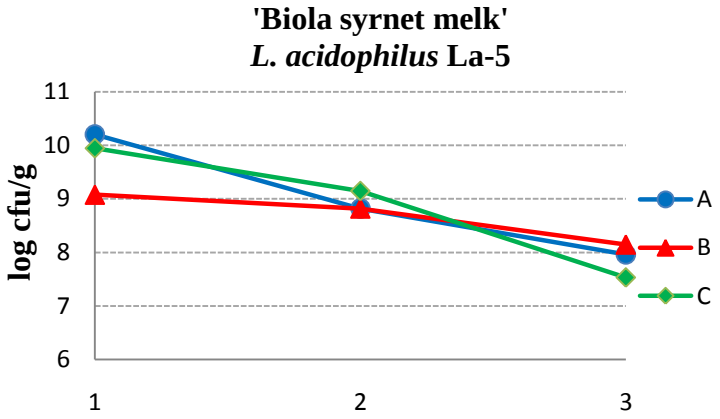


Figure 4. 3 Viable cell counts of *L. acidophilus* La-5 in the product 'Biola Syrnet Lettmelk Naturell' (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling)

The initial viable cell counts of *L. acidophilus* La-5 in the product 'Biola Syrnet Lettmelk Naturell' were between 9.08 and 10.2 log cfu/g. Marked variations were observed between the three productions of 'Biola Syrnet Lettmelk Naturell'. The number of *L. acidophilus* La-5 declined by about 2-log units in 'Biola Syrnet Lettmelk Naturell' from all three productions. *L. acidophilus* La-5 in 'Biola Syrnet Lettmelk Naturell' from production B had the lowest counts of 9.08 log cfu/g, but its viable count was more stable during three weeks' storage and was the highest of the three productions at the end of the shelf-life.

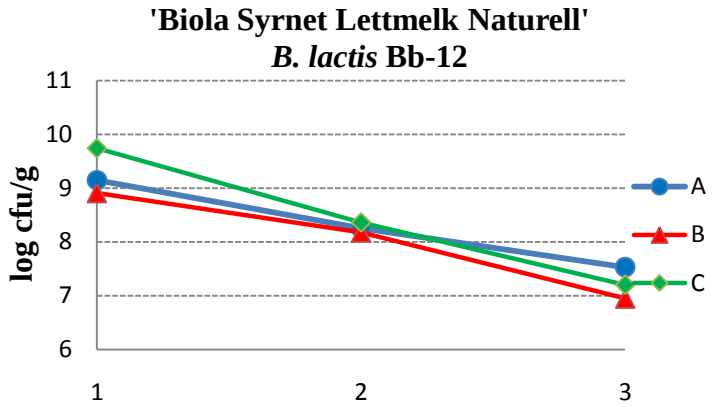


Figure 4. 4 Viable cell counts of *L. lactis* Bb-12 in the product 'Biola Syrnet Lettmelk Naturell'. (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling)

The initial viable cell counts of *B. lactis* Bb-12 in the product 'Biola Syrnet Lettmelk Naturell' from three productions were between 8.91 and 9.74 log cfu/g. Consistent declines in the number of *B. lactis* Bb-12 of about 2-log units were observed in ''Biola Syrnet Lettmelk Naturell' from all three productions. No obvious variations between the three productions of 'Biola Syrnet Lettmelk Naturell' were observed. The viable cell counts of *B. lactis* Bb-12 decreased markedly, but were nevertheless between 6.95-7.53 log cfu/g after three weeks of storage. The decreasing rates of viable counts of *B. lactis* Bb-12 in 'Biola Syrnet Lettmelk Naturell' from three different productions were similar.

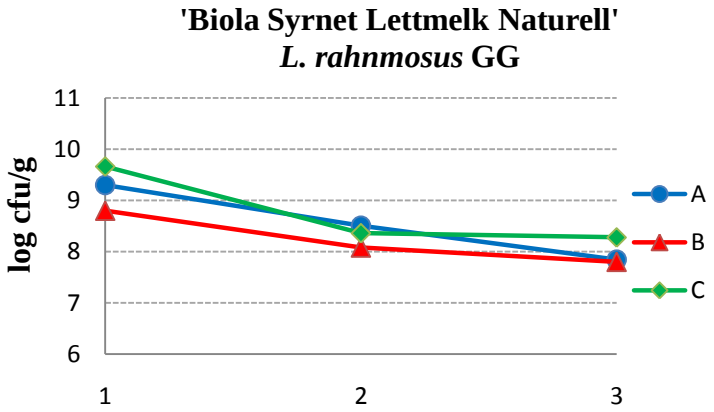


Figure 4. 5 Viable cell counts of *L. rhamnosus* GG in the product 'Biola Syrnet Lettmelk Naturell' (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial viable cell counts of *L. rhamnosus* GG in the product 'Biola Syrnet Lettmelk Naturell' were between 9.30 and 9.66 log cfu/g. Slight and consistent declines in the number of *L .rhamnosus* GG of about 1-log unit were observed in 'Biola Syrnet Lettmelk Naturell' from all three production dates. Variations between the three

productions of ‘Biola Syrnet Lettmelk Naturell’ were small. Viable cell counts of *L .rhamnosus* GG in ‘Biola Syrnet Lettmelk Naturell’ from production C were most stable and it decreased slightly from 9.66 log cfu/g to 8.28 log cfu/g. *L .rhamnosus* GG in ‘Biola Syrnet Lettmelk Naturell’ showed viable cell counts of above 7.80-8.28 log cfu/g following three weeks of storage.

4.1.5 Plate counts of product ‘Biola Pluss Yoghurt Mild Naturell’

The viable cell counts of *L. acidophilus* La-5, *B. lactis* Bb-12 and *L.rhamnosus* GG, *S. thermophilus, L. delbrueckii* subsp. *bulgaricus* in the product ‘Biola Pluss Yoghurt Mild Naturell’ are shown in figures 4.6, 4.7, 4.8, 4.9 and 4.10.

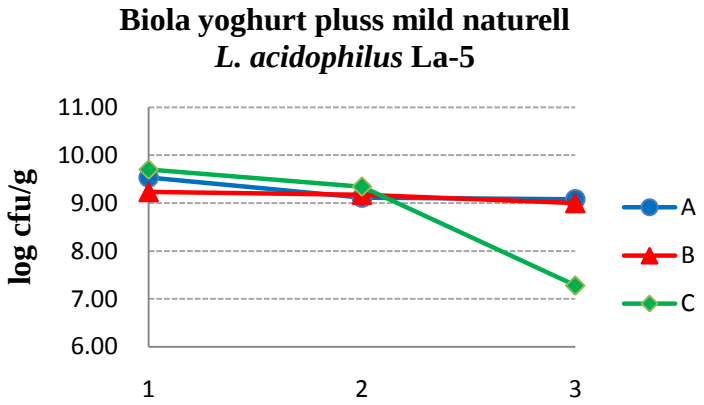


Figure 4. 6 Viable cell counts of *L.acidophilus* La-5 in the product ‘Biola Pluss Yoghurt Mild Naturell’ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling)

The initial viable cell counts of *L. acidophilus* La-5 in the product ‘Biola Pluss Yoghurt Mild Naturell’ were between 9.23 and 9.70 log cfu/ g. The number of *L. acidophilus* La-5 in ‘Biola Pluss Yoghurt Mild Naturell’ from production C declined by about 2-log units. Marked variations between the three productions of ‘Biola Pluss Yoghurt Mild Naturell’ can be seen in figure 4.6. The viable cell counts of *L. acidophilus* La-5 in ‘Biola Pluss Yoghurt Mild Naturell’ from production A and B were stable, decreasing slightly within a level of 9 log cfu/g. The viable cell counts of *L. acidophilus* La-5 in ‘‘Biola Pluss Yoghurt Mild Naturell’ from production C decreased markedly (especially between the second and third sampling) from initial viable cell count of 9.70 log cfu/g to 7.28 log cfu/g during five weeks of storage.

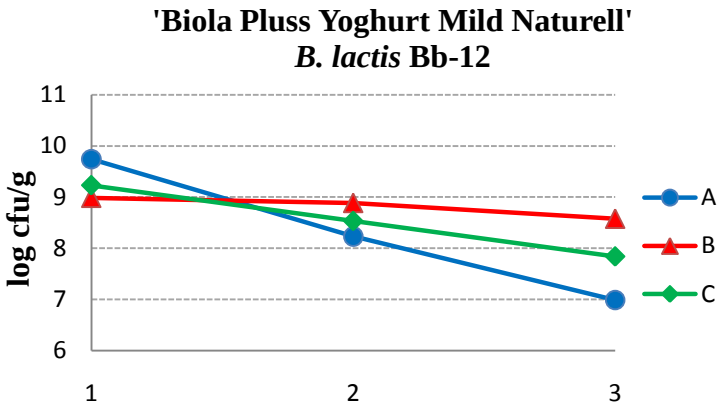


Figure 4. 7 Viable cell counts of *B. lactis* Bb-12 in the product 'Biola Pluss Yoghurt Mild Naturell' (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

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The initial viable cell counts of *B. lactis* Bb-12 in the product 'Biola Pluss Yoghurt Mild Naturell' were between 8.99 and 9.74 log cfu/ g. Consistent declines in the number of *B. lactis* Bb-12 of about 2-log units were observed in 'Biola Pluss Yoghurt Mild Naturell' from all three productions. Small variations between the three productions of 'Biola Pluss Yoghurt Mild Naturell' can be seen in figure 4.7. The viable cell counts of *B. lactis* Bb-12 in 'Biola Pluss Yoghurt Mild Naturell' from production B were stable, maintaining above 8.58 log cfu/g. *B. lactis* Bb-12 in 'Biola Pluss Yoghurt Mild Naturell' retained its viable cell counts above 6.99 log cfu/g to 8.83 log cfu /g after five weeks of storage.

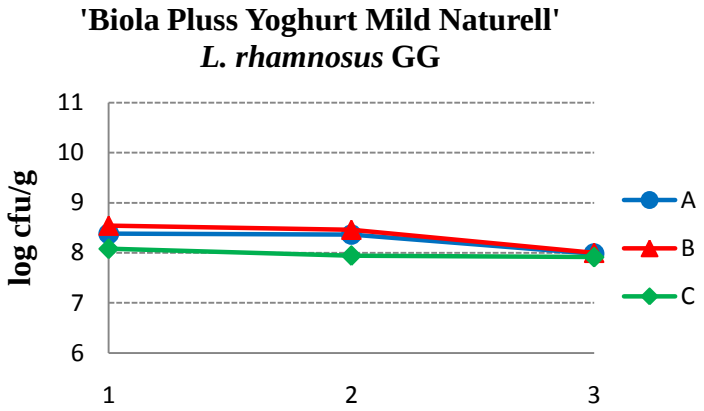

Figure 4. 8 Viable cell counts of *L .rhamnosus* GG in the product 'Biola Pluss Yoghurt Mild Naturell' (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial viable cell counts of *L .rhamnosus* GG in the product 'Biola Pluss Yoghurt Mild Naturell' were between 8.08 and 8.38 log cfu/g. Only a very slight reduction in the number of *L .rhamnosus* GG of about 1-log unit was observed in 'Biola Pluss Yoghurt Mild Naturell' from all three production dates and variations between the three

productions were very small. The viable counts of *L .rhamnosus* GG in ‘Biola Pluss Yoghurt Mild Naturell’ were stable. All products maintained above 8 log cfu/g after five weeks of storage.

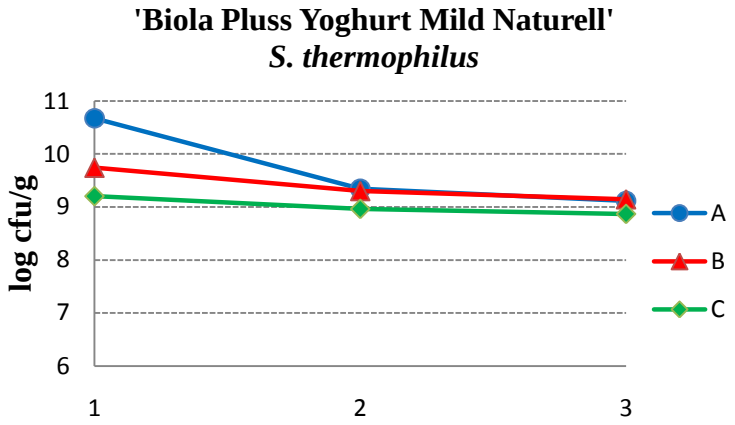

Figure 4. 9 Viable cell counts of *S. thermophilus* in the product ‘Biola Yoghurt Pluss Mild Naturell’ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The viable counts of *S. thermophilus* in the product ‘Biola Pluss Yoghurt Mild Naturell’ were relatively high comparing with other lactic acid bacteria and its initial viable cell counts were between 9.20 and 10.67 log cfu/ g. Slight declines in the number of *S. thermophilus* were observed on different levels in ‘Biola Pluss Yoghurt Mild Naturell’ from all three different productions. *S. thermophilus* showed viable cell counts in ‘Biola Pluss Yoghurt Mild Naturell’ above 8.87 to 9.15 log cfu / g following five weeks of storage.

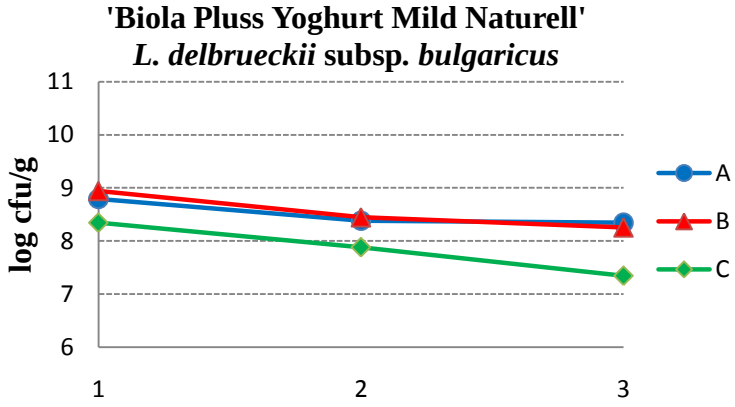

Figure 4. 10 Viable cell counts of *L. delbrueckii* subsp. *bulgaricus* in the product ‘Biola Pluss Yoghurt Mild Naturell’ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The viable counts of *L. delbrueckii* subsp. *bulgaricus* were relatively low compared to the other lactic acid bacteria in the product ‘Biola Pluss Yoghurt Mild Naturell’ and

its initial viable cell counts were between 8.79 and 8.94 log cfu/ g. A decline of about 1 log unit in the number of *L. delbrueckii* subsp. *bulgaricus* was observed in products from all three different production dates.

4.2 pH

4.2.1 pH changes in ‘Cultura Naturell’

Figure 4.11 shows the pH changes in the product ‘Cultura Naturell’ during storage at 4 ℃.

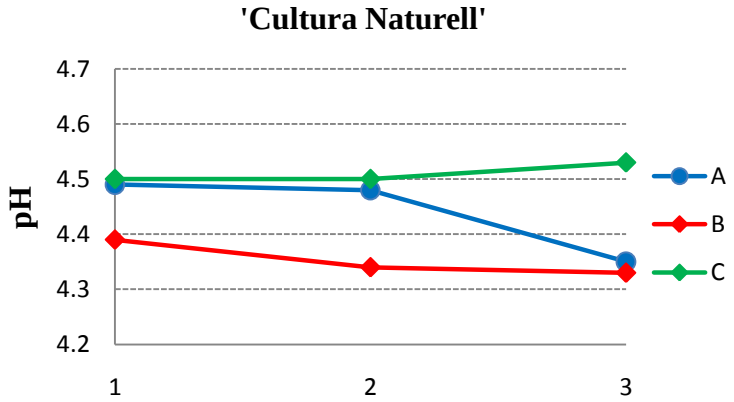

Figure 4. 11 pH changes in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling)

The initial pH in the ‘Cultura Naturell’ was between 4.39 and 4.50 and did not change markedly during storage. The pH in ‘Cultura Naturell’ from production A and B decreased slightly from 4.49 to 4.35 and from 4.39 to 4.34 respectively.

4.2.2 pH changes in ‘Biola Syrnet Lettmelk Naturell’

Figure 4.12 shows pH changes in the product ‘Biola Syrnet Lettmelk Naturell’ during storage at 4 ℃.

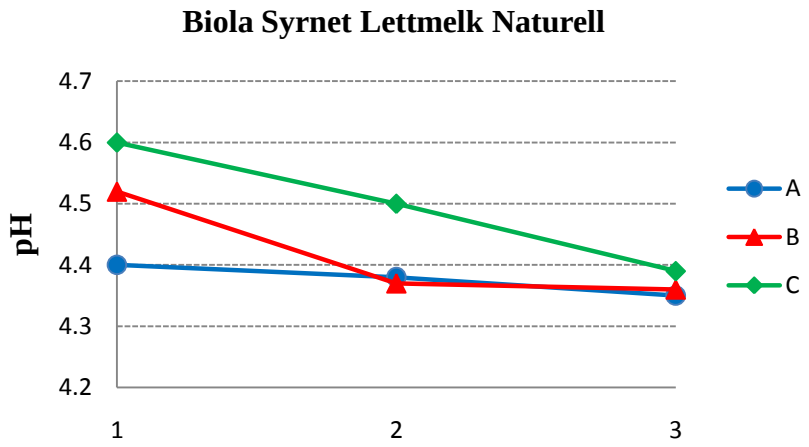

Figure 4. 12 pH changes in the product ‘Biola Syrnet Lettmelk Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

pH decreased in samples from all three productions of ‘Biola Syrnet Lettmelk Naturell’ , which had an initial pH between 4.4 and 4.6. Consistent decreases of pH were measured from all three different production dates. Marked variations between the three productions of ‘Biola Syrnet Lettmelk Naturell’ can be seen in figure 4.12. The pH in ‘Biola Syrnet Lettmelk Naturell’ from production A was relatively stable and decreased slightly from 4.4 to 4.35, whereas ‘Biola Syrnet Lettmelk Naturell’ from production B dropped markedly from 4.52 to 4.35 and pH of product from production C dropped markedly from 4.60 to 4.38.

4.2.3 pH changes in‘Biola Pluss Yoghurt Mild Naturell’

Figure 4.13 shows the changes of pH in ‘Biola Pluss Yoghurt Mild Naturell’ during storage at 4 ℃.

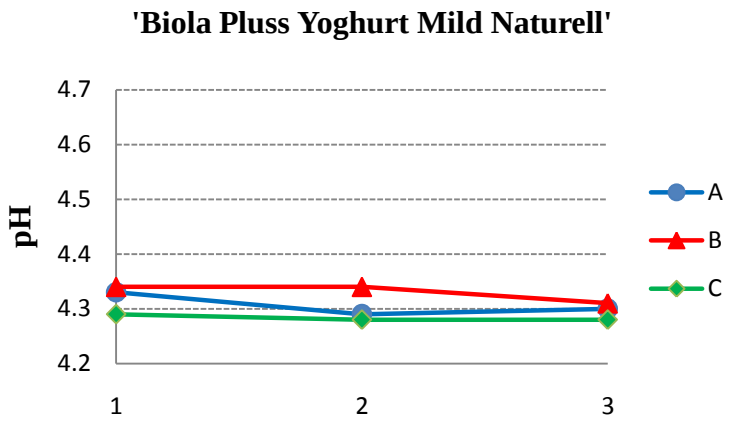

Figure 4. 13 pH changes in 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

Of the three products investigated, 'Biola Pluss Yoghurt Mild Naturell' had the lowest initial pH between 4.29 and 4.34. Only very slight variations of pH were observed between the three productions. 'Biola Pluss Yoghurt Mild Naturell' from production A and B had slight pH drop during five weeks of storage. 'Biola Pluss Yoghurt Mild Naturell' from production C was stable during storage, maintaining a pH of 4.28.

4.3 Viscosity

4.3.1 Viscosity of 'Cultura Naturell'

Figure 4.14 shows the viscosity of product 'Cultura Naturell' during storage at 4 ℃

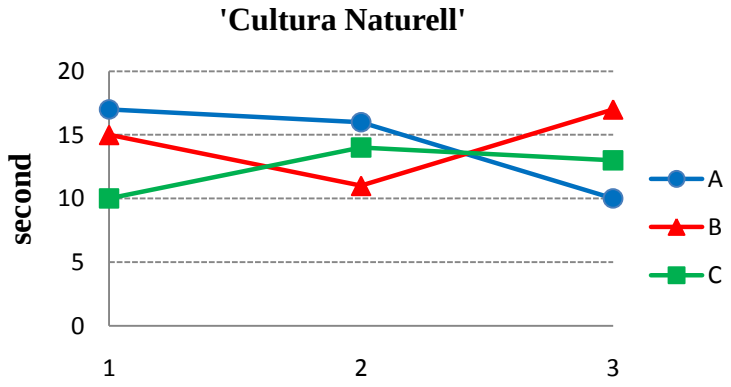

Figure 4. 14 Viscosity changes of 'Cultura Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling)

The time required by 'Culture Naturell' to pass the funnel varied from 10s to 20s and

unexplainable trends were observed in products from production B and C.

4.3.2 Viscosity changes of ‘Biola Syrnet Lettmelk Naturell’

Figure 4.15 shows the viscosity changes of product ‘Biola Syrnet Lettmelk Naturell’ during storage at 4 ℃.

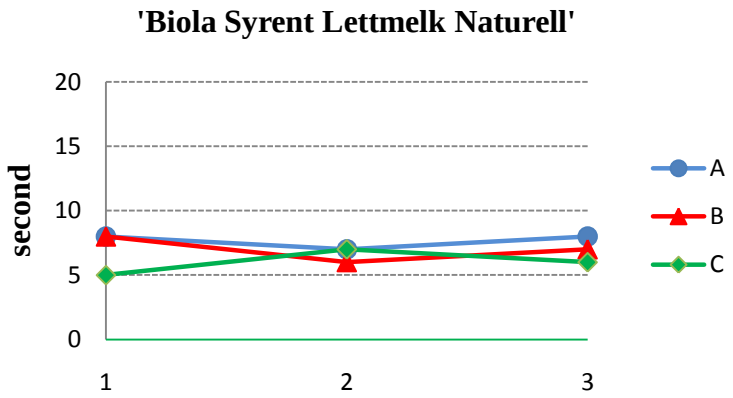

Figure 4. 15 Viscosity changes of the product ‘Biola Syrnet Lettmelk Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling)

The time required by ‘Biola Syrnet Lettmelk Naturell’ to pass through the funnel was stable with only small changes indicating a stable viscosity of ‘Biola Syrnet Lettmelk Naturell’ during storage.

4.2.3 Viscosity changes of ‘Biola Pluss Yoghurt Mild Naturell’ during storage at 4 ℃.

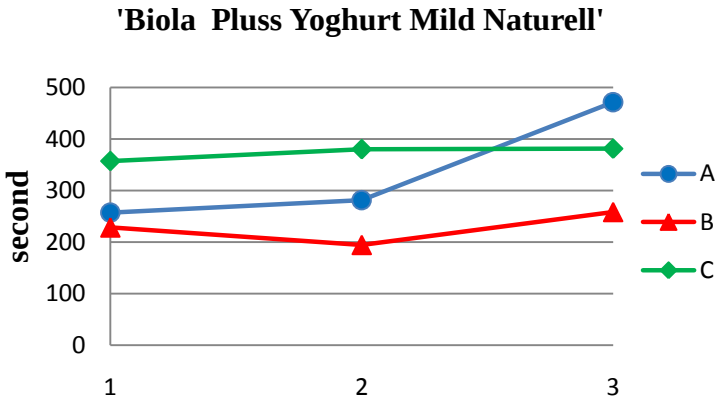

Figure 4. 16 Viscosity changes of the product ‘Biola Pluss Yoghurt Mild Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling)

Time required by ‘Biola Pluss Yoghurt Mild Naturell’ to pass the funnel is relatively long indicating high viscosity of ‘Biola Pluss Yoghurt Mild Naturell’. Consistent increases of viscosity were observed on ‘Biola Pluss Yoghurt Mild Naturell’ from all three different productions. The viscosity of ‘Biola Pluss Yoghurt Mild Naturell’ from production A increased dramatically after the second sampling.

4.4 Volatile compounds (HSGC)

4.4.1 Development of volatile compounds in ‘Cultura Naturell’

Figures 4.17, 4.18, 4.19, 4.20, 4.21, and 4.22 show the development of volatile compounds in the product ‘Cultura Naturell’ during storage at 4 ℃.

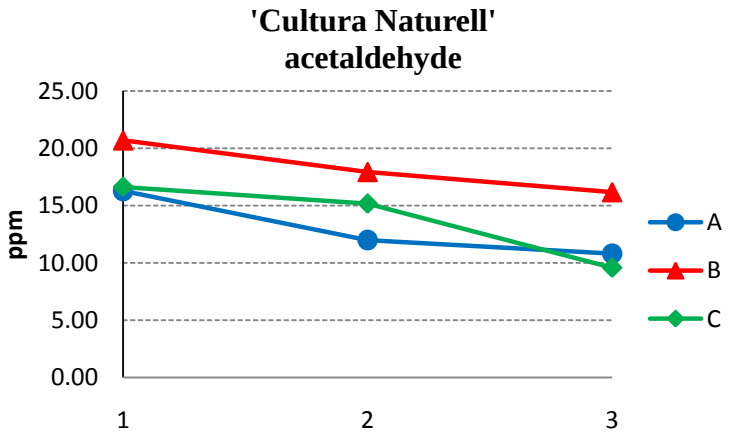

Figure 4. 17 Changes in the level of acetaldehyde in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of acetaldehyde in ‘Cultura Naturell’ were between 16 - 20 ppm. Decreases of acetaldehyde were observed in ‘Cultura Naturell’ from all three production dates. ‘Cultura Naturell’ from production B had obviously higher amount of acetaldehyde than ‘Cultura Naturell’ from productions A and C.

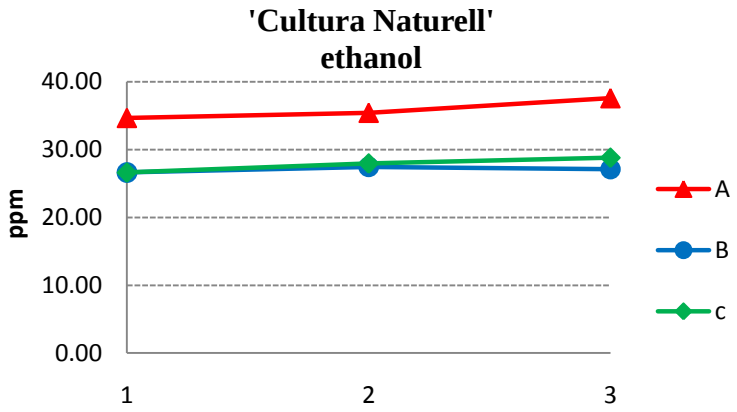

Figure 4. 18 Changes in the level of ethanol in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of ethanol in ‘Cultura Naturell’ were between 26 and 32 ppm. Slight increases of ethanol during storage were observed in ‘Cultura Naturell’ from all three different production dates. ‘Cultura Naturell’ from production A had clearly higher amount of ethanol than ‘Cultura Naturell’ from productions B and C.

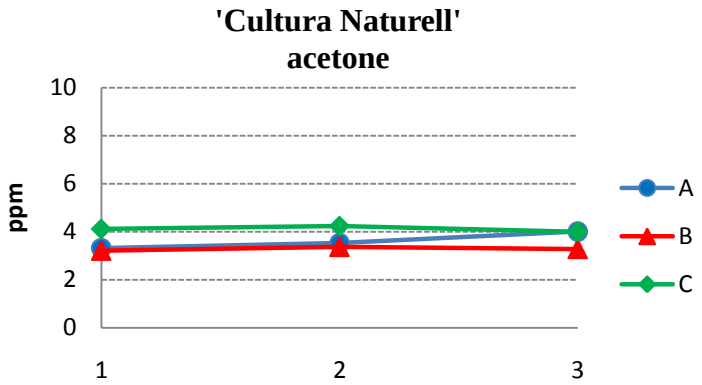

Figure 4. 19 Changes in the level of acetone in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of acetone in ‘Cultura Naturell’ were between 3.2 ppm and 4.2 ppm. No large variations between the three productions of ‘Cultura Naturell’ can be seen in figure 4.19. The amount of acetone was stable during three weeks of storage. ‘Cultura Naturell’ from production C had a slightly higher amount of acetone than samples from productions A and B.

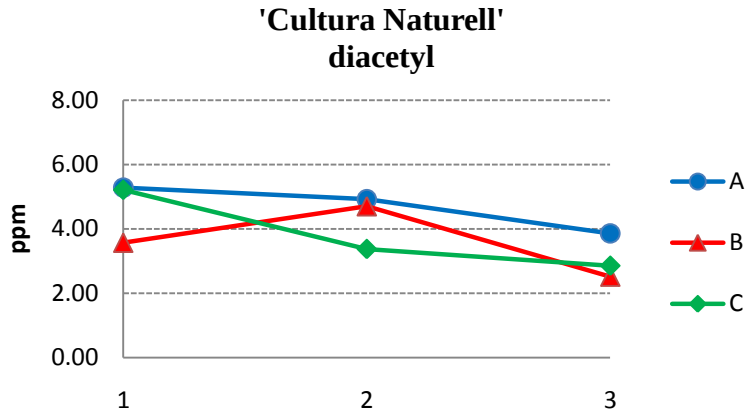

Figure 4. 20 Changes in the level of diacetyl in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of diacetyl in ‘Cultura Naturell’ were between 2.5 and 5.3 ppm. A general trend for decrease in diacetyl concentration was observed in ‘Cultura Naturell’ from all three productions except for a slight increase of diacetyl in ‘Cultura Naturell’ from production B between the first and second sampling. ‘Cultura Naturell’ from production A had a slightly higher amount of diacetyl than samples from productions B and C.

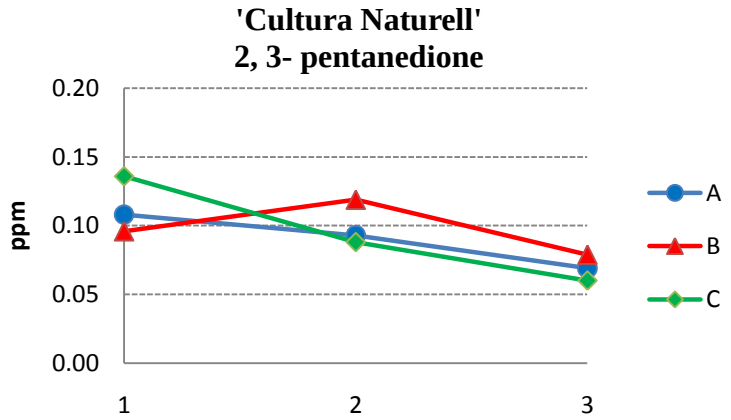

Figure 4. 21 Changes in the level of 2, 3- pentanedione in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

Trace amounts of 2, 3- pentanedione were found in ‘Cultura Naturell’ and its initial concentrations were between 0.06 ppm and 0.11 ppm. A slight decrease of 2, 3- pentanedione was found in samples from all three different productions except for a small increase of 2, 3- pentanedione in ‘Cultura Naturell’ from production B between the first and second sampling.

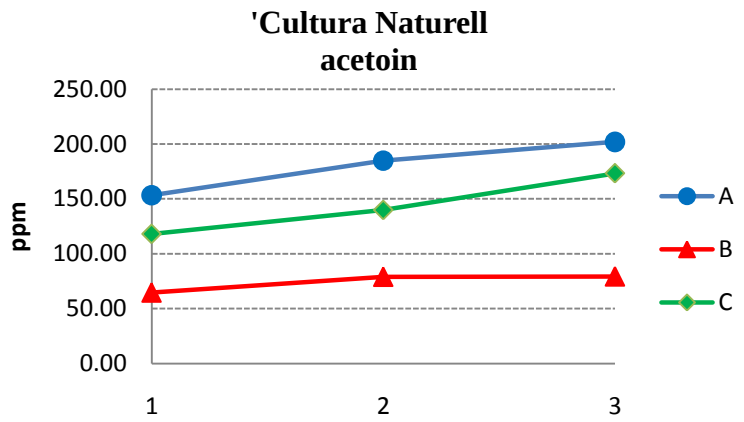

Figure 4. 22 Changes in the level of acetoin in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of acetoin found in ‘Cultura Naturell’ were between 65 and 202 ppm and there are large variations between the three productions. Amount of acetoin in ‘Cultura Naturell’ increased during three weeks of storage. Acetoin was highest in samples from production A and it decreased from 150 ppm to 200 ppm during storage.

4.4.2 Development of volatile compounds in ‘Biola Syrnet Lettmelk Naturell’

Figure 4.23, 4.24, 4.25, 4.26, 4.27 and 4.28 show the development of volatile compounds in the product ‘Biola Syrnet Lettmelk Naturell’ during storage at 4 ℃.

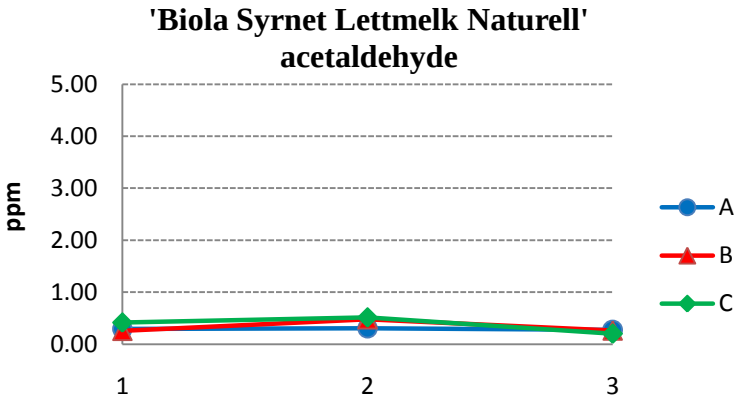

Figure 4. 23 Changes in the level of acetaldehyde in the product ‘Biola Syrnet Lettmelk Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

Trace amounts of acetaldehyde was found in ‘Biola Syrnet Lettmelk Naturell’ and its initial concentrations were between 0.3 and 0.5 ppm. No large variations between the three productions of ‘Biola Syrnet Lettmelk Naturell’ can be seen in figure 4.23.The

amount of acetaldehyde in ‘Biola Syrnet Lettmelk Naturell’ was stable, maintaining at a level under 1 ppm during three weeks of storage.

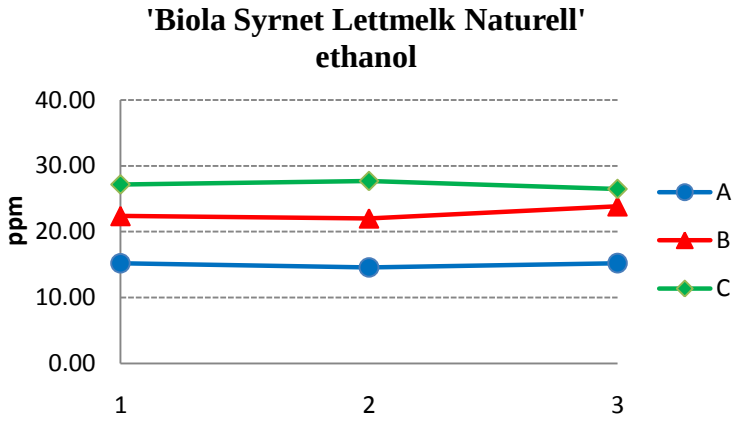

Figure 4. 24 Changes in the level of ethanol in the product ‘Biola Syrnet Lettmelk Naturell’ under storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of ethanol in ‘Biola Syrnet Lettmelk Naturell’ were between 18 and 27 ppm. Large variations between the three productions were found. However, the concentration of ethanol in ‘Biola Syrnet Lettmelk Naturell’ was stable. ‘Biola Syrnet Lettmelk Naturell’ from production C had a much higher concentration of ethanol than ‘‘Biola Syrnet Lettmelk Naturell’ from productions A and B

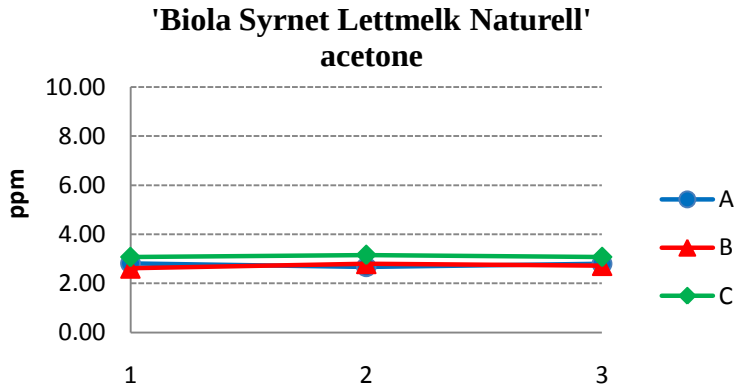

Figure 4. 25 Changes in the level of acetone in the product ‘Biola Syrnet Lettmelk Naturell’ under storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of acetone in the production ‘Biola Syrnet Lettmelk Naturell’ were between 2.8 ppm to 3.1 ppm. There were no large variations between the three productions. The amount of acetone was table during three weeks of storage.

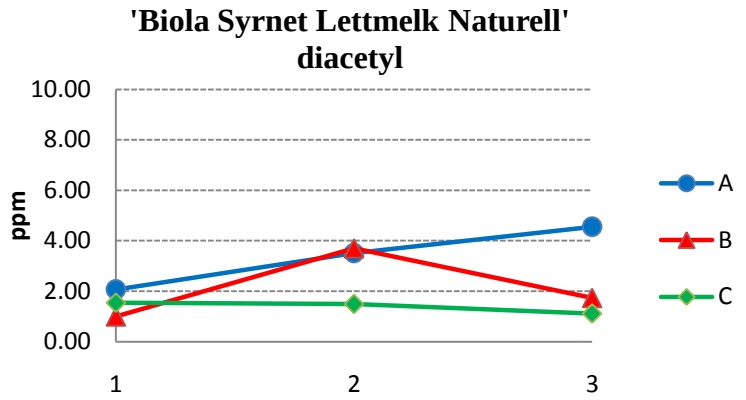

Figure 4. 26 Changes in the level of diacetyl in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of diacetyl in the production 'Biola Syrnet Lettmelk Naturell' were between 1 and 2 ppm. Changes in the level of diacetyl during storage were small, and different trends were observed in samples from the three different productions. 'Biola Syrnet Lettmelk Naturell' from production A had the highest level of diacetyl of the three.

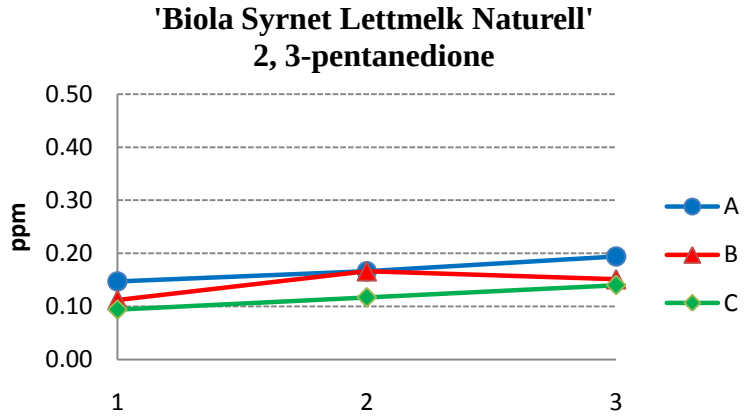

Figure 4. 27 Changes in the level of 2, 3-pentanedione in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

Trace amount of 2, 3- pentanedione was found in the product 'Biola Syrnet Lettmelk Naturell' and its initial concentrations were between 0.11 and 0.19. No big variations of the three productions are shown in figure 4.27. Amounts of 2, 3- pentanedione in 'Biola Syrnet Lettmelk Naturell' were stable, maintaining at a level between 0.1 and 0.2 ppm.

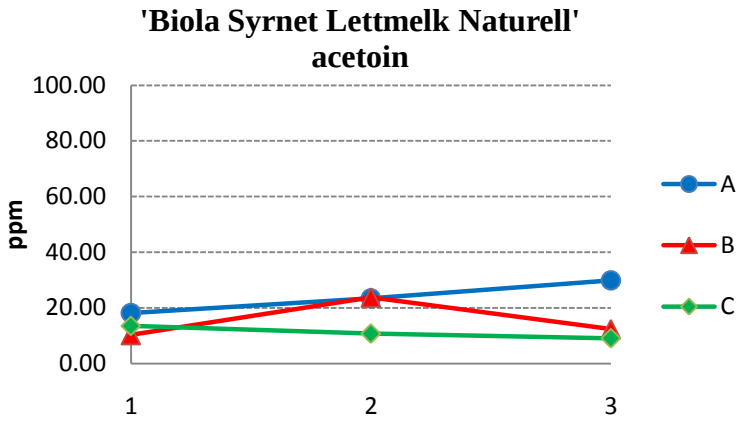

Figure 4. 28 Changes in the level of acetoin in the product 'Biola Syrnet Lettmelk Naturell' under storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of acetoin in the production 'Biola Syrnet Lettmelk Naturell' were between 10 and 18 ppm. Changes in the level of acetoin in the three productions of 'Biola Syrnet Lettmelk Naturell' did not follow a regular rule and no particular trends were observed for the three different productions of 'Biola Syrnet Lettmelk Naturell'. 'Biola Syrnet Lettmelk Naturell' from production A had the highest level of acetoin of the three.

4.4.3 Development of volatile compounds in 'Biola Pluss Yoghurt Mild Naturell'

Figure 4.29, 4.30, 4.31, 4.32, 4.33 and 4.34 show the development of volatile compounds in the product 'Biola Pluss Yoghurt Mild Naturell'

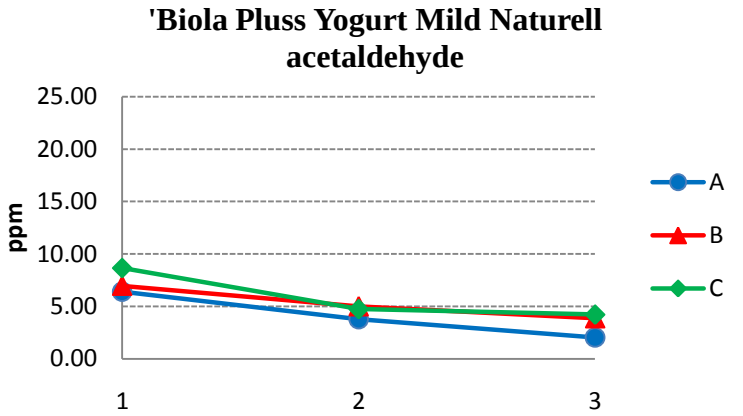

Figure 4. 29 Changes in the level of acetaldehyde in the product 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of acetaldehyde in the product 'Biola Pluss Yoghurt Mild Naturell' were between 6.4 and 8.6 ppm. The amount of acetaldehyde in 'Biola Pluss

Yoghurt Mild Naturell' deceased slightly during storage and variations between the three productions of 'Biola Pluss Yoghurt Mild Naturell' were small.

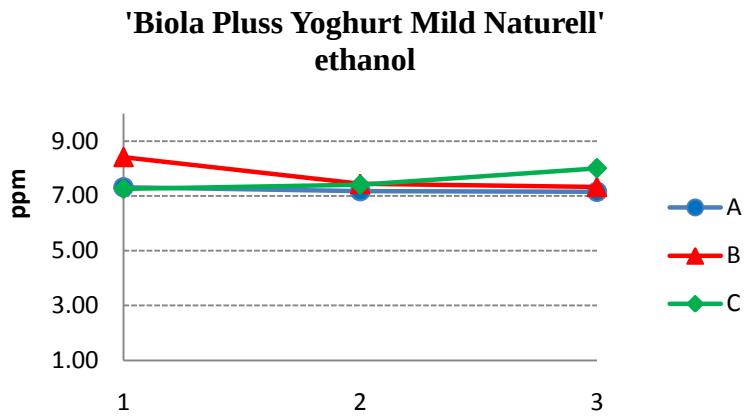

Figure 4. 30 Changes in the level of ethanol in the product 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

Compared to 'Cultura Naturell' and 'Biola Syrnet Lettmelk Naturell', amounts of ethanol in 'Biola Pluss Yoghurt Mild Naturell' were relatively low with initial concentrations between 7.2 and 8.4ppm. Variations between the three productions of 'Biola Pluss Yoghurt Mild Naturell' were small and the concentrations were stable during storage.

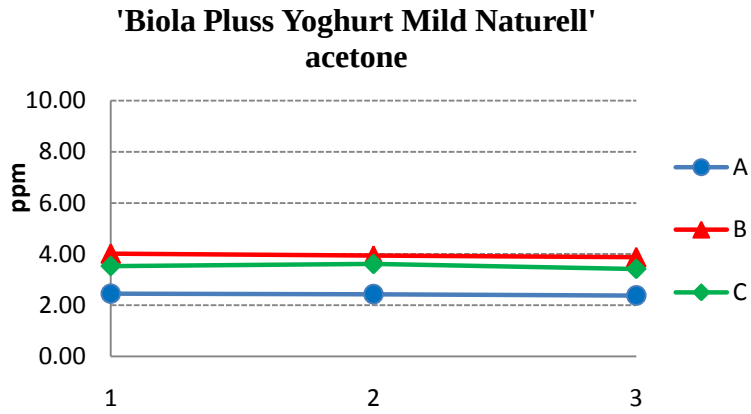

Figure 4. 31 Changes in the level of acetone in the product 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of acetone in 'Biola Pluss Yoghurt Mild Naturell' were between 2.4 to 4 ppm. Variations between the three productions of 'Biola Pluss Yoghurt Mild Naturell' were small and the concentration of acetone was stable during storage.

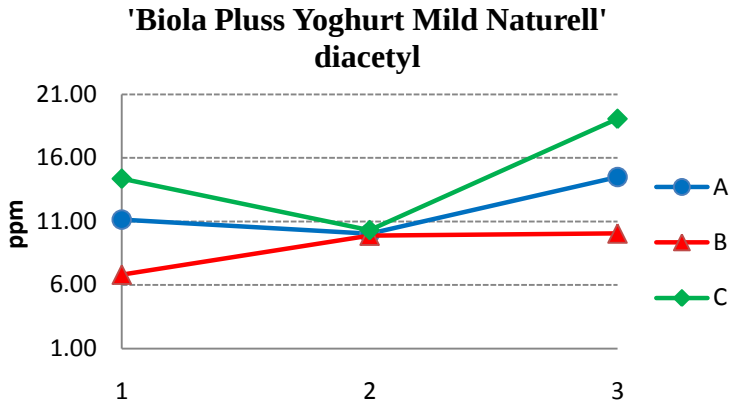

Figure 4. 32 Changes in the level of diacetyl in the product 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of diacetyl in 'Biola Pluss Yoghurt Mild Naturell' were between 6.8 and 14.4 ppm with considerable variations between the three productions, as shown in figure 4.32.Changes of diacetyl in 'Biola Pluss Yoghurt Mild Naturell' did not follow a regular rule, but its amount in general increased slightly during five weeks of storage. 'Biola Pluss Yoghurt Mild Naturell' from production B had the lowest amount of diacetyl of the three which increased slightly from 6.8 to 10 ppm during storage.

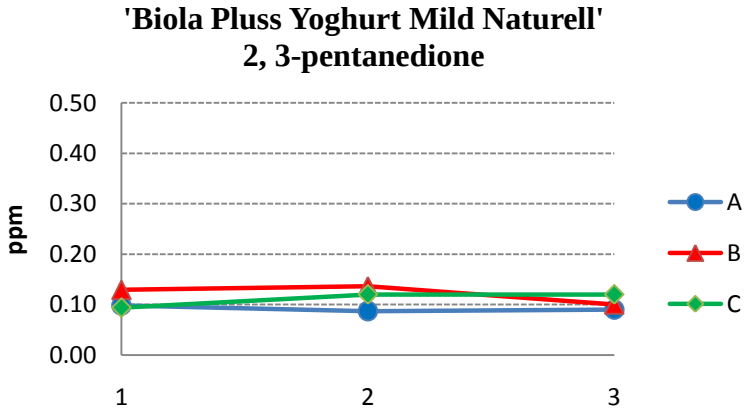

Figure 4. 33 Changes in the level of 2, 3-petandione in the product 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

Trace amount of 2, 3- pentanedione was found in 'Biola Pluss Yoghurt Mild Naturell' and its initial concentrations were between 0.1 and 0.2 ppm. Variations between the three productions were very small and the amounts of 2, 3- pentanedione were stable during storage.

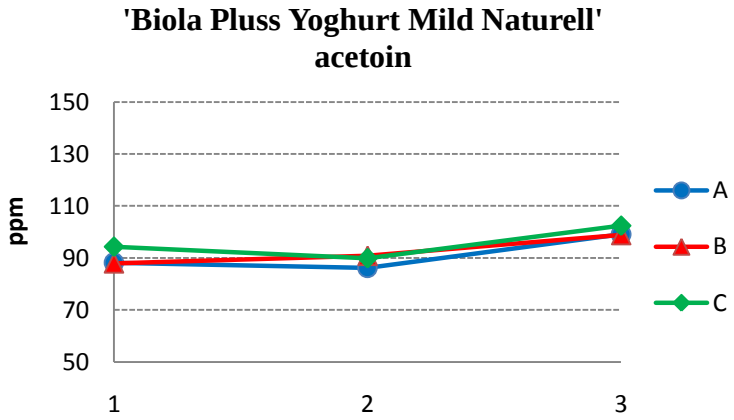

Figure 4. 34 Changes in the level of acetoin in the product 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of acetoin in 'Biola Pluss Yoghurt Mild Naturell' were between 88 and 94 ppm. Variations between the three productions were very small and the amounts of acetoin in 'Biola Pluss Yoghurt Mild Naturell' increased slightly during five weeks of storage.

4.5 Organic acids (HPLC)

4.5.1 Development of organic acids in 'Cultura Naturell'

Figure 4.35, 4.36, 4.37, 4.38, 4.39, 4.40, 4.41, 4.42, 4.43, and 4.44 show the development of organic acids in the product 'Cultura Naturell'

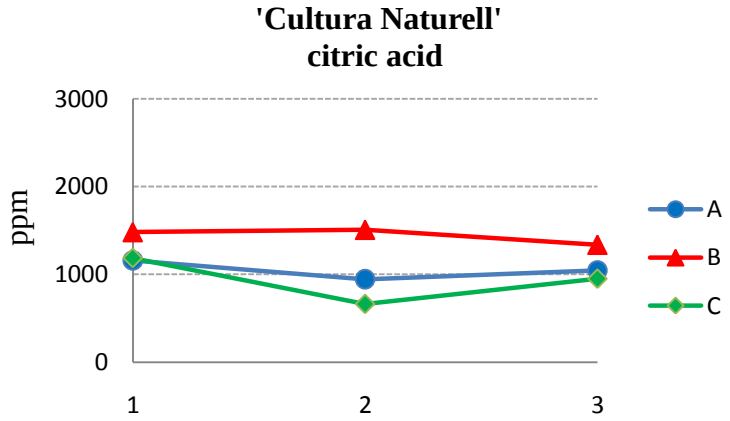

Figure 4. 35 Changes in the level of citric acid in the product 'Cultura Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of citric acid in 'Cultura Naturell' during storage were

between 1159 and 1481 ppm and showed considerable variation between the three productions. Changes in the level of citric acid did not follow a defined trend, but its amount in general decreased during two weeks' storage. 'Cultura Naturell' from production B contained the highest concentration of citric acid.

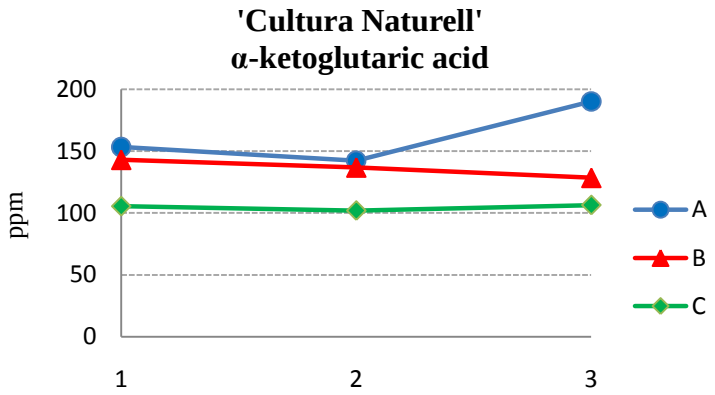

Figure 4. 36 Changes in the level of α-ketoglutaric acid in the product 'Cultura Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of α-ketoglutaric acid during storage in the product 'Cultura Naturell' were between 105 and 153 ppm and varied considerably between the three productions. Apart from an increase product A, the levels did not change during storage.

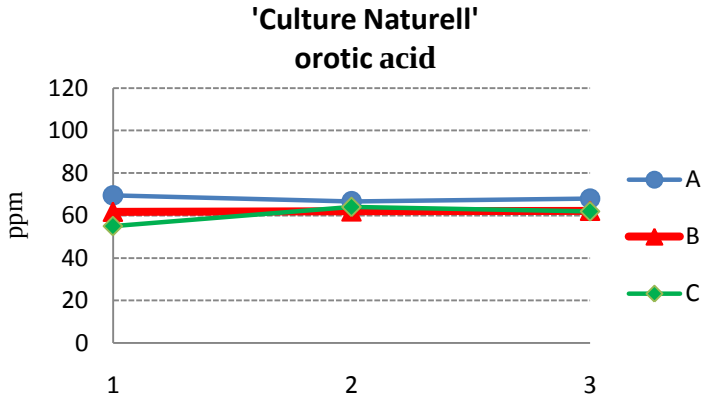

Figure 4. 37 Changes in the level of orotic acid in the product 'Cultura Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of orotic acid in the product 'Cultura Naturell' were between 59 and 66 ppm. No big variations between the three productions of 'Cultura Naturell' were shown and the levels were stable during storage.

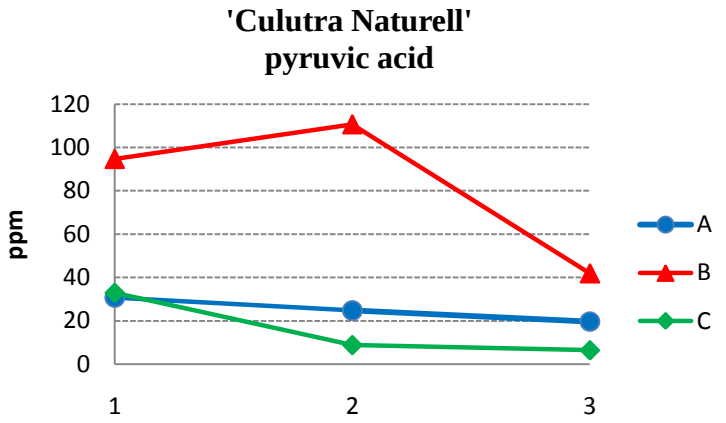

Figure 4. 38 Changes in the level of pyruvic acid in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of pyruvic acid in the product ‘Cultura Naturell’ were between 31 and 95 ppm. Big variations between the three productions of ‘Cultura Naturell’ were found and the concentration decreased in all products during storage. ‘Cultura Naturell’ from production B had much higher pyruvic acid amount, which decreased markedly from 95 to 42 ppm during three weeks’ storage.

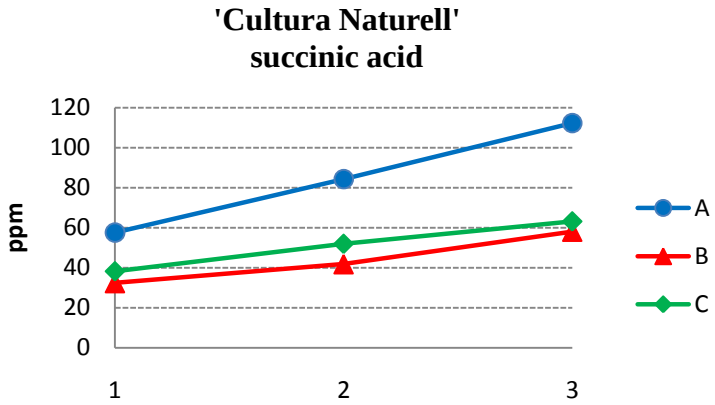

Figure 4. 39 Changes in the level of succinic acid in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of succinic acid in the product ‘Cultura Naturell’ were between 38 and 58 ppm. Great increases in the level of succinic acid were observed on ‘Cultura Naturell’ from all three productions. ‘Cultura Naturell’ from production A had highest amount of succinic acid, which increased most markedly during three weeks’ storage from 58 ppm to 112 ppm.

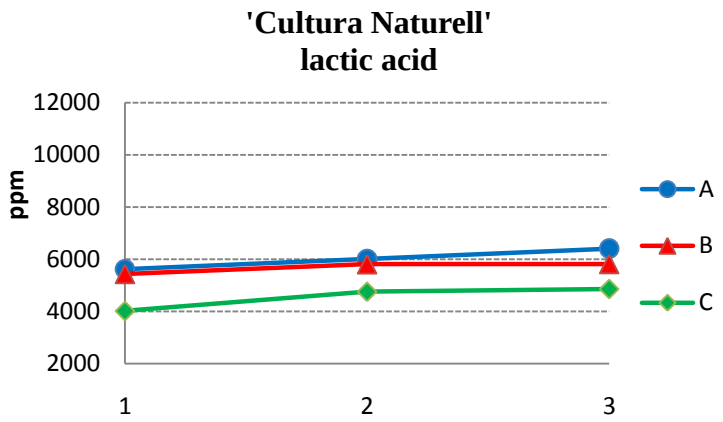

Figure 4. 40 Changes in the level of lactic acid in the product ‘Cultura Naturell’ under storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of lactic acid in the product ‘Cultura Naturell’ were between 4014 and 5615 ppm. About 1000 ppm increases were found in ‘Cultura Naturell’ from production A and C but only about 400 ppm increase was found in samples from production B after three weeks’ storage. ‘Cultura Naturell’ from production C had a much lower lactic acid concentration than from production A and B.

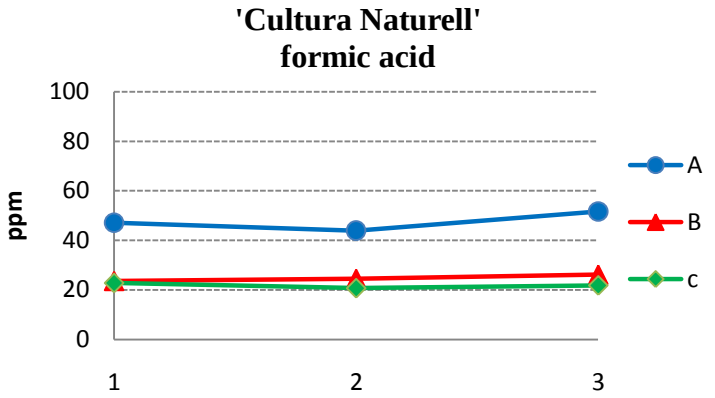

Figure 4. 41 Changes in the level of formic acid in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of formic acid in the product ‘Cultura Naturell’ were between 23 and 47 ppm. Big variations between the three productions of ‘Cultura Naturell’ were found and production A had much higher formic acid concentration than production B and C, which were similar. The amounts of formic acid in ‘Cultura Naturell’ were stable during storage.

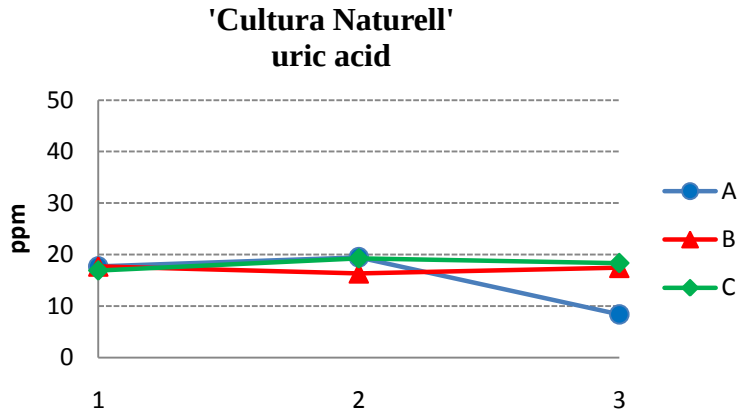

Figure 4. 42 Changes in the level of uric acid in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of uric acid in the product ‘Cultura Naturell’ were round 17 ppm and its amounts were stable during storage except for a decrease in ‘Culture Naturell’ from production A between the second and third sampling.

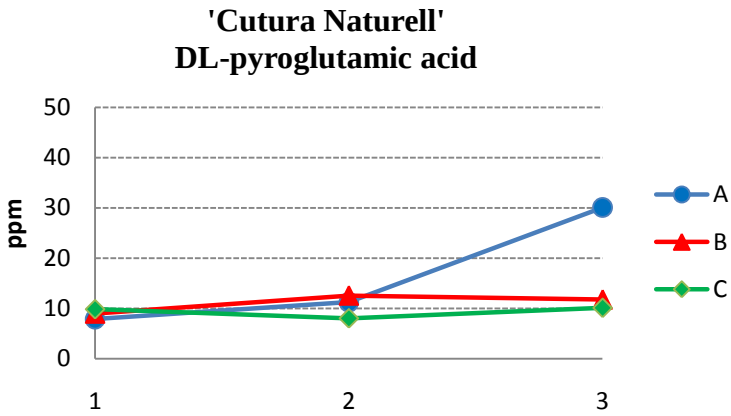

Figure 4. 43 Changes in the level of DL-pyroglutamic acid in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of DL-pyroglutamic acid in the product ‘Cultura Naturell’ were round 10 ppm and its amounts were stable except for an obvious increase in the third sample from production A during storage.

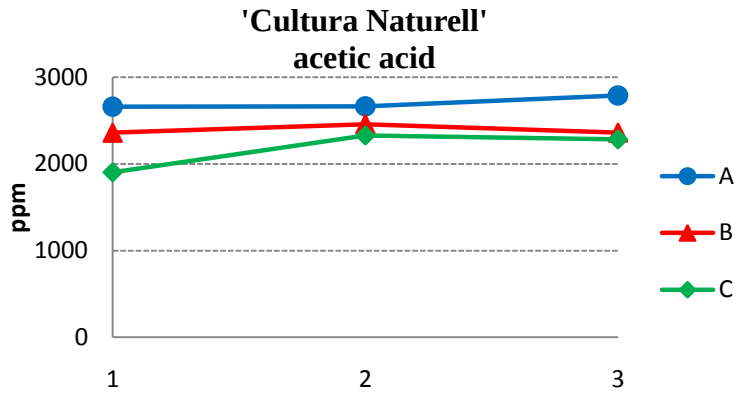

Figure 4. 44 Changes in the level of acetic acid in the product ‘Cultura Naturell’ under storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of acetic acid in the product ‘Cultura Naturell’ were between 1903 and 2662 ppm. Large variations between the three productions of ‘Cultura Naturell’ are shown in figure 4.44. The amount of acetic acid in general increased slightly during storage. Production A of ‘Cultura Naturell’ contained highest amount of acetic acid between 2662 and 2790 ppm.

4.5.2 Development of organic acids in ‘Biola Syrnet Lettmelk Naturell’

Figure 4.45 4.46, 4.47, 4.48, 4.49, 4.50, 4.51, 4.52, 4.53 and 4.54 show the changes of organic acids in the product ‘Biola Syrnet Lettmelk Naturell’

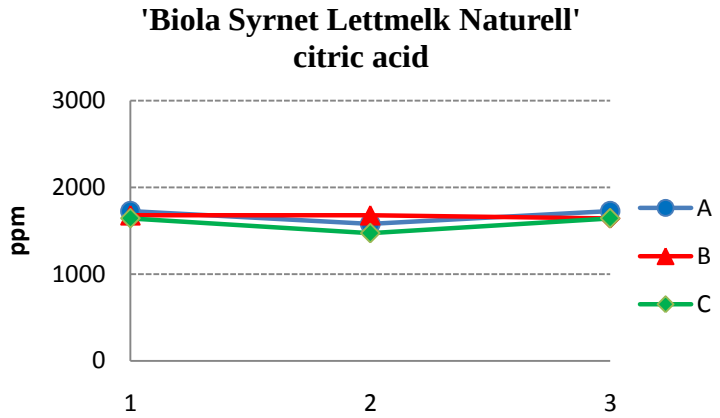

Figure 4. 45 Changes in the level of citric acid in the product ‘Biola Syrnet Lettmelk Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of citric acid in the product ‘Biola Syrnet Lettmelk Naturell’ were between 1642 and 1724 ppm. No big variations were found between the three

productions of ‘Biola Syrnet Lettmelk Naturell’ as shown in figure 4.45, and the levels were stable during three weeks’ storage.

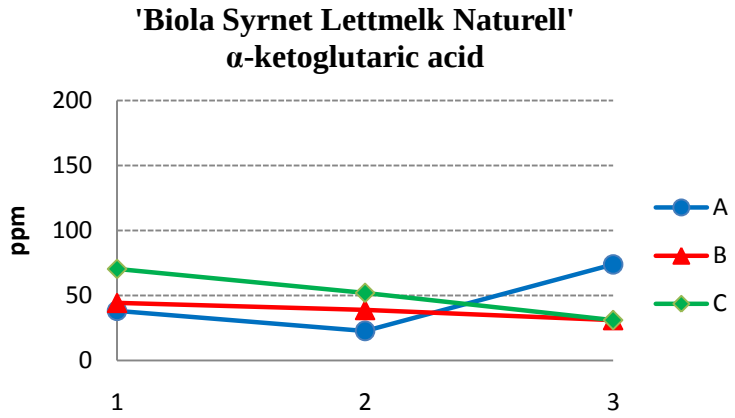

Figure 4. 46 Changes in the level of citric acid in the product ‘Biola Syrnet Lettmelk Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of α-ketoglutaric acid in ‘Biola Syrnet Lettmelk Naturell’ were between 38 and 70 ppm. Big variations between the three productions of ‘Biola Syrnet Lettmelk Naturell’ are shown in figure 4.46. The amounts of a-ketoglutaric in ‘Biola Syrnet Lettmelk Naturell’ in general decrease during three weeks of storage except for an increase in ‘Biola Syrnet Lettmelk Naturell’ from production A between the second and third sampling.

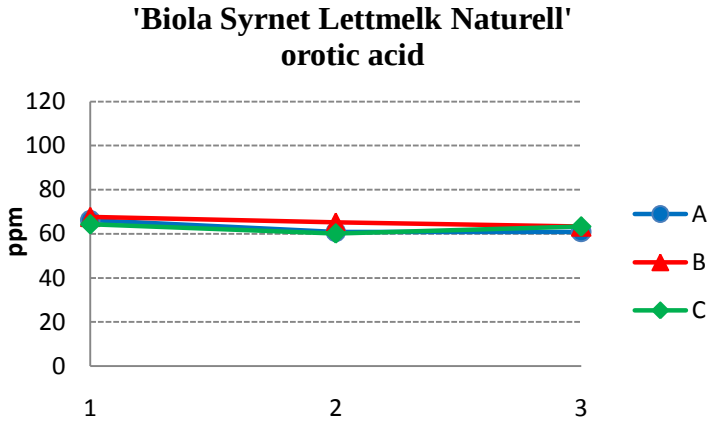

Figure 4. 47 Changes in the level of citric acid in the product ‘Biola Syrnet Lettmelk Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of orotic acid in the product ‘Biola Syrnet Lettmelk Naturell’ were between 66 and 68 ppm. No big variations between the three productions of ‘Biola Syrnet Lettmelk Naturell’ were found as shown in figure 4.47 and the levels remained the same during storage.

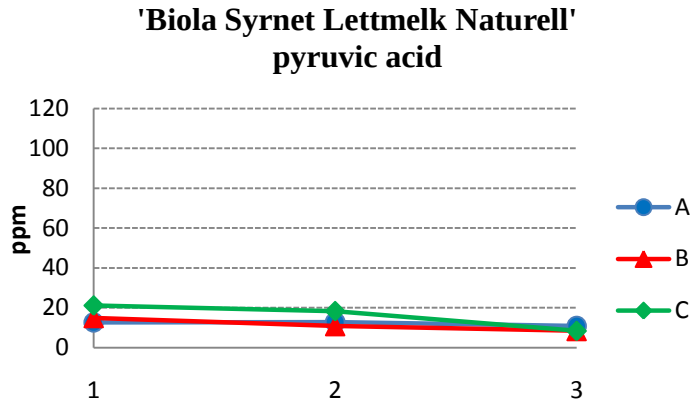

Figure 4. 48 Changes in the level of citric acid in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of pyruvic acid in the product 'Biola Syrnet Lettmelk Naturell' were between 13 and 21 ppm. No big variations between the three productions of 'Biola Syrnet Lettmelk Naturell' are shown in figure 4.48. The amounts of pyruvic acid in 'Biola Syrnet Lettmelk Naturell' decreased slightly during storage.

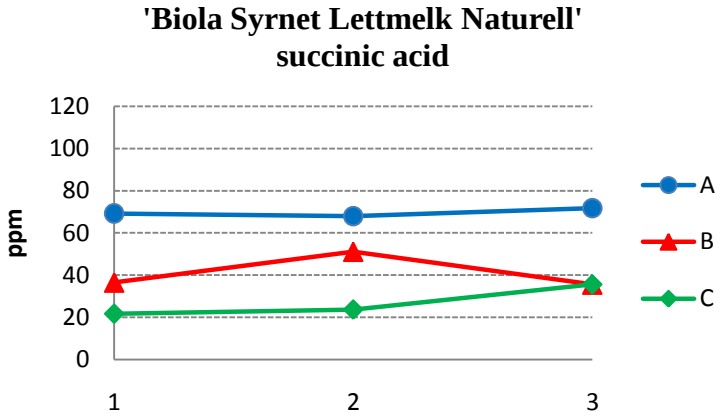

Figure 4. 49 Changes in the level of citric acid in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of succinic acid in the product 'Biola Syrnet Lettmelk Naturell' were between 22 and 69 ppm with large variations between the three productions as shown in figure 4.49. The amounts of succinic acid in 'Biola Syrnet Lettmelk Naturell' slightly increased during three weeks' storage except for a slight decrease in 'Biola Syrnet Lettmelk Naturell' from production B between the second and third sampling.

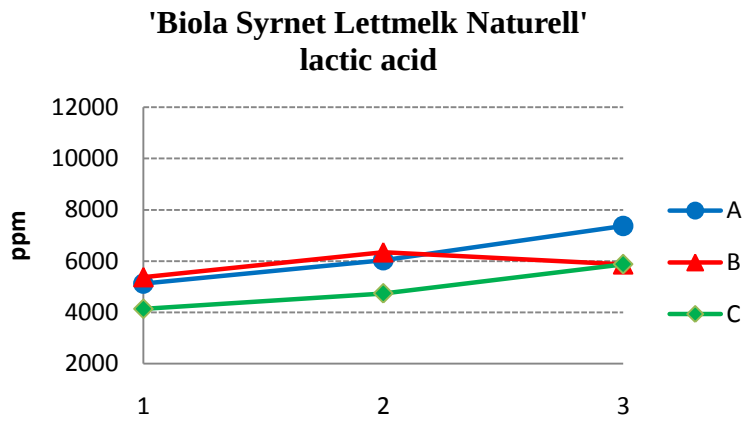

Figure 4. 50 Changes in the level of citric acid in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of lactic acid in the product 'Biola Syrnet Lettmelk Naturell' were between 4138 and 5378 ppm, and varied greatly between productions. Lactic acid increased greatly during storage.

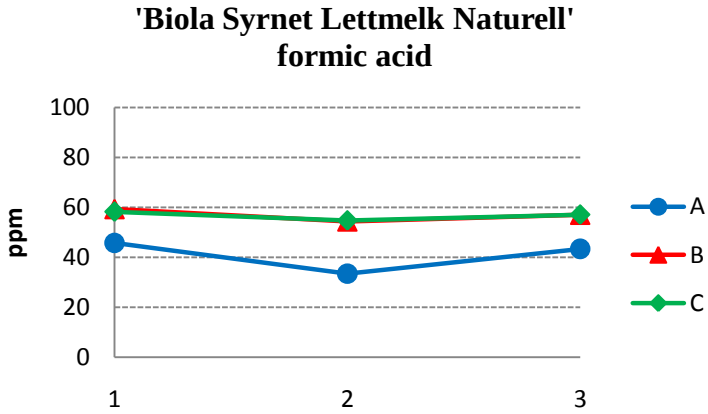

Figure 4. 51 Changes in the level of formic acid in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of formic acid in the product 'Biola Syrnet Lettmelk Naturell' were between 46 and 59 ppm. Big variations between the three productions of 'Biola Syrnet Lettmelk Naturell' are shown in figure 4.51. 'Biola Syrnet Lettmelk Naturell' from production A had the lowest concentration of formic acid. The amounts of formic acid in 'Biola Syrnet Lettmelk Naturell' were stable during three weeks' storage.

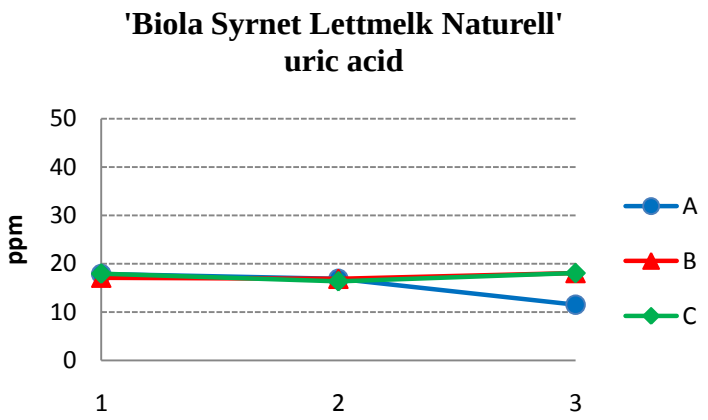

Figure 4. 52 Changes in the level of uric acid in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of uric acid in the product 'Biola Syrnet Lettmelk Naturell' were round 17 ppm. No big variations between the three productions of 'Biola Syrnet Lettmelk Naturell' and the amounts of uric acid were stable during three weeks' storage except for a decrease in the sample from production A at the third sampling time.

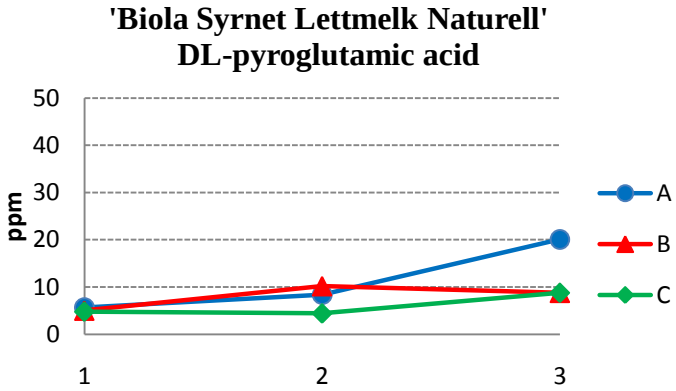

Figure 4. 53 Changes in the level of DL-pyroglutamic acid in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of DL-pyroglutamic acid in the product 'Biola Syrnet Lettmelk Naturell' were round 5 ppm and increased during storage. Variations between the three productions of 'Biola Syrnet Lettmelk Naturell' were small.

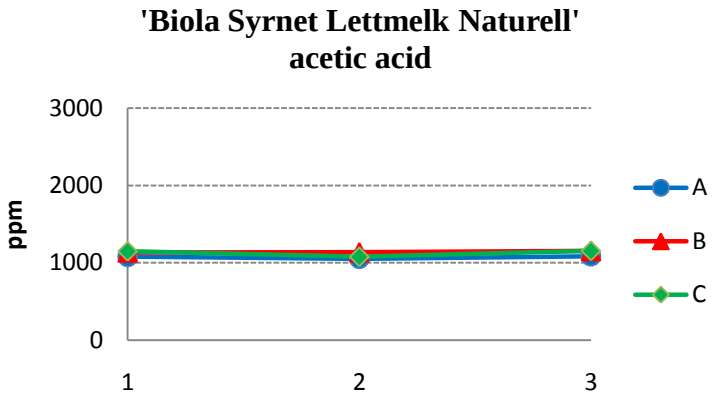

Figure 4. 54 Changes in the level of acetic acid in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of acetic acid in the product 'Biola Syrnet Lettmelk Naturell' were round 1100 ppm, considerably lower than in the product 'Cultura Naturell'. No variations between the three productions of 'Biola Syrnet Lettmelk Naturell' were found as shown in figure 4.54 and the levels were stable during storage.

4.5.3 Development of organic acids in 'Biola Yoghurt Pluss Mild Naturell'

Figure 4.55, 4.56, 4.57, 4.58, 4.59, 4.60, 4.61, 4.62, 4.63, and 4.64 show the development of organic acids in product 'Biola Yoghurt Pluss Mild Naturell' under storage at 4 ℃.

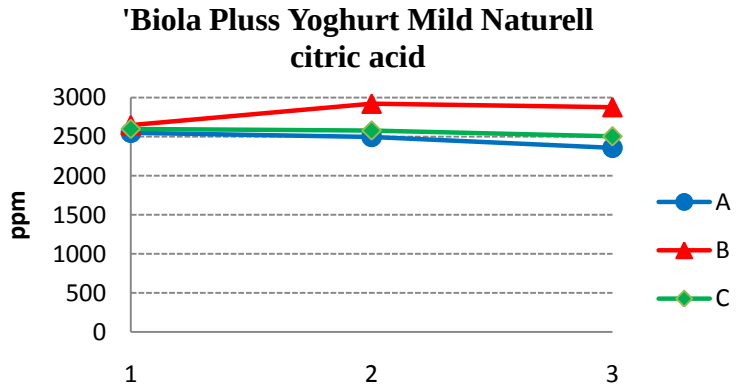

Figure 4. 55 Changes in the level of citric acid in the product 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

High concentrations of citric acid were observed in the production 'Biola Pluss Yoghurt Mild Naturell' with initial concentrations round 2600 ppm. The amounts of citric acid in 'Biola Pluss Yoghurt Mild Naturell' from production A and C were stable during five

weeks of storage, while the amount of citric acid in ‘Biola Pluss Yoghurt Mild Naturell’ from production B appeared to unexplainable increase slightly from 2648 to 2877 ppm during storage.

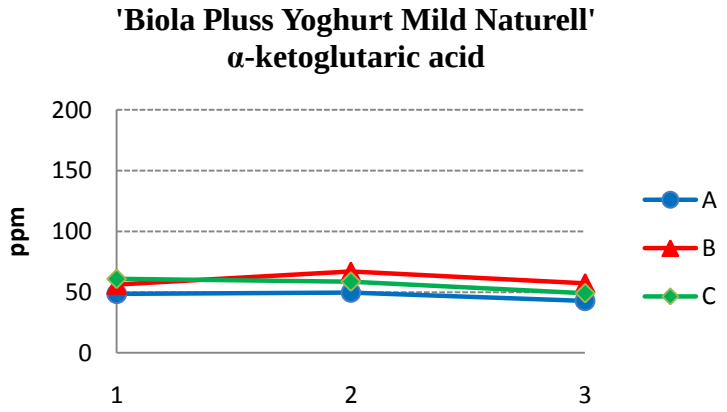

Figure 4. 56 Changes in the level of α-ketoglutaric acid in the product ‘Biola Pluss Yoghurt Mild Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of α-ketoglutaric acid in ‘Biola Pluss Yoghurt Mild Naturell’ were between 49- 61 ppm. No big variations between the three productions of ‘Biola Pluss Yoghurt Mild Naturell’ are shown in figure 4.56. The amounts of a-ketoglutaric acid in ‘Biola Pluss Yoghurt Mild Naturell’ were stable and no obvious changes were observed during three weeks’ storage.

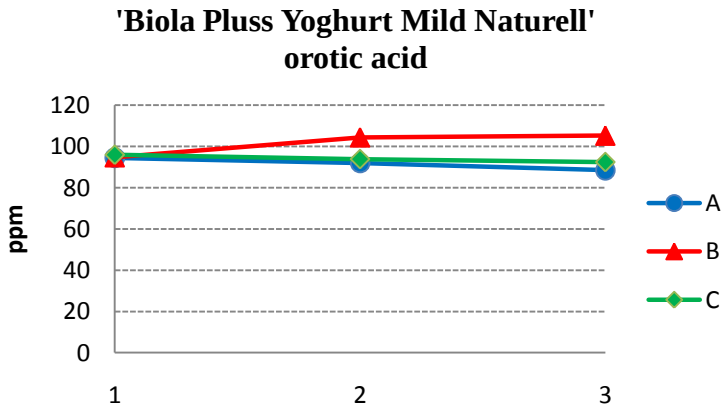

Figure 4. 57 Changes in the level of orotic acid in the product ‘Biola Pluss Yoghurt Mild Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of orotic acid in the product ‘Biola Pluss Yoghurt Mild Naturell’ were round 95 ppm. The amounts of orotic acid in ‘Biola Pluss Yoghurt Mild Naturell’ from production A and C were stable during five weeks of storage, while its amount in production B increased from 95 to 105 ppm during storage.

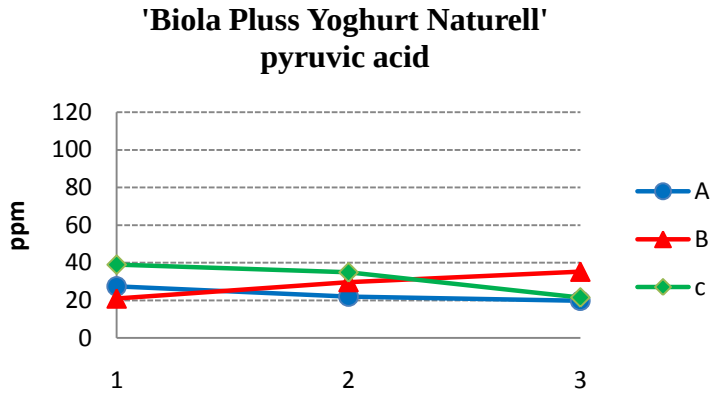

Figure 4. 58 Changes in the level of pyruvic acid in the product 'Biola Pluss Yoghurt Mild Naturell' under storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of pyruvic acid in the product 'Biola Pluss Yoghurt Mild Naturell' were between 21 and 39 ppm and varied between the three productions of 'Biola Pluss Yoghurt Mild Naturell' are shown in figure 4.58. Changes in the level of pyruvic acid in 'Biola Pluss Yoghurt Mild Naturell' did not follow a regular rule during storage, but its amounts in general were stable, maintaining at a level between 20 and 40 ppm.

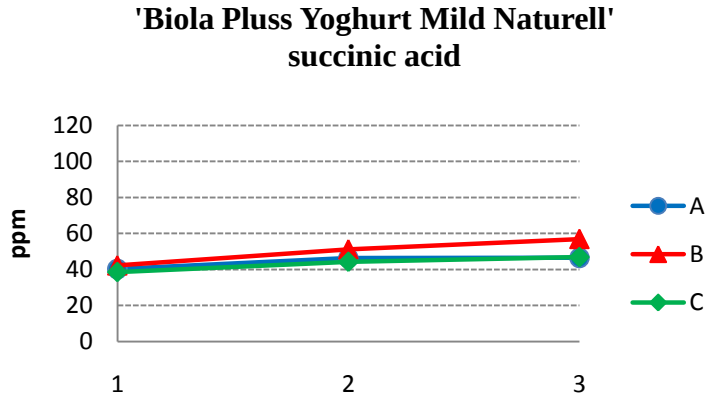

Figure 4. 59 Changes in the level of succinic acid in the product 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of succinic acid in the product 'Biola Pluss Yoghurt Mild Naturell' were round 40 ppm. No big variations between the three productions of 'Biola Pluss Yoghurt Mild Naturell' are shown in figure 4.59. The amounts of succinic acid in 'Biola Pluss Yoghurt Mild Naturell' in general slightly increase during three weeks' storage. Succinic acid in 'Biola Pluss Yoghurt Mild Naturell' from production B increased most markedly from 40 ppm to almost 60 ppm.

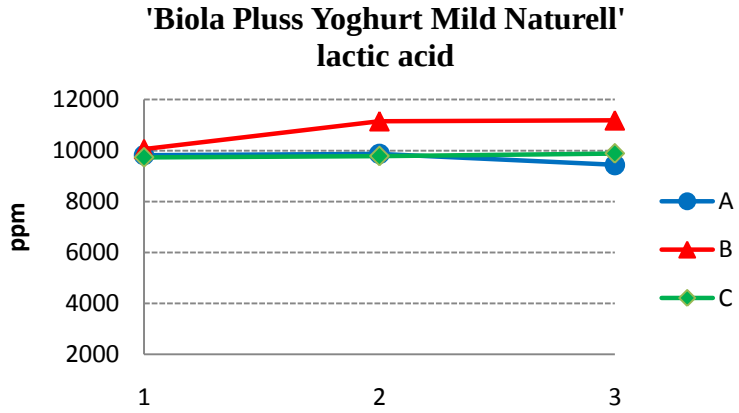

Figure 4. 60 Changes in the level of lactic acid in the product 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

High amounts of lactic acid were observed in the product 'Biola Pluss Yoghurt Mild Naturell' with initial concentrations approximately 10000 ppm. The amounts of lactic acid in 'Biola Pluss Yoghurt Mild Naturell' were stable during five weeks' storage except for an increase in 'Biola Pluss Yoghurt Mild Naturell' from production B from 10063 to 11188 ppm.

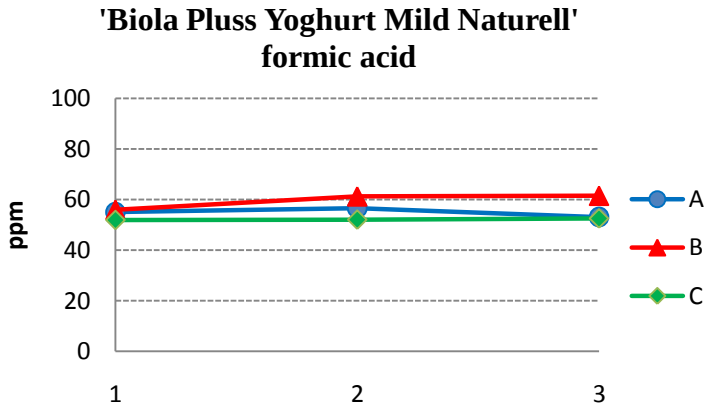

Figure 4. 61 Changes in the level of formic acid in the product 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of formic acid in the product 'Biola Pluss Yoghurt Mild Naturell' were between 52 and 56 ppm. No big variations between the three productions of 'Biola Pluss Yoghurt Mild Naturell' are shown in figure 4.61. The amounts of formic acid in 'Biola Pluss Yoghurt Mild Naturell' were stable during three weeks' storage, maintaining at a level round 55 ppm during storage.

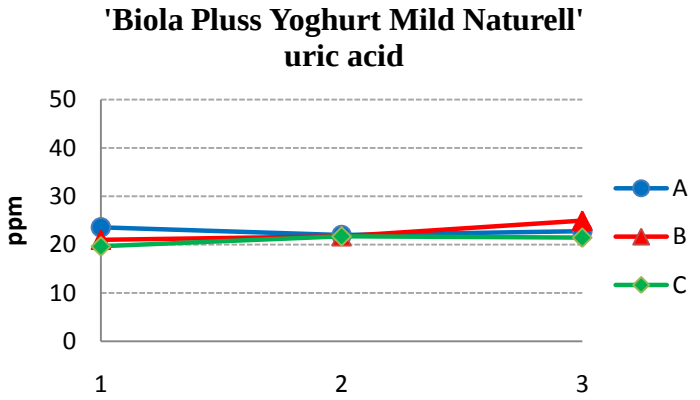

Figure 4. 62 Changes in the level of uric acid in the product ‘Biola Pluss Yoghurt Mild Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of uric acid in the product ‘Biola Pluss Yoghurt Mild Naturell’ were round 22 ppm. No big variations between the three productions of ‘Biola Pluss Yoghurt Mild Naturell’ are shown in figure 4.62. The amounts of uric acid in ‘Biola Pluss Yoghurt Mild Naturell’ were stable during storage maintaining at a level round 22 ppm.

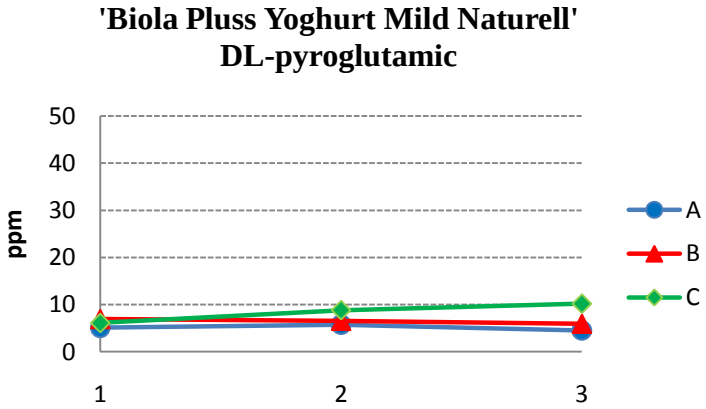

Figure 4. 63 Changes in the level of DL-pyroglutamic acid in the product ‘Biola Pluss Yoghurt Mild Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of DL-pyroglutamic acid in the product ‘Biola Syrnet Lettmelk Naturell’ were round 6 ppm. Variations between the three productions of ‘Biola Pluss Yoghurt Mild Naturell’ were small. The amounts of DL-pyroglutamic acid in ‘Biola Syrnet Lettmelk Naturell’ were stable except for a slight increase in ‘Biola Pluss Yoghurt Mild Naturell’ from production C during five weeks’ storage.

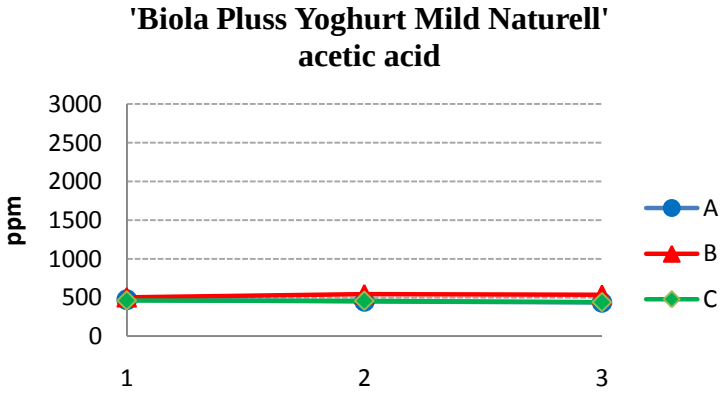

Figure 4. 64 Changes in the level of acetic acid in the product 'Biola Pluss Yoghurt Mild Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The initial concentrations of acetic acid in the product in 'Biola Syrnet Lettmelk Naturell' were round 500 ppm. No obvious variations between the three productions of 'Biola Syrnet Lettmelk Naturell' were found as shown in figure 4.64 and the amounts of acetic acid were stable during storage.

4.6 Carbohydrate (HPLC)

4.6.1 Carbohydrate changes in 'Cultura Naturell'

Figure 4.65, 4.66 and 4.67 shows carbohydrate changes in 'Cultura Naturell' during storage at 4 ℃.

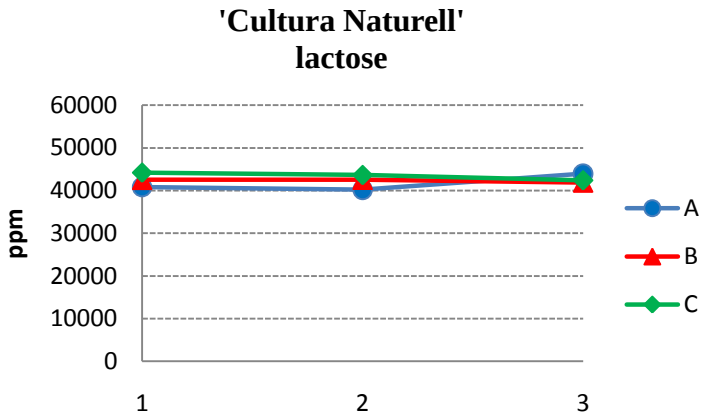

Figure 4. 65 Changes in the level of lactose in the product 'Cultura Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

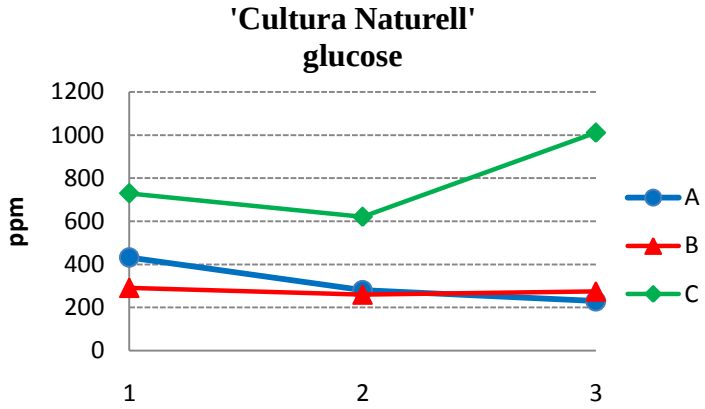

Figure 4. 66 Changes in the level of glucose in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

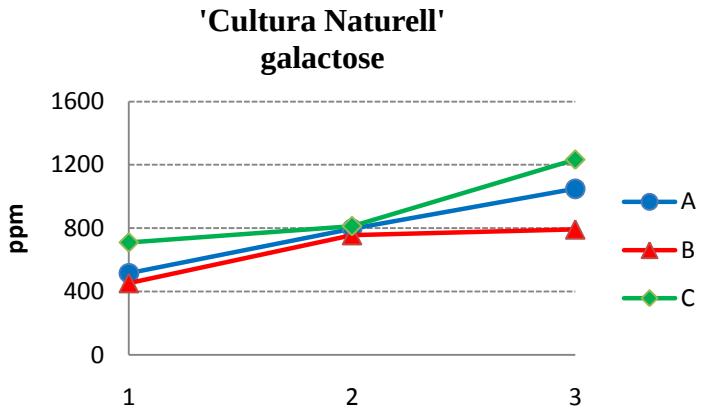

Figure 4. 67 Changes in the level of galactose in the product ‘Cultura Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The carbohydrates lactose, galactose and glucose were detected in the product ‘Cultura Naturell’. The initial concentrations of lactose were between 40800 and 44200 ppm, the initial concentrations of galactose were between 453 and 715 ppm and the initial concentrations of glucose were between 292 and 730 ppm. Variations between the three productions of ‘Cultura Naturell’ are shown in figure 4.65, 4.66 and 4.67. The amounts of lactose were relatively stable during three weeks’ storage. Compared to lactose, amounts of galactose were much lower and increased markedly during storage. Amounts of glucose in general decreased during storage except for an obvious increase in ‘Culture Naturell’ from production C between the second and third sampling.

4.6.2 Carbohydrate changes in ‘Biola Syrnet Lettmelk Naturell’

Figure 4.68, 4.69, 4.70 and 4.71 show the changes of carbohydrates in the product ‘Biola Syrnet Lettmelk Naturell’ during storage at 4℃.

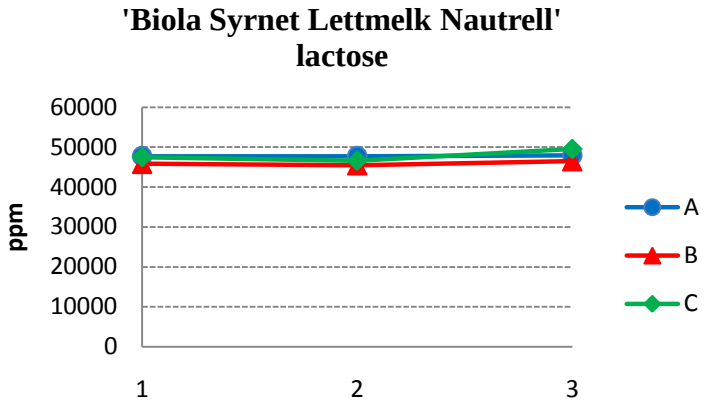

Figure 4. 68 Changes in the level of lactose in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

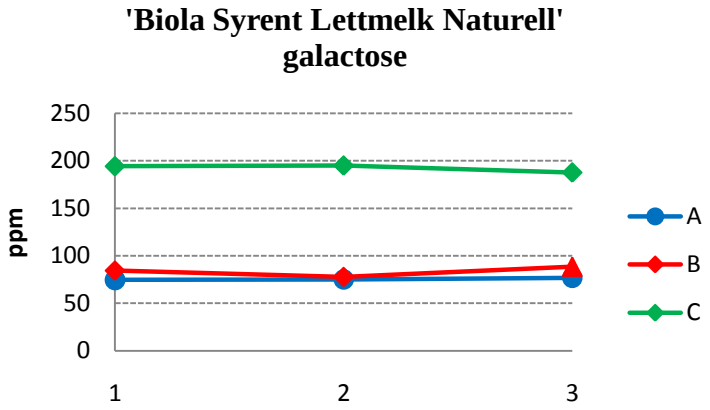

Figure 4. 69 Changes in the level of galactose in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

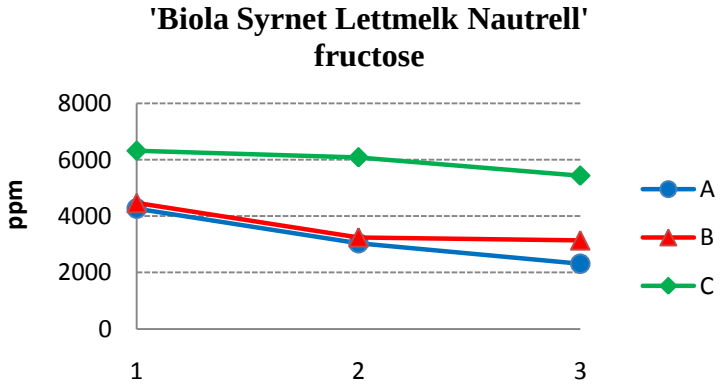

Figure 4. 70 Changes in the level of fructose in the product 'Biola Syrnet Lettmelk Naturell' during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

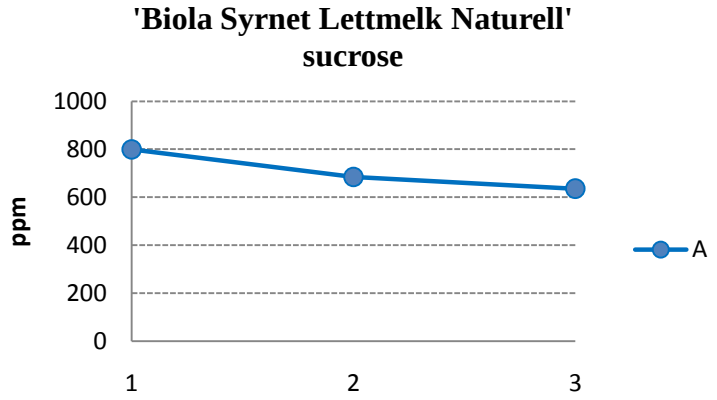

Figure 4. 71 Changes in the level of sucrose in the product ‘Biola Syrnet Lettmelk Naturell’during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The carbohydrates lactose, galactose, and fructose were detected in ‘Biola Syrnet Lettmelk Naturell’ from all three productions. The initial concentrations of lactose were between 45820 and 47750 ppm. The initial concentrations of galactose were between 75 and 194 ppm and the initial concentrations of fructose were between 4270 and 6318 ppm. Sucrose was detected only in ‘Biola Syrnet Lettmelk Naturell’ from production A with an initial concentration of 800 ppm. The amounts of lactose and galactose were stable during storage and the amounts of fructose and sucrose obviously decreased. ‘Biola Syrnet Lettmelk Naturell’ from production C had higher amount of fructose and galactose than ‘Biola Syrnet Lettmelk Naturell’ from production A and B.

4.6.2 Carbohydrate changes in ‘Biola Pluss Yoghurt Mild Naturell’

Figure 4.72, 4.73, and 4.74 show the changes of carbohydrates in the product ‘Biola Pluss Yoghurt Mild Naturell’ during storage at 4℃.

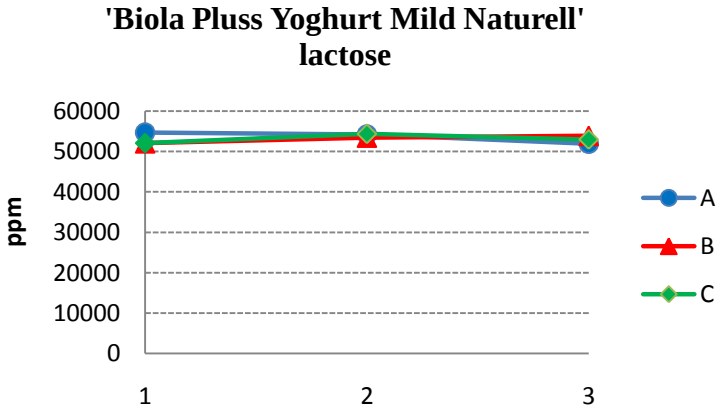

Figure 4. 72 Changes in the level of lactose in the product ‘Biola Pluss Yoghurt Mild Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

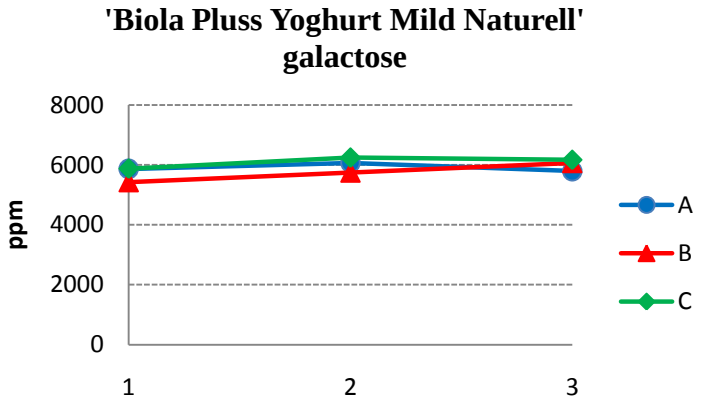

Figure 4. 73 Changes in the level of galactose in the product ‘Biola Pluss Yoghurt Mild Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

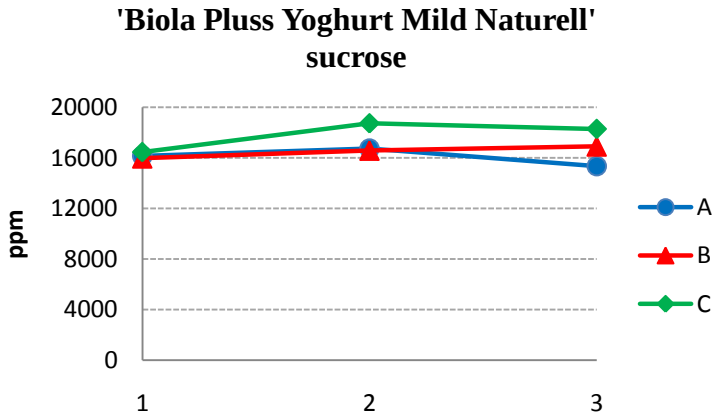

Figure 4. 74 Changes in the level of fructose in the product ‘Biola Pluss Yoghurt Mild Naturell’ during storage at 4 ℃ (A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling).

The carbohydrates lactose, galactose and sucrose were detected in ‘Biola Pluss Yoghurt Mild Naturell’ from all three productions. The initial concentrations of lactose were between 52085 and 54702 ppm and he initial concentrations of galactose were between 5425 and 5871 ppm. The amounts of sucrose were high with initial concentrations between 15953 and 16440 ppm. The amounts of lactose, galactose and sucrose were stable regarding their high levels during storage.

4.7 Sensory analysis

4.7.1 Sensory properties of ’Cultura Naturell’

Figure 4.75, 4.76 and 4.77 show the changes of sensory properties of the product ‘Cultura Naturell’ during storage at 4 ℃.

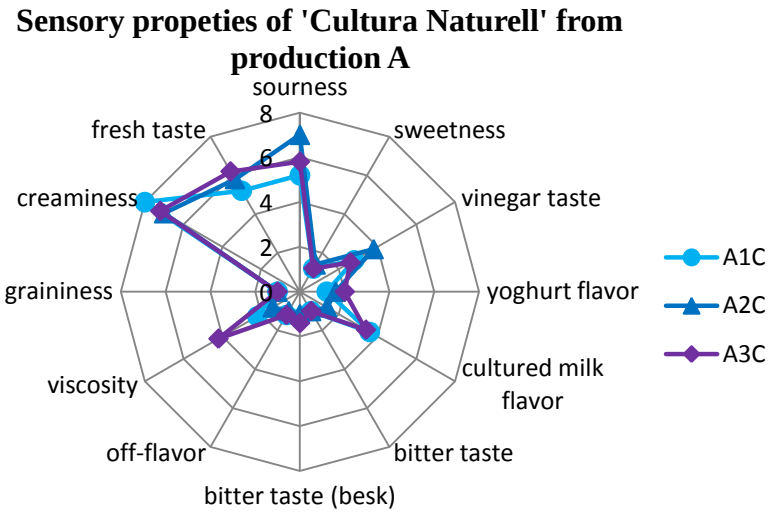

Figure 4. 75 Changes of the sensory properties of ‘Cultura Naturell’ from production A during storage at 4 ℃.

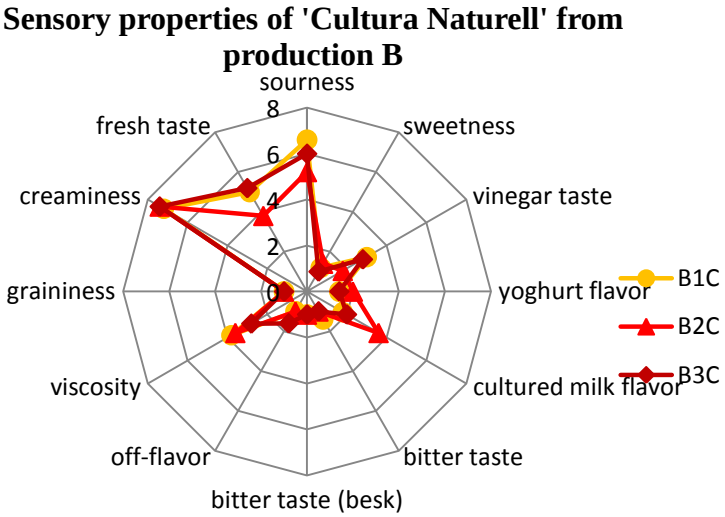

Figure 4. 76 Changes of the sensory properties of ‘Cultura Naturell’ from production B. during storage at 4 ℃.

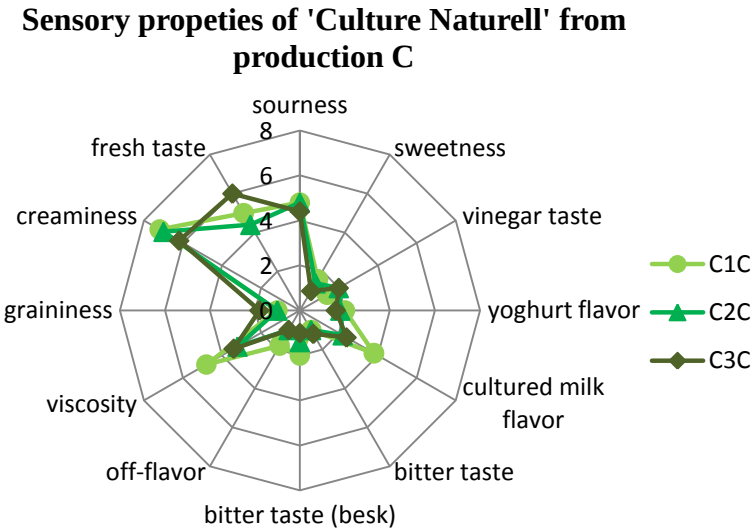

Figure 4. 77 Changes of the sensory properties of ‘Cultura Naturell’ from production C during storage at 4 ℃.

Figures 4.75, 4.76 and 4.77 show that a few sensory descriptors (including bitter taste, creaminess, after taste, yoghurt flavor, and vinegar taste) did not vary in the samples either between productions or during storage. Yogurt flavor, sweetness and vinegar taste were not detected by the sensory panel in product. Consistent decrease of cultured milk flavor and creaminess were detected in ‘Cultura Naturell’. The changes of viscosity, fresh taste and sourness were irregular.

4.6.2 Sensory properties of ‘Biola Syrnet Lettmelk Naturell’

Figure 4.78, 4.79 and 4.80 show the changes of sensory properties of product ‘Biola Syrnet Lettmelk Naturell’ during storage at 4 ℃.

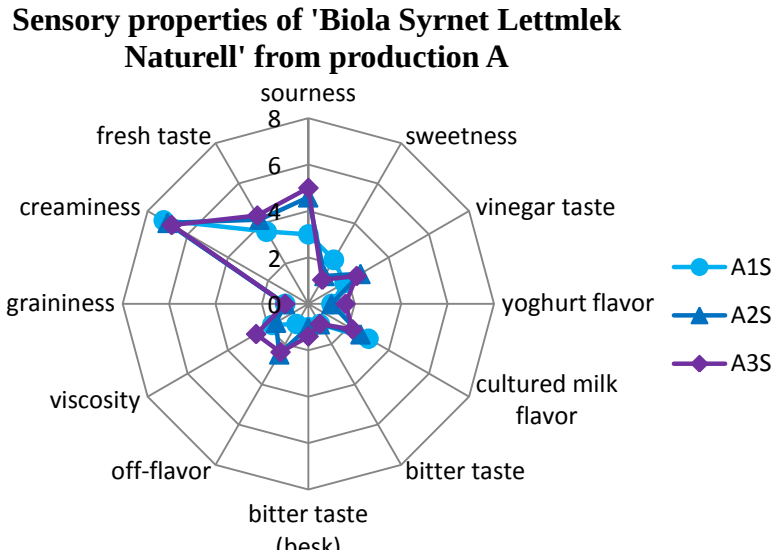

Figure 4. 78 Changes of the sensory properties of ‘Biola Syrnet Lettmelk Naturell’ from production A during storage at 4 ℃.

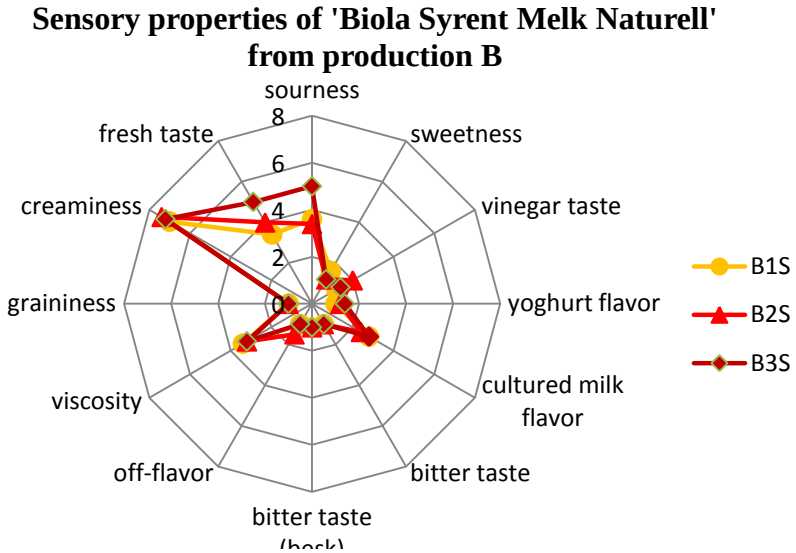

Figure 4. 79 Changes of sensory properties of ‘Biola Syrnet Lettmelk Naturell’ from production B during storage at 4 ℃.

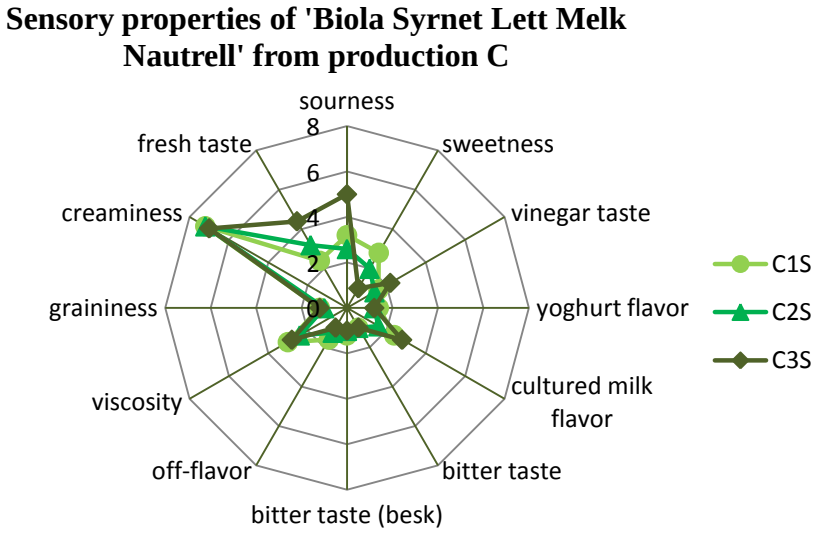

Figure 4. 80 Changes of the sensory properties of ‘Biola Syrnet Lettmelk Naturell’ from production C during storage at 4 ℃.

Figures 4.78, 4.79 and 4.80 show that a few sensory descriptors including bitter taste, creaminess, viscosity, after taste, cultured milk flavor, yoghurt flavor, and vinegar taste did not vary in the samples either between productions or during storage. Yogurt flavor, sweetness and vinegar taste were not particularly noted by the sensory panel in the product ‘Biola Syrnet Lettmelk Naturell’ but consistent increases of sourness and fresh taste in ‘Biola Syrnet Lettmelk Naturell’ were detected as storage time increased. A decrease in sweetness was detected by the sensory panel ‘Biola Syrnet Lettmelk Naturell’ during storage.

4.6.3 Sensory properties of ‘Biola Pluss Yoghurt Mild Naturell’

Figures 4.81, 4.82 and 4.83 show the changes of sensory properties of the product ‘Biola Pluss Yoghurt Mild Naturell’during storage at 4 ℃.

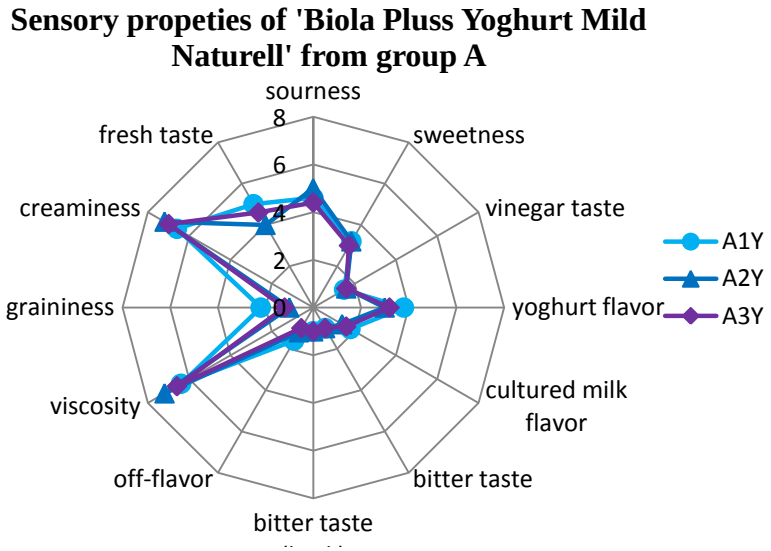

Figure 4. 81 Changes of the sensory properties of ‘Biola Pluss Yoghurt Mild Naturell’ from production A during storage at 4 ℃.

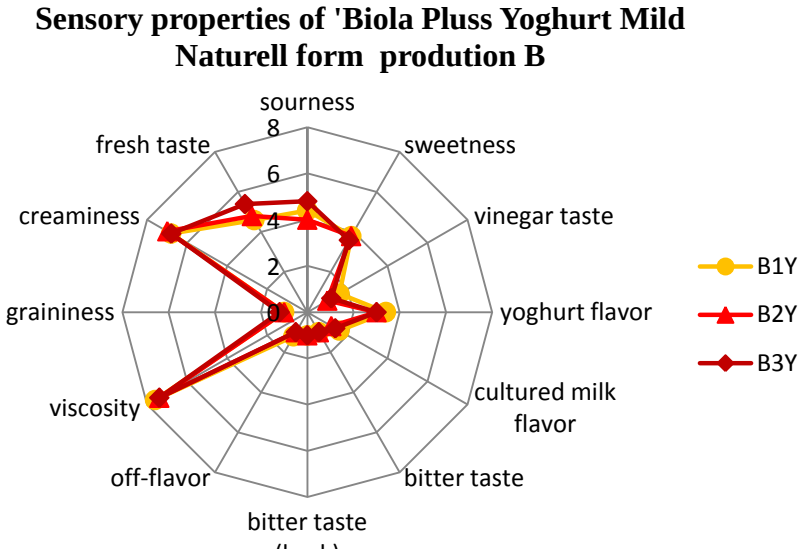

Figure 4. 82 Changes of the sensory properties of ‘Biola Pluss Yoghurt Mild Naturell’ from production B during storage at 4 ℃.

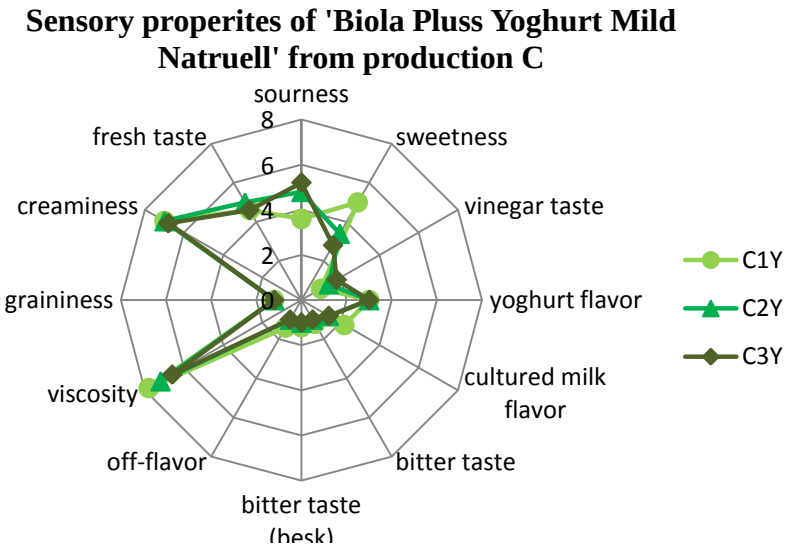

Figure 4. 83 Changes of the sensory properties of ‘Biola Pluss Yoghurt Mild Naturell’ from production C during storage at 4 ℃.

Figures 4.81, 4.82 and 4.83 show that a few sensory descriptors including bitter taste, creaminess, viscosity, after taste, cultured milk flavor, yoghurt flavor, and vinegar taste did not vary between samples. Bitter taste, cultured milk flavor and vinegar taste were not obviously detected by the sensory panel in the product ‘Biola Pluss Yoghurt Mild Naturell’. An increase in sourness was detected by the sensory panel during storage. The changes of fresh taste and sweetness were irregular.

4.8 PCA

A correlation loadings plot from the principal component analysis (PCA) of the significant sensory attributes for all 27 samples is presented in figure 4.84.

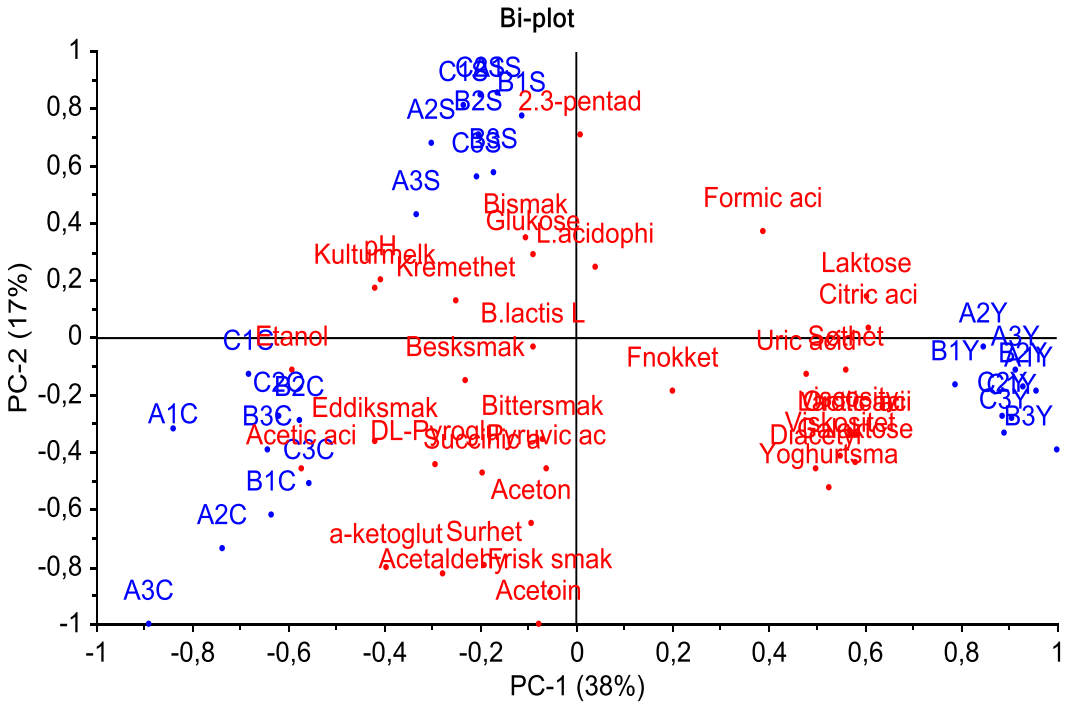

Figure 4. 84 Bi-plot from PCA of all 27 samples (C, S, Y= ‘Culture Naturell’, ‘Biola Syrnet Lettmelk Naturell’ and ‘Biola Pluss Yoghurt Mild Naturell’; A, B, C = production A, production B and production C; 1, 2, 3 = first sampling, second sampling and third sampling)

According to figure 4.84, variables and samples are overlaid on the same graph and 27 samples can be clearly divided into three different groups representing the three products studied the loading of the first and the second principal components showed that samples of ‘Cultura Naturell’ were positively correlated with ethanol, acetic acid and vinegar taste (placed to the left in figure 4.84). Samples of ‘Biola Syrnet Lettmelk Naturell’ were positively correlated with 2, 3- pentanedione, pH and off-flavor (placed on the top in figure 4.48); Samples of ‘Biola Pluss Yoghurt Mild Naturell’ were positively correlated with lactose, citric acid, yoghurt taste, viscosity, diacetyl and sucrose.

5.0 DISCUSSION

5.1 Product 'Cultura Naturell|'

TINE 'Cultura Naturell' is a fermented low-fat milk product with 1.5 % fat, fermented with a bacteria culture composed of *L. acidophilus* La-5 and *B. lactis* Bb-12. These two bacteria exist naturally in the digestive system of humans. However, their number decreases as people getting older or are exposed under stress and poor diet, causing disruption of microbial balance in digestive system (Sander, 2000). In order to restore intestinal balance and maintain digestive health, certain amounts of beneficial bacteria should be boosted through dietary supplements and foods. It is recommended that 10^6 cfu /g probiotic bacteria should be present in a probiotic fermented milk products (Rybka & Kailasapathy, 1995). This standard was also developed by the fermented milks and Lactic Acid Bacteria Beverages Association in Japan which claimed that a minimum of 10^7 cfu /g viable *Bifidobacteria* cells should be present in fresh dairy products (Ishibashi & Shimamura,1993). In order to compensate for the number of probiotic bacteria that die during transit trough the GIT, consumers are recommended to intake 100 g per day of probiotic milk products containing totally10^9 cfu probiotic bacteria (Rybka & Kailasapathy, 1995).These studies assume that customers can expect the beneficial effect of probiotic bacteria in fermented milk products only when the number of these probiotic bacteria are maintained at level equivalent to or higher than the level they claimed in their studies.

The viable cell counts of both *L. acidophilus* La-5 and *B. lactis* Bb-12 in TINE 'Cultura Naturell' during storage at 4 ℃ maintained a level above 10^7 cfu/ g during its designated shelf-life. This indicates that, in this product, the number and viability of probiotic bacteria is satisfactory and consumption of TINE 'Cultura Naturell' could therefore be suitable for therapeutic purpose throughout the designated shelf-life.

The survival of lactic acid bacteria in fermented milk products is affected by many different factors. Some studies concentrating on the viability of probiotic lactic acid bacteria show a crucial factor affecting the survival of *L. acidophilus* and *Bifidobacterium* is the low pH of the environment and that their number decreases dramatically under acidic conditions, especially for *Bifidobacterium* (Conway *et al.*, 1987; Hood & Zottola, 1988). Compared to *Bifidobacterium, L. acidophilus* is more acid-tolerant. The growth of *B. lactis* Bb-12 ceases at pH 5.0 (Costello, 1993) whereas the growth of *L. acidophilus* ceases at pH 4.0 (Playne, 1993). Both *L. acidophilus* and *Bifidobacteria* show poor growth in milk because of lack of sufficient amino acids/peptides in milk. (Laniewska-Trokenheim *et al.*, 2010). Therefore, in practice, they are often used in combination of other LAB (Hunger & Peitersen, 1993; Montes *et al.*, 1995). The number of both *L. acidophilus* La-5 and *B. lactis* Bb-12 in TINE

‘Cultura Naturell’ was relatively stable between first sampling and second sampling and their number declined dramatically, by about 2 log units between the second sampling and third sampling. The decrease in pH of ‘Cultura Naturell’ during storage could be a reason for this. On the other hand, the prolonged exposure to low pH may have caused the observed reduction in cell numbers.

The pH of ‘Cultura Naturell’ from production A decreased most markedly between the second sampling and third sampling and this is in line with the result observed from viability of probiotic bacteria that the decrease of both *L.acidophilus* La-5 and *B. lactis* Bb-12 were greatest in ‘Cultura Naturell’ from production A. By observing figure 4.2 (Viable cell counts of *B. lactis* Bb-12) and figure 4.11(pH of ‘Cultura Naturell’), more relationship between pH and *B. lactis* Bb-12 can be found. A stable pH of product from production B corresponds to a stable number of *B. lactis* Bb-12 in the product from production B. An obvious decline in the pH of the product from production A corresponds to a considerable decrease of *B. lactis* Bb-12 in the product from production A. However, a similar relationship cannot be observed between the viable cell counts of *L. acidophilus* La-5 and the pH of ‘Cultura Naturell’. This result suggests that pH is an important, but not the only factor that affects the viability of probiotic bacteria. The effect of pH is more significant on those probiotic bacteria that are more sensitive to pH of the environment (less acid-tolerant), such as *B. lactis* Bb-12. A decrease of *B. lactis* Bb-12 may also be attributed to hydrogen peroxide produced by *L. acidophilus* La-5. Other factors such as dissolved oxygen content, nutrition, storage temperature, and level of acetic and lactic acid have been reported to be the reasons for decline of probiotic bacteria in fermented milk products (Gilliland & Speck, 1977; Hull *et al.*, 1984; Martin & Chou, 1992; Kneifel *et al.*, 1993; Rybka & Kailasapathy, 1995.)

In the case of viscosity, ‘Cultura Naturell’ had a very low viscosity as shown by the short time required for the product to run through the SMR funnel. Obvious changes were observed in the viscosity of ‘Cultura Naturell’. During milk fermentation, protein in milk undergoes a structure changing process. Casein micelles agglomerate, forming a three-dimensional network, called an acid gel. This acid gel strongly depends on the raw material, processing and fermentation. An acid gel is a weak gel in comparison with a rennet coagulated gel. Heat treatment or increased solid content positively contributes to acid gel structure. In drinking type fermented milk and stirred yogurt, these products undergo a series of processes such as mixing, pumping, cooling and filling after fermentation. The acid gel structure is partially broken down during these processes into a concentrated dispersion of small pieces of gel made of protein particles, despite that it is still desirable to retain this net work in the form of viscosity (Van Marle, 1998).These pieces of gel retain whey fraction and will, to a certain degree, form a new net work during storage due to inter-particle interactions resulting in improvement of viscosity (Renan *et al.*, 2008). Several factors have been reported to affect the firmness and texture of the gel. Seasonal variation of milk composition has an important influence on the texture of the yoghurt (Tamime & Robinson, 2000). Total solid content in milk suspension positively affected the firmness of yoghurt coagulum in linear way

(Kristo *et al.*, 2003). Fermented milk product with a higher concentration of total solids starts to form gel earlier and prolongs the time of fermentation probably because of its buffering effect. Since more nutrition is available for lactic acid bacteria, the viability and the acid production is improved. Fat content affects the texture of fermented milk in a similar way. Full fat fermented milk is suggested to exhibit firmer gel structure than low fat fermented milk. The weak gel texture of fermented skim milk is a potential problem which that has to be faced by manufacturer (Xu *et al.*, 2008). The gel structure is also largely affected by processing. The firmness of yoghurt is positively related to slow gelation (Jumah *et al.*, 2001). Generally, higher pre-heating and prolonged preheating time contributes to firmer gel. Higher fermentation temperature increase gelation rate which results in more porous gel structure and high level of syneresis (Kristo *et al.*, 2003) and it is also reported that low fermentation temperature results in gel structure with higher viscosity. In addition, concentration of starter culture inoculums and their ability to produce EPS are also factors that affect the texture of fermented milk products (Beal *et al.*, 1999).

The development of viscosity in 'Cultura Naturell' varied somewhat according to production batch. In productions B and C, the viscosity increased during storage, whereas in production A, the viscosity decreased. A decrease in viscosity can result from proteolysis during storage. With proteolysis, milk protein is hydrolyzed by proteolytic enzymes resulting in the liberation of different peptides and free amino acids into serum and the gel structure is hence weakened (Tamime & Robinson, 2000). However, it is also possible that particular sample was poorly mixed before viscosity measurement. The gel produced during the fermentation of 'Cultura Naturell' is a coarse gel, due to the high temperature of incubation. Furthermore, this product is not dry-matter enriched. As discussed above, these two factors combined make the gel very liable to spontaneous syneresis during storage and a gradual settling out of the casein at the bottom of the product whilst the upper layer often has a very low viscosity.

In the case of volatile compounds, despite that more than 90 volatile compounds have been identified in the fermented milk products, only few of them were proved to be of sensory importance. Some studies shows that only acetaldehyde, ethanol, acetone, diacetyl and 2-butanone have great influence on the desired flavor (Badings & Neeter, 1980; Tamime & Deeth, 1980; Marshall, 1984; Ulberth, 1991; Kneifel *et al.*, 1992; Ulberth & Kneifel, 1992; Marshall, 1993; Tamime& Robinson, 2000). Certain volatiles, such as diacetyl, affect the product flavor greatly even in small amount; other volatiles such as acetoin, do not affect the flavor much even in high amount (Cheng, 2011). According to (Yuguchi *et al.*, 1989), within a certain range, good flavor of cultured milk positively correlated with the concentrations of acetaldehyde, diacetyl, succinic acid and lactic acid, while it negatively correlated with the concentration of acetone, ethanol and acetic acid.

In 'Cultura Naturell', large amounts of acetaldehyde, ethanol, acetone, and acetoin and relatively low amounts of diacetyl and 2, 3-pentadione were detected. Acetaldehyde is

considered to be the most important constituent of yoghurt aroma. The milk fermented by *L. acidophilus* or *Bifidobacteria* spp. is reported to be lack of flavor because that both of them possess an alcohol dehydrogenase which converts the acetaldehyde to ethanol. However, results in this study seem to be in contrast with this literature date. 16-22 ppm of acetaldehyde was found in 'Cultura Naturell' indicating a limited ability of both bacteria strains to reduce acetaldehyde to ethanol. Despite that TINE 'Cultura Naturell' is not called yoghurt, its relatively high amounts of acetaldehyde could be expected to give yoghurt taste in this product. However, this was not supported by the result of sensory assessment as the panel agreed that no yoghurt taste was prevalent.

In fermented milk products, acetaldehyde can be formed through the metabolism of carbohydrate, protein and nucleic acid in the presence of different enzymes. Figure 5.1 shows the possible metabolic pathway for formation of acetaldehyde. Firstly, it can be produced through glycolysis of glucose. In the presence of pyruvate decarboxylase or pyruvate oxidase, acetaldehyde is formed directly from pyruvate. Alternatively, it can be formed indirectly through the formation of an intermediate product acetyl coenzyme A in the presence of enzyme pyruvate dehydrogenase or pyruvate formatelyase. Secondly, it can be formed from several amino acids producing pyruvate as intermediate product and threonine can be directly converted into acetaldehyde and glycine in the presence of threonine aldolase (TA) (Chaves, *et al.*, 2002)

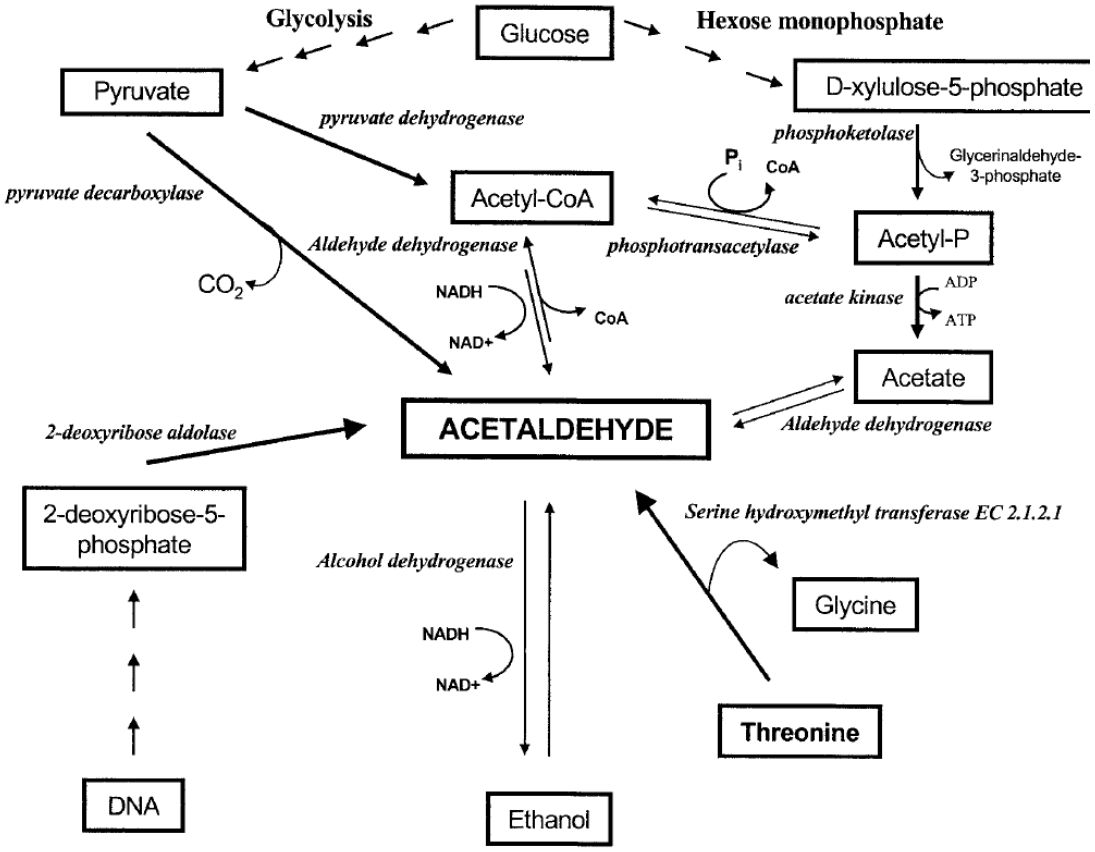

Figure 5.1 metabolic pathways that lead to acetaldehyde formation (Chaves, *et al.*, 2002)

For *L. acidophilus*, activities of pyruvate oxidase, phosphotransacetilase, acetate kinase, aldehyde dehydrogenase, 2-deoxiriboaldolase, α-carboxilase and threonine aldolase were found (Hickey *et al.*, 1983; Gonzalez *et al.*, 1994). A decrease of acetaldehyde during storage was observed in the products from all three productions. This is because at lower temperatures, acetaldehyde can be degraded to ethanol by *L. acidophilus* La-5 and *B. lactis* Bb-12 during storage and this was supported by the increases in ethanol observed at the later stages of storage. Ethanol can be commonly found in fermented milk products, especially in Kefir. It is an end product in the hetero-fermentative breakdown of glucose and catabolism of amino acids (Urbach, 1995; Güler-Akin & Akin *et al.*, 2007).

Diacetyl gives delicate flavor to fermented milk products even in small amount and is produced chemically from the unstable compound α-acetolactate through oxidative decarboxylation (De Man, 1959). A report shows that low amount of diacetyl was detected in milk fermented with *L. acidophilus* La-5 and no diacetyl was detected in milk fermented with *B. lactis* Bb-12 (Østlie *et al.*, 2003) indicating that *L. acidophilus* La-5 is responsible for the production of diacetyl in 'Cultura Naturell'. There are different views on the role of diacetyl in yoghurt flavor formation, some studies claimed that diacetyl will be dominant in profile of yoghurt flavor only when acetaldehyde is in low concentration; others claimed that diacetyl is always important element in profile of yoghurt flavor. Results from this study shows that despite the high amount of acetaldehyde and diacetyl, yoghurt flavor was not detected in 'Cultura Naturell' by the sensory panel. This is probably because that more elements play important role in formation of yoghurt flavor. In some studies, 2, 3-pentanedione is also mentioned as key aroma compound yoghurt flavor and pH in these studies are claimed to dominantly influence the sensory perception of sensory assessors. There were only trace amount of 2, 3-pentadione detected in 'Cultura Naturell', which could be reason for lack of sensory detection of yoghurt flavor.

Diacetyl decreased during storage in products from all three productions. This is probably because that diacetyl under storage was converted in to acetoin by the enzyme diacetyl reductase during storage and acetoin can be, in further step, reduced to butanediol by the same enzyme (Collins, 1972; Østlie *et al.*, 2003). The decrease of diacetyl during storage is in concordance with the increase of the acetoin in the later stages of storage in 'Cultura Naturell'.

The concentration of acetoin in 'Cultura Naturell' is extremely high. A study shows that *L.acidophilus* can produce diacetyl and acetoin from pyruvate but the amount of acetoin is much greater than that of diacetyl especially at 37 ℃ and 45 ℃ (Benito de Cardenas *et al.*, 1991). This is because that α-acetolactate decarboxylase can decarboxylates α-acetolactate to acetoin actively (Monnet *et al.*, 1994). However, it is also reported in a study that no diacetyl acetoin was detected in milk fermented by *L. acidophilus* probably because they were further reduced to 2, 3 butandiol. Rates of

diacetyl and acetoin production depend upon the rate of citrate metabolism and *B. lactis* Bb-12 does not produce diacetyl (Østlie *et al.*, 2003).

Concentrations of acetone detected in fresh 'Cultura Naturell' were between 3.2 ppm and 4.2pm. Acetone naturally exits in milk varying from 0.8-2.7ppm and increase in acetone concentration during refrigerated storage have been observed in yogurts made from cow's milk (Gaafar, 1992; Kang *et al.*, 1988; Kondratenko & Gyosheva, 1978; Kwak, 1995). But its concentration in 'Cultura Naturell' was stable during storage.

Changing in the level of organic acids in 'Cultura Naturell' during storage was not marked. Lactic acid is reported to give refreshing tart flavor to fermented milk products (Panagiotidis & Tzia, 2001) and is an important component of yoghurt flavor. (Ott *et al.*, 2000; Rash, 1990). *L. acidophilus* La-5 ferments lactose through the homofermentative path way and produce lactate. *B. lactis* Bb-12 ferments lactose through the bifidus pathway and produce lactate and acetate in the ratio of 3: 2 (Kandler & Weiss, 1986; Scardovi, 1986). Metabolism of lactose depends not only on the lactose available, but also pH. When pH is decreased to a certain level, metabolism of lactose will be stopped before all lactose is exhausted (Narvhus *et al.*, 1998). Concentrations of lactic acid in fresh 'Cultura Naturell' were between 4000 and 5600 ppm and slightly increase was observed during storage, probably due to post-acidification. The source of lactic acid is lactose and an increase of lactic acid should correspond with a reduction of lactose. This is supported by results from this study.

Acetic acid is an important compound produced by some lactic starter cultures (Alonso & Fraga, 2001; Beshkova et al., 1998; Tamime & Robinson, 2000). It exists also naturally in trace amount in raw milk and at high concentrations will give a vinegar taste that is not appreciated by customers. Concentrations of acetic acid in fresh 'Cultura Naturell' were between 1903 and 2662 ppm. The high amount of acetic acid in 'Cultura Naturell' is because of the metabolism of *B. lactis* Bb-12. As mentioned above, *Bifidobacteria* spp. is reported to degrade hexose exclusively through a particular metabolic pathway, termed the 'bifid shunt', by the fructose-6-phosphate phosphoketolase (F6PK). This pathway yields 1.5 mol of acetate and 1 mol of lactate from 1 mol fermented glucose (de Vries & Stouthamer, 1967; Scardovi, 1986). It is also reported that the ratio of lactate to acetate formed by bifidobacteria may vary depending on the carbon source utilized and also on the species examined (Palframan *et al.*, 2003).The amount of acetic acid has been reported to significantly increase during storage probably because of metabolism of *B. lactis* Bb-12. In addition, higher amount of acetic acid was observed on milk fermented by *B. lactis* Bb-12 than that of by *L. lactobacillus* La-5 (Østlie *et al.*, 2003).

Pyruvic acid is an intermediate metabolite in different metabolic pathway. Pyruvate in high concentration is reported to be toxic for the bacteria cells and is not expect in high concentration in the final product (Fernandes-Garcia & McGregor, 1994). Changing of

pyruvic acid indicates a potential bacterial activity. Reduction of pyruvic acid was observed during storage in 'Cultura Naturell'.

Citric acid naturally exists in raw milk. It is reported that concentration of citric acid in raw milk is 1967-2223 ppm (Østlie *et al*., 2002). The concentrations of citric acid detected in 'Cultura Naturell' were between 1100 and 1400 ppm indicating that about 800 ppm citric acid was metabolized by lactic acid bacteria during fermentation. It is reported *L. acidophilus* La-5 could metabolite citrate, while *B. lactis* Bb-12 dose not metabolite citrate (Østlie *et al*.2003). According to this, the decrease of citric acid in 'Cultura Naturell' during was probably because of metabolism of *L. acidophilus La-5*.

Orotic acid is an intermediate product in the synthesis of nucleotides and a growth factor for yoghurt starter culture (Its amount in raw milk varies greatly depending on the cow's origin, diet and it increases during development of lactation (Motyl *et al.*,1991; Gaia *et al.*, 2000). The concentration of orotic acid in fermented milk products depends on fermentation and the soluble whey solids in the product (Tamime & Robinson, 2000). A study shows that concentration of orotic acid in raw milk is around 80 ppm and a decrease of about 48% in the level of orotic acid was detected in the manufacturing and storage of yoghurt (Okonkwo & Kinsella , 1969; Arla, 1982). Decrease in the level of orotic acid from 80 to 40-50 ppm during fermentation by *L.acidophilus* La-5, *B. lactis* Bb-12 and *L. rhamnosus* GG were reported (Østlie *et al.*, 2003). The concentrations of orotic acid in 'Cultura Naturell' were between 59 and 66 ppm indicating that less amount of orotic acid is reduced in 'Cultura Naturell' fermented by combination of *L.acidophilus* La-5 and *B. lactis* Bb-12.

Succinic is commonly produced by homofermentative lactobacilli isolated from the intestine of some animal as the end product of glucose and citrate metabolism. It is also reported that succinic acid could be produced by bifidobacterium probably by converting phosphoenolpyruvate (PEP) into oxaloacetate (van der Meulen *et al.*, 2006). In addition, increase in the level of succinic acid from 750 to 1017–1960 ppm was also found in milk fermented with *L. acidophilus* La-5, *B. lactis* Bb-12 and *L. rhamnosus* GG (Østlie *et al*., 2003). However, succinic acid detected in 'Cultura Naturell' was lower with initial concentration between 38 and 58 ppm and its amount increased markedly during storage indicating activity of *L. acidophilus* La-5, *B. lactis* Bb-12 and *L. rhamnosus* GG during storage.

Uric acid was reported to be stable during fermentation of milk by *L.acidophilus* La-5, *B. lactis* Bb-12 and *L. rhamnosus* GG at 16 mg/ kg (Østlie *et al.*, 2003). The concentrations of uric acid in 'Cultura Naturell' were round 17 ppm and its amount did not change markedly during storage, which concur with the result reported.

Three different sugars (lactose, glucose and galactose) were found in 'Cultura Naturell'. Lactose is the source for lactic acid. It exists naturally in raw milk with

a typical concentration of 46000 ppm (Walstra *et al.*, 2006) but can vary much depending on season, lactation and source. The concentrations of lactose in 'Cultura Naturell' were between 40800 and 44200 ppm. At the beginning of the metabolism, lactose is firstly transported into bacteria cell and then degraded into glucose and galactose, and 1 mol lactose can generate 1 mol glucose and 1 mol galactose. Since that 'Cultura Naturell' is not dry-matter enriched, about 1800 ppm to 5200 ppm of lactose is metabolized.

The initial concentrations of glucose in 'Cultura Naturell' during storage were between 300 to 730 ppm and the initial concentrations of galactose were between 450 and 700 ppm indicating utilization of both glucose and galactose by LAB during fermentation in 'Cultura Naturell'. Glucose is the main source for further metabolism and it could be utilized by almost all kinds of lactic acid bacteria. However, galactose cannot be utilized further by many thermophilic lactic acid bacteria and galactose is instead secreted as an exchange molecule for the transport of lactose into the cell. Hickey *et al.* (1983) showed that *L. acidophilus* strains metabolized only the glucose moiety and approximately 1 mol galactose was released into the growth medium for each mole of lactose utilized. This indicates that *B. lactis* Bb-12 is responsible for the utilization of galactose in 'Cultura Naturell. Decrease of glucose during storage indicates some bacteria activities. Accumulation of the galactose in the samples during storage indicates that utilization of galactose by *B. lactis* Bb-12 was greatly suppressed probably because of low temperature and its decreased viable cell counts. The increased lactic acid corresponds well with the decrease of glucose during storage.

'Cultura Naturell' was perceived by the assessors to be the sourest of the three products, which is in accordance with the fact that largest amount of acetic acid was found in the product. Acetic acid has a much sourer taste than lactic acid. According to result of sensory assessment, 'Cultura Naturell' from production C had lowest sourness which in accordance with its lower amount of lactic and acetic acid and its higher pH than 'Cultura Naturell' from production A and B. Increased amount of lactic acid and acetic acid and decrease of pH indicating that product was becoming sourer during storage, but these little changes were not correctly detected by the sensory panel. According to the sensory result of 'Cultura Naturell', sourness judged by assessors in sensory panel are inconsistent with the result of organic acid and pH. Sweetness, bitter taste, off-flavor was as expected not detected. Fresh taste increased it is probably because of increase of ethanol and acid production. In addition, inconsistent results were observed with regards to viscosity, cultured milk flavor.

5.2 Product 'Biola Syrnet Lettmelk Naturell'

TINE 'Biola Syrnet Lettmelk Naturell' is a fermented low-fat milk product with 1.5 % fat fermented with bacteria culture composed of *L.acidophilus* La-5, *B. lactis* Bb-12 and *L. rhamnosus* GG. During the storage of 'Biola Syrnet Lettmelk Naturell', all of The three probiotic bacteria strains maintained a level higher than the suggested level of

10^6 cfu/g and this indicates that in TINE 'Biola Syrnet Lettmelk Naturell' the number And viable probiotic bacteria is satisfactory and consumption of TINE 'Biola Syrnet Lettmelk Naturell' would be suitable for therapeutic purpose.

Unlike 'Cultura Naturell', decreases of *L. acidophilus* La-5, *B. lactis* Bb-12 and *L. rhamnosus* GG of about 2 log units were observed from the beginning of the storage. The decrease of *B. lactis* Bb-12 was most notable of the three and was more pronounced than in 'Cultura Naturell'. *L. rhamnosus* GG showed the highest viable cell counts above 10^8 cfu/ g. The good viability of this bacteria strain in dairy products has been shown in many studies (Nighswonger *et al.*, 1996; Schillinger, 1999, Helland *et al.*, 2004). As already mentioned above, pH is an important reason for decrease of the number of probiotic bacteria. As shown in figure 4.12, the pH of 'Biola Syrnet Lettmelk Naturell' decreased markedly in the beginning of the storage explaining the rapid decrease of viable cell counts of bacteria in the beginning of storage.

the pH of products from production B and production C was highest in the beginning and it slightly decreased during storage. Obvious decrease in pH of product from production A was not detected. It can be depicted from Figure 4.3, 4.4, 4.5 and 4.12 that there is a positive relation between viable cell counts and pH. The increased production of hydrogen peroxide by lactobacilli potentially due to the addition of *L.rhamnosus* GG is probably another reason for the decrease of *B. lactis* Bb-12. Other factors such as oxygen content, and nutrition could also be reasons for decline of probiotic bacteria in 'Biola Syrnet Lettmelk Naturell'.

In the case of viscosity, 'Biola Syrnet Lettmelk Naturell' had even lower viscosity than 'Culture Naturell' as shown by the short time required for the product to run through the SMR funnel. The viscosity of 'Biola Syrnet Lettmelk Naturell' increased during storage. As discussed above, its low concentrations of total milk solid and fat content are the dominant reasons for its lower viscosity. In addition, its low viscosity is probably because of its relatively high pH (around 4.6) indicating that the product was not sufficiently acidified resulting in a weak protein gel structure.

In the case of volatile compounds, amounts of ethanol and acetoin detected in 'Biola Syrnet Lettmelk Naturell' were relatively higher than other volatile compounds, such as acetaldehyde, acetone, diacetyl, and 2, 3-pentadione. The level of acetaldehyde in fermented milk products depends on the ability of bacteria strains to produce acetaldehyde and to further reduce it to ethanol (Gonzalez *et al.*, 1994).The level of acetaldehyde is notably low in 'Biola Syrnet Lettmelk Naturell' indicating a high utilization of acetaldehyde under fermentation or a lack of ability to produce this compound. According to results of 'Cultura Naturell', *L.acidophilus* La-5 and *B. lactis* Bb-12 in combination produce large amount of acetaldehyde but they have limited abilities to reduce all acetaldehyde to ethanol despite that both of them possess alcohol dehydrogenase during fermentation. This implies that not only does *L.rhamnosus* GG possess alcohol dehydrogenase, but it also can actively reduce acetaldehyde to ethanol

in large amount (Marshall & Cole, 1983). Total concentrations of ethanol and acetaldehyde in 'Biola Syrnet Lettmelk' were lower than in 'Cultura Naturell' indicating that less acetaldehyde was produced with the introduction of *L. rhamnosus GG*. No marked changes of acetaldehyde and ethanol were observed during storage since the level of acetaldehyde was low at the beginning of the storage. ¨

As discussed above, *L. acidophilus* La-5 is reported to produce diacetyl, mainly from citric acid and *L.rhamnosus* GG produces also diacetyl despite that it is citrate negative (Østlie *et al.*, 2003).

The concentrations of lactic acid in 'Biola Syrnet Lettmelk Naturell' were between 4138 and 5378 ppm, which was similar to its concentrations in 'Culture Naturell'. Similarity was found in the level of lactose between 'Cultura Naturell' (between 40800 and 44200 ppm) and 'Biola Syrnet Lettmelk Naturell' (between 45820 and 47750 ppm).

The levels of orotic acid, succinic acid, uric acid and α- ketoglutaric acid in 'Biola Syrnet Lettmelk Naturell' were stable. Similar amounts of these organic acids was found in the products 'Cultura Naturell' and 'Biola Syrnet Lettmelk Naturell' .Compared to 'Cultura Naturell', production of acetic acid and pyruvic acid was very low and their amounts were stable under storage. It is reported that acetic acid could be produced both by *L. acidophilus* La-5 and *B. lactis* Bb-12 and its amount is especially high in milk fermented by *B. lactis* Bb-12. However no acetic acid was detected in milk fermented with *L.rhamnosus* GG (Østlie *et al.*, 2003). The concentration of acetic acid in 'Biola Syrnet Lettmelk Naturell' is 1000 ppm, which was much lower than it in 'Culture Naturell'. Since the viable cell counts of *L. acidophilus* La-5 and *B. lactis* Bb-12 in 'Culture Naturell' and 'Biola Syrnet Lettmelk Naturell' are similar, this probably because that the addition of *L. rhamnosus* GG suppressed the acetic production or it is able to metabolize acetate.

It was also observed that pH of 'Biola Syrnet Lettmelk Naturell' was relatively higher than that of 'Culture Naturell' and pH variations between the three productions of 'Biola syrnet Lettmelk Naturell' are large. Since amounts of lactate in 'Culture Naturell' and in 'Biola Syrnet Lettmelk Naturell' were similar, the higher pH of 'Biola syrnet Lettmelk Naturell' is probably because its low amount of acetic acid.

It is reported that bifidobacteria is capable to utilize glucose, galactose, fructose and lactose. *L. acidophilus* is reported to ferment lactose, glucose, galactose, sucrose and fructose in different degree. In a study concentration on acid production of *L. acidophilus* in media containing these five sugars, maximum acid production was found in media with glucose and fructose. Minimum acid production was found in media with galactose and only trace amount of the galactose was utilized by *L. acidophilus* strains, which indicates its restricted ability to ferment galactose (Srinivas *et al.*, 1990). No glucose was detected in 'Biola Syrnet Lettmelk Naturell'. It may be assumed that glucose was completely utilized by *L. acidophilus* La-5 and *B. lactis*

Bb-12 during fermentation. As mentioned above, *L. rhamnosus* GG cannot metabolize lactose and sucrose, but it can utilize glucose and fructose (Goldin *et al.*, 1992; Ouwehand *et al.*, 2002). Concentrations of galactose in 'Biola Syrnet Lettmelk Naturell' were lower than it in 'Culture Naturell'. This is probably because that in the metabolism of lactose, it is firstly transported into cell cytoplasm of *L. acidophilus* La-5 and B. lactis Bb-12 and cleaved into glucose and galactose. Glucose is directly utilized in the cell of *L. acidophilus* La-5 and *B. lactis* Bb-12, while galactose is partially excreted into serum and can be utilized by *L. rhamnosus* GG. Interestingly, 4000 to 6000 ppm fructose was detected in 'Biola Syrnet Lettmelk Naturell'. Addition of fructose was not claimed on the label of the product 'Biola Syrnet Lettmelk Naturell' and fructose would in no way be produced as an end product of lactose fermentation. All three bacteria strains are capable of fermenting fructose, and the observed decrease of fructose during storage was probably because of metabolism of these bacteria strains. Similarly, 800 ppm of sucrose was detected only in 'Biola Syrnet Lettmelk Naturell' from production A, which is also unexplainable. It is reported at addition of sucrose into skim milk can enhance acid production by *L.acidophilus* (Agrawal *et al.*, 1986).

In the case of sensory analysis, changes of sourness during storage were also detected by the assessors. According to their opinion, 'Biola Syrnet Lettmelk Naturell' became sourer during storage and this is supported by the decrease of pH and the increase of organic acids during storage. No sweetness and bitter taste were detected by sensory panel. The sensory panel gives relative low scores for fresh taste but the freshness increased during storage probably because of marked increase of lactic acid. 'Biola Syrnet Lettmelk Naturell' was perceived by the sensory assessors to have the lowest viscosity. However, its score for creaminess is as high as 'Culture Naturell' and 'Biola Pluss Yoghurt Mild Naturell'. No big changes of creaminess and viscosity were detected in 'Biola Syrnet Lettmelk Naturell'. Fishy like off flavor was detected by sensory panel in 'Biola Syrnet Lettmelk Naturell' and syneresis was observed in 'Biola Syrnet Lettmelk Naturell' from all productions in the end of the storage.

5.3 Product 'Biola Pluss Yoghurt Mild Naturell'

In 'Biola Pluss Yoghurt Mild Naturell', probiotic bacteria strains *L.acidophilus* La-5, *B. lactis* Bb-12 and *L. rhamnosus* GG are added in additional to yoghurt culture. The milk is fortified with extra SNF (2.5%). Sucrose (2%) is added to enhance the sweetness of TINE Biola Yoghurt. Inulin (2.9%), which has been proved to improve the viability of some probiotic bacteria strains (Donkor *et al.*, 2007) is added claimed to enhance the fiber content in yogurt.

According to result of viable cell counts of probiotic bacteria in 'TINE 'Biola Pluss Yoghurt Mild Naturell', all of the three probiotic bacteria maintained a level higher than the suggested value of 10^6 cfu / g indicating that in this product the number and viability of probiotic bacteria is satisfactory and regular consumption of TINE 'Biola Pluss

Yoghurt Mild Naturell' could give therapeutic benefits.

It has been reported that *L.acidophilus* and *Bifidobacterium* spp. are unstable in yoghurt probably because their antagonist relationship with yoghurt culture. It is reported that the viability of *L. acidophilus* was affected by the presence of *L. delbrueckii* subsp. *bulgaricus* (Dave & Shah, 1996). *L. acidophilus* when added to the set yoghurt after manufacture lost almost all viability within several days. But when added with yoghurt culture under processing, the loss of viability was significantly deceased and it could survive in sufficient levels of 10^6 cfu/ g up to 28 days (Costello, 1993; Ishibashi & Shimamura, 1993; Rybka & Kailasapathy, 1995). Other studies show that pH< 4.4 results in 3-4 log reduction of *L.acidophilus* within 15 days. However, despite that pH of 'Biola Pluss Yoghurt Mild Naturell' ranged from 4.30-4.35 and was lower than that of 'Cultura Naturell' and 'Biola Syrnet Lettmelk Naturell', the number of *L.acidophilus* La-5 showed a higher stability than in 'Culture Naturell' and 'Biola Syrnet Lettmelk Naturell'.. It is reported that *L.acidophilus* survives better than the yoghurt starter culture organisms (Shah & Jelen 1990; Lankaputhra & shah, 1995) and this is also suggested by the results in this study.

L. rhamnosus GG was particularly stable; the changes in viable cell counts during storage were almost negligible. *B. lactis* Bb-12 was reported to have higher stability when it is used in fermented milk product in the presence of *L. delbrueckii* subsp. *bulgaricus* but that is not supported by the results in this study (Dave & Shah, 1996). It is important to point out that dissolved oxygen content affects the viability of probiotic bacteria during storage, generally from two different ways. Firstly, oxygen is toxic for bacteria cells which require strict anaerobic condition for their growth. Secondly, it accelerates production of hydrogen peroxide especially in the presence of *L. delbrueckii* subsp. *bulgaricus* which has been proved to inhibit *Bifidobacterium* spp. (Dave & shah, 1996). The oxygen content is determined by the material of package (permeability of package). Champagne *et al.*, (2005) showed that oxygen content increases in plastic cups in storage compared to glass bottles. It is reported that the viability of *L. acidophilus* La-5 and *B. lactis* Bb-12 was improved when the dissolved oxygen in the product was low (Dave & Shah, 1996).Use of plastic cups for 'Biola Pluss Yoghurt Mild Naturell' instead of paper carton used for 'Cultura Naturell' and 'Biola Syrnet Lettmelk Naturell' could be the reason for different stability of *L.acidophilus* La-5, *B. lactis* Bb-12 and *L. rhamnosus* GG. Viability of all five lactic acid bacteria used in the production of 'Biola Pluss Yoghurt Mild Naturell' was positively related to pH indicating the importance of pH in determination of the viability of bacteria.

In general, the viability of probiotic bacteria seems to be better in the yoghurt. This is probably because of that their growth was stimulated by free amino acid released through the proteolytic activity of the yoghurt starter culture (Donkor *et al.*, 2007).

The viscosity of 'Biola Pluss Yoghurt Mild Naturell' was much greater than the other

two products studied due to the addition of SNF and the higher fat content. Expected increases of viscosity were observed. The reason for this has already discussed above. Production of exocellular texturising agents (exopolysaccharides) by *S. thermophilu*s is possible reason for viscosity enhancement.

In the case of volatile compounds, the concentration of acetaldehyde in 'Biola Pluss Yoghurt Mild Naturell' was between 6.4 and 8.6 ppm, which was higher than that in 'Biola Syrnet Lettmelk Naturell' but lower than that in 'Culture Naturell'. The main source for acetaldehyde in yoghurt is from threonine through threonine aldolase activity of *L. delbrueckii* subsp. *bulgaricus* (Tamime & Robinson, 2003). The low concentration of acetaldehyde in 'Biola Pluss Yoghurt Mild Naturell' is probably because of *L. rhamnosus* GG which is suggested by earlier results to possess an active alcohol dehydrogenase which reduces acetaldehyde to ethanol in large amount. Decreases in the level of acetaldehyde during storage has been observed in yoghurt made from milk (14.8 to 13.1 ppm), milk fortified with skimmed milk powder (SMP) (22.8 to 16.5 ppm) and UF milk (25.0 to 20.6 ppm) (Estevez *et al.*, 1988) and decrease in the level of acetaldehyde was also found in TINE 'Biola Pluss Yoghurt Mild Naturell' from about 7 to 5 ppm.

The concentrations of diacetyl in 'Biola Pluss Yoghurt Naturell Mild' were between 6.8 and 14.4 ppm, higher than that in 'Cultura Naturell' and 'Biola Syrnet Lettmelk Naturell'. In some studies (Rasic & Kurmann, 1978), *S. thermophilus* was recognized as the only producer of diacetyl in classical yoghurt. In other studies, *L. delbrueckii* subsp. *bulgaricus* was reported to play a leading role in the diacetyl production in classical yoghurt but the mechanism is not described in any literature (Dutta *et al.*, 1973; Beshkova *et al.*, 1998). In addition, diacetyl is also a important product of citrate metabolism and can be produced by alternative metabolic pathways (Østlie *et al.*, 2003).

Concentrations of acetoin in 'Biola Pluss Yoghurt Mild Naturell' were between 88 and 94 ppm. Acetoin cannot be produced by *L. delbrueckii* subsp. *bulgaricus.* However, synthesis of acetoin by *S. thermophilus* is more active when *L. delbrueckii* subsp. *bulgaricus* is present (Beshkova *et al.*, 1998).

The concentrations of acetone in 'Biola Pluss Yoghurt Mild Naturell' were between 2.4 to 4 ppm, a little higher than it is in 'Cultura Naturell' and 'Biola Syrnet Lettmelk Naturell'. It is reported that concentration of acetone in raw milk varies from 0.8-2.7 ppm and in classical yoghurt, its amount varies from 0.3 – 4 ppm mainly due to lactobacilli (Hild, 1979; Ott *et al.*, 1999). The results indicates that trace amount of acetione was produced during fermentation of 'Biola Pluss Yoghurt Mild Naturell' probably because of yoghurt culture.

The amount of organic acids did not change markedly in 'Biola Pluss Yoghurt Mild Naturell' during storage. 'Biola Pluss Yoghurt Mild Naturell' has much higher

concentration of lactic acid than ‘Cultura Naturell’ and ‘Biola Syrnet Lettmelk Naturell’. This is because that yoghurt culture has much larger acidification ability than probiotic lactic acid bacteria and addition of yoghurt culture will accelerate fermentation process. The addition of dry matter increases the buffer capacity so that more acid will be produced to reach a certain pH Increase of lactic during storage corresponds well with reduction of carbohydrates in ‘Biola Pluss Yoghurt Mild Naturell’. High concentration of lactic acid was in accordance with the low pH of ‘Biola Pluss Yoghurt Mild Naturell’.

The concentrations of citric acid in ‘Biola Pluss Yoghurt Mild Naturell’ were round 2500 ppm, which was much higher than its natural amount in raw milk. These is because that ‘Biola Pluss Yoghurt Mild Naturell’ is fortified with SNF mainly composed of casein, lactose, whey protein and citrate.
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In the case of carbohydrates, large amount of lactose and sucrose was found in ‘Biola Pluss Yoghurt Mild Naturell’ due to the addition of SNF and sucrose during processing. No glucose was found in yoghurt because it was completely utilized by lactic bacteria during fermentation. Accumulation of galactose was observed in ‘Biola Pluss Yoghurt Mild Naturell’ due to the restrict ability of yoghurt culture strains to metabolize galactose. Hickey *et al.* (1983) showed that *L. acidophilus* strains metabolized only the glucose moiety of lactose and *L. delbrueckii* subsp. *bulgaricus* metabolized only the glucose moiety of lactose. However, *L. delbrueckii* subsp. *bulgaricus* were able to metabolize the galactose moiety of lactose when lactose was limited in medium. In addition, no fructose was found in Biola Pluss Yoghurt Mild Naturell’ indicating a high ability of these bacteria strains to metabolize fructose.

In the case of sensory properties, assessors in the sensory panel perceived that ‘Biola Pluss Yoghurt Mild Naturell’ became sourer during storage and this is in accordance with increase of acid and decrease of pH. As expected, yoghurt taste and sweetness were detected by the sensory assessors. A big reduction of sweetness was detected in product of production C was unexpected because it was observed that no obvious decrease in the level of sucrose. As expected, off flavor and bitter taste were not detected indicating a good sensory quality of TINE ‘Biola Pluss Yoghurt Mild Naturell’.

5.4 Products comparisons

As shown by results of PCA, the commercial probiotic products ‘Culture Naturell’, ‘Biola Syrnet Lettmelk Naturell’ and ‘Biola Pluss Yoghurt Naturell Mild’ have different characteristics regarding to bacteria strains, pH, volatile compounds, organic acids and sensory properties.

In the case of viable cell counts, the difference between the three different products was not obvious. Good viability of the probiotic bacteria was observed in all three products. *L. acidophilus* La-5 showed a better viability in ‘Biola Pluss Yoghurt Mild Naturell’

despite the low pH in yoghurt; *B. lactis* Bb-12 showed a better viability in 'Cultura Naturell'. As discussed above, *B. lactis* Bb-12 was most sensitive to pH and can be poisoned by hydrogen peroxide produced when bacteria strains able to produce hydrogen peroxide are incorporated. *L. rhamnosus* GG showed a better stability in 'Biola Pluss Yoghurt Mild Naturell' than in 'Biola Syrnet Lettmelk Naturell'.

In the case of pH, 'Biola Pluss Yoghurt Mild Naturell' has lowest pH this is because of large acidification ability of yoghurt culture and its high SNF concentration. 'Cultura Naturell' is in middle, its relatively low pH is largely caused by its high concentration of acetic acid produced by *L. acidophilus* La-5 and *B. lactis* Bb-12. 'Biola Syrnet Lettmelk Naturell' has the highest pH. According to the results, the concentrations of lactic acid in 'Culture Naturell' and 'Biola Syrnet Lettmelk Naturell' were similar, but great difference was observed on the concentration of acetic acid. It is seems that when *L. rhamnosus* GG is added, the ability of starter culture blends to produce acetic acid will be suppressed.

In the case of viscosity, 'Biola Pluss Yoghurt Mild Naturell' has by far the highest viscosity because of its high concentration of milk solids and fat. 'Culture Naturell' and 'Biola Syrnet Lettmelk Naturell' had low viscosity because their low concentration of milk solid and fat. 'Biola Syrnet Lettmelk Naturell' had even lower viscosity than 'Culture Naturell' probably because of its higher pH.

In the case of volatile compounds, big differences between the three products were observed on the concentration of acetaldehyde, diacetyl, acetoin and ethanol. 'Cultura Naturell' had highest concentration of acetaldehyde. Acetaldehyde is an intermediate compounds in the production of ethanol. In the presence of yoghurt culture, it could be produced through threonine aldolase activity. According to the results, 'Cultura Naturell' had highest level of acetaldehyde, it probably because threonine aldolase activity of *L. acidophilus* La-5. The concentration of acetaldehyde in 'Biola Syrnet Lettmelk Naturell' and 'Biola Pluss Yoghurt Mild Naturell' were much lower. It is probably because of presence of *L. rhamnosus* GG which can reduce acetaldehyde in large amount. In 'Biola Pluss Yoghurt Mild Naturell', it can be speculated that yoghurt culture is responsible for the production of diacetyl. In 'Cultura Naturell' and 'Biola Syrnet Lettmelk naturell', diacetyl is produced mainly by *L. acidophilus* La-5 through citrate metabolism but its concentration is lower than in 'Biola Pluss Yoghurt Mild Naturell'. The production of acetoin is closely related to diacetyl and the presence of diacetyl reductase.

In the case of organic acid, big differences of the three products were observed with respect to citric acid, lactic acid and acetic acid. The concentration of citric acid depends largely on citrate metabolism and content of milk solid. 'Biola Pluss Yoghurt Mild Naturell' had highest level of citric acid this is because it is fortified with extra milk solid. Citric acid in 'Biola Syrnet Lettmelk Naturell' was lower than in 'Culture Naturell'. This is probably because that the ability of *L. acidophilus* La-5 to metabolize

citric acid was suppressed in the presence of *L. rhamnosus* GG.

In the case of carbohydrate, highest accumulation of galactose was found in 'Cultura Naturell' indicating restricted ability of *L. acidophilus* La-5 to utilize galactose. Sucrose was found in 'Biola Pluss Yoghurt Mild Naturell' because it is added as sweetener during processing. Fructose as an end product of degradation of sucrose was also found in same product. Sucrose and fructose was found in 'Biola Syrnet Lettmelk Naturell' without being declared on the label.

Regarding the sensory properties of the sensory properties, sensory panel was most sensitive to sourness and sweetness. Differences of some properties between the three products were not correctly detected. According to result, sensory panel could not detect any viscosity changes of the three products during storage. Similarly, no creaminess difference sbetween the three products were detected. The concept creaminess is often associated to a high fat content, a viscous, slippery, greasy and mouth coating texture. Some studies claim a relationship between creaminess, thickness and smoothness (Harman *et al.*, 2000 Guinard & Mazzucchelli, 1996). Despite that the fat content, milk solid content and viscosity of the three products were very different, they were still perceived by sensory panel to have the same creaminess.

The performances of *L. acidophilus* La-5, *B. lactis Bb-12* and *L. rhamnosus* GG, as commercial probiotic bacteria strains, are satisfactory in TINE probiotic fermented milk. All of them showed a good viability during fermentation and storage and do not cause post- acidification of the product in large extent. *L. rhamnosus GG* is a relatively new commercial probiotic bacteria strain and its high viability in dairy fermented product has been suggested both in this study and other studies. However, the information that has been published, concerning its metabolic pathway and its sensory contributes to the final products is very limited. As shown in the result of this study, the introduction of *L. rhamnosus* GG in addition to *L. acidophilus* La-5 and *B. lactis Bb-12* results in the product 'Biola Syrnet Lettmelk Naturell', which has different characteristics than 'Cultura Naturell'. Compared to 'Cultura Naturell', 'Biola Syrnet Lettmelk Naturell' has higher pH and lower viscosity. In addition, off-flavor was detected by the sensory panel exclusively in 'Biola Syrnet Lettmelk Naturell'. It is still not clear whether these changes are caused by the introduction of *L. rhamnosus* GG.

Probiotic bacteria strains have increasingly been incorporated into food products. This study lay the groundwork for a good understanding of TINE probiotic fermented products concerning their viable cell counts, pH, viscosity, organic acids, carbohydrates, volatile compounds and sensory properties. Despite that certain differences were observed between the three products, reasons causing these differences were not studied and discussed deeply. The study presented in this thesis seems to have raised more questions, especially about *L. rhamnosus* GG:

1) Despite that the metabolism pattern of *L. rhamnosus* GG in milk is reported by (Østlie, *et al.*, 2003), but its interactions with other lactic acid bacteria strains was not reported.
2) Whether the introduction of *L. rhamnosus* GG is the reason for the high pH, low viscosity and off-flavor of 'Biola Syrnet Lettmelk Naturell' is worth to be studied.
3) The sensory contributes of *L. rhamnosus* GG to the probiotic fermented milk products could be an interesting topic.
4) It would be desirable to know concentrations of interesting metabolic products for a good reference product of a probiotic product.
5) Influences of fruit flesh, flavor additives and preservatives were not taken into account in this study and further work has to be done to extent the topic.

6.0 CONCLUSION

The quality of TINE probiotic fermented milk was stable during storage within designated shelf-life. The viable cell counts of the probiotic bacteria strains in TINE probiotic fermented milk product are satisfactory. Products produced with different bacteria, raw material and chemical ingredients show a series of unique characteristics. Variations between different productions of product were detected but they seem not to affect the customers' sensory perception on the products.

7.0 REFERENCES

Agrawal, V., Usha, M. S., Mital, B. K. (1986). Preparation and evaluation of acidophilus milk. *Asian Journal of Dairy Research 5:* 33-38.

Alnes, L. (2011). Personal communication, TINE.

Alonso, L., Fraga, M. J. (2001). Simple and rapid analysis for quantization of the most important volatile flavor compounds in yogurt by headspace gas chromatography–mass spectrometry. *Journal of Chromatography Science 39:* 297–300.

Anderson, J. W., Gilliland, S. E. (1999). Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus* L1 on Serum Cholesterol in Hypercholesterolemic Humans. Journal. *American College of Nutrition 18:*43–50.

Arla, L. A. (1982). Effect of fermentation on B vitamin content of milk in Sweeden. *Journal of Dairy Science 65:*353.

Axelsson, L. (1998). Lactic acid bacteria: classification and physiology, In: S. Salminen and A. Von Wright (eds). *Lactic Acid Bacteria: Microbiology and Functional Aspects, 2nd ed. Marcel Dekker, Inc, New York.*

Badings, H. T., Neeter, R. (1980). Recent advances in the study of aroma compounds of milk and dairy products. *Netherlands milk and dairy journal 34:* 9–30.

Beal, C., Skokanova, J., latrille, E., Martin, N., Corrieu, G. (1999). Combined effects of culture conditions and storage time on acidification and viscosity of stirred yoghurt. Journal of Dairy Science 82: 673-681.

Benito de Ca ŕdenas, I.L., Ledesma, O., Oliver, G., (1989). Effect of pyruvate on diacetyl and acetoin production by *Lactobacillus casei* and *Lactobacillus plantarum*. *Milchwissenschaft 44:* 347– 350.

Beshkova, D., Simova, E., Frengova, G., Simov, Z. (1998). Production of flavour compounds by yogurt starter cultures. *Journal Industrial Microbiology and Biotechnology 20:* 180–186.

Bridge, P. B., Sneath, P. H. A. (1983). Numerical taxonomy of Streptococcus. *Journal of General Microbiology 129:* 565– 597.

Bouvier, M. (2001). Effects of consumption of a milk fermented by the probiotic *Bifidobacterium animalis* DN 173 010 on colonic transit time in healthy humans. *Bioscience and Microflora 20(2):* 43-48, 2001.

van de Casteele, S., Vanheuverzwijn, T., Ruyssen, T., Van Assche, P., Swings, J., Huys, G. (2005). Evaluation of culture media for selective enumeration of probiotic strains of lactobacilli and bifidobacteria in combination with yoghurt or cheese starters. *International Dairy Journal 16:* 1470–1476.

Cayot, P., Schenker ,F., Houze, G., Sulmont-Rosse, C., Colas, B. (2008).Creaminess in relation to consistency and particle size in stirred fat-free yogurt. *International Dairy Journal 18(3):*303-311.

Champagne, C. P., Gardner, N., Roy, D. (2005). Challenges in the addition of probiotic cultures to foods. *Critical Reviews in food science and Nutrition45:* 61-84.

Chen, J., Zhang, Y. P., Xiao, G. X., (1999). A preliminary clinical study of bifidobacteria preparation in the treatment of post-burn diarrhea in children, *Annals of Burns and Fire Disasters 7(1):* 42-47.

Chaves, A. C. S. D., Fernandez, M., Lerayer, A. L. S., Mierau, I., Kleerebezem, M., Hugenholtz, Z. (2002). Metabolic engineering of acetaldehyde production by *Streptococcus thermophilus*. *Applied and Environmental Microbiology, 68 (11):* 5656–5662

Cheng, H. F. (2011). Volatile Flavor Compounds in Yogurt: A Review. *Critical Reviews in Food Science and Nutrition 50:*938–950 (2010)

Chiang, B. L., Sheih, Y. H., Wang, L. H., Liao, C. K., Gill, H. S. (2001). Enhancement of immunity in the elderly by dietary supplementation with the probiotic Bifidobacterium lactis HN019. *American Journal of Clinical Nutrition 74 (6):* 833-839.

Christopher, M. D., Reddy, V. P., Venkateswarlu, K. (2008). Viability during storage of two *Bifidobacterium bifidum* strains in set and stirred flavored yoghurts containing whey protein concentrate. *National Product Radiance 8(1):* 25-31.

Chr. Hansen (2001). *Lactobacillus reuteri* in fermented milk products –Method for counting probiotic bacteria, *Technical Bulletin F-5.* Chr. Hansen Denmark.

Chr. Hansen (2007a). Enumeration of *L. Acidophilus* in fermented milk products – Guidelines, *Technical Bulletin P-10*. Christian Hansen, Denmark.

Chr. Hansen (2007b). Enumeration of *Bifidobacteria* in fermented milk products –Guideline, *Technical Bulletin P-11*. Chr. Hansen Denmark.

Coeuret, V., Gueguen, M., Vernoux, J. P. (2004). Numbers and strains of lactobacilli in some probiotic products, *International Journal of Food Microbiology 97:*

147-156.

Collins, E. B., (1972). Biosynthesis of flavor compounds by microorganisms. *Journal of Dairy Science 55:* 1022-1028.

Conway, P. L., Gorbach, S. L., Goldin, B. R. (1987). Survival of lactic acid bacteria in the human stomach and adhension to intestinal cells. *Journal of Dairy Science 70: 1-12.*

Costello, M. (1993). Probiotic Foods. t*he Food Industry Conference Proceedings, Sydney Convention and Exhibition Centre . Publ. FoodPro-93, Sydney.*

Coultate, T. P. (2002). Food: the chemistry of its components. 4nd ed. *published by the Royal Society of Chemistry*.

D'Souza, A. L., Rajkumar, C., Cooke J., Bulpitt, C. J. (2002). Probiotics in prevention of antibiotic associated diarrhea: meta-analysis. *British Medical Journal 324:* 1341–1345.

Dave, R. I., Shah, N. P. (1996). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *International Dairy Journal 7*: (31-41)

Dave, R. I., Shah, N. P. (1997). Effect of cystein on the viability of yoghurt and probiotic bacteria in yoghurts made with commercial starter cultures. *Internationl Dairy Journal 7:* 537- 545.

De Man, J. C. (1959). The formation of diacetyl and acetoin from α-acetolactic acid. *Recueil des Travaux Chimiques des Pays-bas 78:* 480-486.

Donkor, O. N., Nilmini, S. L. I., Stolic, P., Vasiljevic,T. Shah, N. P. (2007), Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. International Dairy Journal 17: 657–665.

Dutta, S., Kuila, R. Ranganathan, B. (1973). Effect of different heat treatments of milk on acid and flavour production by five single straincultures. *Milchwissenschaft 28:* 321–233.

Estevez, R. C., goicoechea, A., Jimenez-Perez, S. (1988). Aromabildung in joghurt bei unterschiedlichen Herstellungsverfahren. *D. M.Z 24:* 733-735

Fairclough A. C, (2008). Investigation of a novel delivery system for probiotics. *Food Innovation project at Sheffield Hallam University.*

Felis G. E., Dellaglio F. (2007) Taxonomy of lactobacilli and bifidobacteria *Current Issues in Intestinal Microbiology 8(2):*44-61.

Fernandez-Garcia, E., McGregor, J.U. (1994). Determination of organic acids during the fermentation and cold storage of yogurt. *Journal of Dairy Science 77:* 2934– 2939.

Fox, P. F., McSweeney, P. L. H. (1998). Dairy Chemistry and Biochemistry. *Kluwer Academic/Plenum Publishers, New York, NY.*

Fuller, R. (1989). Probiotics in man and animals. *Journal of applied bacteriology (66):*365-378.

Gaia, A., Antonelli, M. L., Biondi, A., vinci, G. (2000). Orotic acid: a milk constituent, enzymatic determination by means of a new microcalorimetric method. *Talanta 52:* 947-952.

Gardini, F., Lanciotti, R., Guerzoni, M. E., Torriani, S. (1999). Evaluation of aroma production and survival of *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus* in fermented milks. *International Dairy Journal 9*: 125-134

Gaafar, A. M. (1992). Volatile flavor compounds of yoghurt, *International Journal of Food Science and Technology 27:* 87–91.

Gibson, G. R. (2007). Functional foods: probiotics and prebiotics. *Culture 28 (2):*1-3.

Gilliland, S. E., Speck, M. L. (1977). Instability of *L.acidophilus* in yoghurt. *Journal of Dairy Science 60:*1394-1398.

Gismondo, M. R., Drago, L., Lombardi, A. (1999). Review of probiotics available to modify gastrointestinal flora. *International Journal of Antimicrobial Agent 12*:287–292.

Goldin, I., Gorhach, S. L., Saxelin, M., Barakat, S., Gualtieri, L., Salminen, S. (1992) Survival of Lactobacillus species (strain GG) in human gastrointestinal tract. *Digestive Diseases Science 3:* 121-128.

Gonzalez, S., Morata de Ambrosini, V., Manca de Nadra, M., Pesce de Ruiz Holgado, A., Oliver, G., (1994). Acetaldehyde production by strains used as probiotics in fermented milk. *Journal of Food Protection 57:* 436–440.

Gueimonde, M., Delgado, S., Mayo, B., Ruas-Madiedo, P., Margolles, A., de los Reyes-Gavil, C. G. (2004). Viability and diversity of probiotic lactobacillus and

bifidobacterium populations included in commercial fermented milks. *Food Research International 37:* 839–850.

Güler-Akin, M. B., Akin, M. S. (2007). Effects of cysteine and different in incubation temperatures on the microflora, chemical composition and sensory characteristics of bio-yoghurt made from goat's milk. *Food Chemistry 100:* 788-793.

Hardie, J. M., Wiley, R. A. (1994). Recent developments in streptococcal taxonomy: their relation to infections. *Reviews in Medical Microbiology, 5 (3):*151-162.

Hartwig, P., McDaniel, M. (1995). Flavor characteristics or lactic, malic, citric, and acetic acids at various pH levels. Journal of Food Science 60:384-388.

Hild, V. (1979). Quantitative determination of some important flavor components in dairy products by the headspace technique. *Milchwissenschaft 34:* 281–283.

Helland M. H., Wicklund, T., Narvhus, J. A. (2004). Growth and metabolism of selected strains of probiotic bacteria, in maize porridge with added malted barley. *International Journal of Food Microbiology 91:* 305-313.

Heller, K. J. (2010). Probiotic bacteria in fermented foods: product characteristics and starter organisms, *American Journal of Clinical Nutrition 73 (2):* 374–379.

Horne, D. S. (1998). Casein interactions: Casting light on the Black Boxes, the structure in dairy products. *International Dairy Journal 8:*171-177.

Hernandez, E. J. G., Estepa, R. G., Rivas, I. R. (1995). Analysis of diacetyl in yogurt by two new spectrophotometric and fluorimetric methods. *Food Chemistry 53:* 315–319.

Hickey, M. W., Hillier, A. J., Jago, G. R. (1983). Transport and metabolism of lactose, glucose and galactose in homofermentative lactobacilli. *Applied and Environment Microbiology 5:* 825-831.

Holt, C., Horne, D. S. (1996). The hairy casein micelle: evolution of the concept and its implication for dairy technology. *Netherlands Milk and Dairy Journal 50:* 85-111.

Hood, S. K., Zottola, A. (1988). Effect of low pH on the ability of *Lactobacillus acidophilus* to survive and adhere to human intestinal cells. *Journal of Food Science 53:* 1514-1516.

Hull, R. R. & Roberts, A. V. (1984). Differential enumeration of *Lactobacillus acidophilus* in yoghurt. *Journal of Dairy Technology 3:* 160-164.

Hunger, W., Peitersen, N. (1993). New technical aspects of the preparation of starter cultures. *Bulletion of the Internationol. Dairy Federation 277:*1-27.

International Dairy Federation. (1981). Joint IDF/ISO/AOAC Group E44.

Ishibashi, N., Shimamura, S. (1993). Bifidobacteria*:* Research and development in japan. F*ood technology 47:* 126, 129-134.

Iwana, H., Masuda, H., Fujisawa, H., Mitsuoka, T. (1993). Isolation and identification of *Bifidobacterium* spp. in commercial yoghurts sod in Europe. *Bifidobacteria Microflora 12:* 39-45.

Jiang, T., Mustapha, A., Savaiano, D. A. (1996). Improvement of lactose digestion in humans by ingestion of unfermented milk containing *Bifidobacterium longum*. *Journal of Dairy Science 79(5):* 750-757.

Jiang, T., Savaiano, D. A., (1997). In Vitro Lactose Fermentation by Human Colonic Bacteria Is Modified by *Lactobacillus acidophilus* Supplementation. *Journal of Nutrition 127(8):* 1489-1495.

Jumah, R. Y., Shaker, R. R., Abu-Jdayil, B. (2001). Effect of milk source on the rheological properties of yoghurt during the gelation process. International Journal of Dairy technology 54: 89-93.

Kailasapathy, K., Chin, J. (1999). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. *Immunology and Cell biology 78:*80-88.

Kaminarides, S., Stamou P., Massouras, T. (2007). Comparison of the characteristics of set type yoghurt made from ovine milk of different fat content. *International Journal of Food Science and Technology 42:* 1019–1028.

Kandler, O., Weiss, N. (1986). Genus Lactobacillus Beijerinck 1901, 212. *Bergey's Manual of Systematic Bacteriology 2:* 1209-1234. Edited by Sneath, P. H. A., Mair, N. S., Sharpe, M. E., Holt, J. G. Baltimore: Williams & Wilkins.

Kang, Y., Frank, J. F., Lillard, D. A. (1988). Gas chromatographic detection of yogurt flavor compounds and changes during refrigerated storage. *Cultured Dairy Products Journal 23:* 6–9.

Klaus, M. (2003). Market and marketing of functional food in Europe. *Journal of Food Engineering 56*: 181-183.

Kneifel,W. (1992). Starter cultures for fermented milks. Nutrition 16: 150–156.

Kneifel, W., Ulberth, F., Erhard, F., Jaros, D. (1992). Aroma profiles and sensory properties of yogurt and yogurt-related products. I. Screening of commercially available starter cultures. Milchwissenschaft 47: 362–365.

Kneifel, W., Jaros, D., Erhard, F. (1993). Microflora and acidification properties of yoghurt and yoghurt related products fermented with commercially available starter cultures. *International Journal of Food Microbiology 18:*179-189.

Kondratenko, M., Gyosheva, B. (1978). Changes in volatile components of Bulgarian yogurt. *Lait 58:* 390–396.

Kristo, E., Biliaderis, C.G., Tzanotakis, N. (2003). Modelling of rheological and acidifiaciton properties of a fermented milk product containing a probiotic strain of *Lactobacillus paracasei*. *International Dairy Journal 13:*517-28.

Kwak, H. S. (1995). Effect of volatile flavor compound on yogurt during refrigerated storage. *Korean Journal of Food Science Technology 27:* 939–943.

Laniewska-Trokenheim, 2010. Patterns of survival and volatile metabolites of selected *lactobacillus* strains during long-term incubation in milk. *Journal of microbiology 49:515-521*.

Lankaputhra, W. E. V., Shah, N. P. (1995). Survival of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in yoghurt. *24th International Dairy Congress,* Melbourne, Australia.

Lee, W. J., Lucey, J. A. (2010). Formation and physical properties of yogurt, Asian *Australasian Journal of Animal Science. 23 (9):* 1127 – 1136.

Lourens-Hattingh A., Viljoen B. C. (2001). Yoghurt as probiotic carrier food. *International Dairy Journal 11 (1–2):*1–17.

Lin, M. Y., Chang F. J. (2000). Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC15708 and *Lactobacillus acidophilus* ATCC 4356. *Digestive Diseases and Sciences 45:* 1617–1622.

Lindsay, R. C, Day, E, A, Sandine, W.E.(1965). Green flavor defect in lactic starter cultures. Journal of Dairy Science 48:863–9.

McSweeney, P. L. H., Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. *Lait 80:* 293–324.

Marshall, V. M. (1984). Flavor development in fermented milks. *I Science Publishers, London, UK. n: Advances in the Microbiology and Biochemistry of Cheese and Fermented Milk. pp. 153–186. Davies, F. L. and Law, B. A., Eds., Elsevier Applied.*

Marshall, V. M. (1993). Starter cultures for milk fermentation and their characteristics. *International Journal of Dairy Technology 46:* 49–56.

Marshall, V. M., Cole, W.M., (1983). Threonine aldolase and alcohol dehydrogenase activities in *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* and their contribution to flavour production in fermented milks. Journal of Dairy Reserach 50: 375– 379.

Marsili, R. T., Ostapenko, H., Simmons, R. E., Green, D. E. (1981). High performance liquid chromatographic determination of organic acids in dairy products. *Journal of Food Science 46:* 52-57.

Martin, J. H., Chou, K. M. (1992). Selection of *Bifidobacterium* spp. for use as dietary adjuncts in cultured dairy foods. I. Tolerance to pH of yoghurt. *Cultured Dairy Product Journal 27(4):* 21, 23-26.

Mataragas, M., Dimitriou, V., Skandamis, P. N., Drosinos, E. H. (2001). Quantifiying the spoilage and Shelf-life of yoghurt with fruits. *Food Microbiology 28:* 611-616.

McDonough, F. E., Hitchins, A. D., Wong, N. P, Wells, P., Bodwell, C. E. (1987). Modification of sweet acidophilus milk to improve utilization by lactose-intolerant persons. *American Journal of Clinical Nutrition 45:* 570-574.

Meance, S., Cayuela, C., Turchet, P. (2001).A fermented milk with a Bifidobacterium probiotic strain DN-173010 shortened oro-fecal gut transit time in elderly. *Microbial Ecology in Health and Disease13:* 217–222.

van de Meulen, R., Adriany, T., Verbrugghe, K., De Vuyst, L., (2006). Kinetic analysis of bifidobacterial metabolism reveals a minor role for succinic acid in the regeneration of NAD^+ through its growth- associated production. *American Society for Microbiology 72 (8):* 5204-5210.

Monnet, C., Schmitt, P., Divies, C. (1994). Diacetyl production in milk by an α-acetolactic acid accumulating strains of *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis*. *Journal of Dairy Science 77:* 2916-2924.

Moriya, J., Fachin, L., Gandara, L. A., Viotto, W. H. (2006). Evaluation of culture media for counts of *Bifidobacterium animalis* subsp. *lactis* Bb-12 in the presence of yoghurt bacteria. *Brazilian Journal of Microbiology 37:* 516-520.

Motyl, T., Krzedminski, J., Podgurmiak, M., Witeszczak, C., Zochowski, P. (1991). Variability of orotic acid concentration in cow's milk. *Endocrine regulations 25 (1-2):* 79-82

Narvhus, J. A. (1996). Probiotiske bakterier-metabolisme i melk. *Meieriposten 12:* 341– 343.

Narvhus, J. A., Østeraas, K., Mutukumira, T., Abrahamsen, R. K. (1998). Production of fermented milk using a malty compound-producing strain of *Lactococcus lactis* subsp. *lactis biova*. Diacetylactis, isolated form Zimbabwean naturally fermented milk. *International Journal of Food Microbiology, 41(1):*73-80.

Narvhus, J. A., Thorvaldsen, K., Abrahamsen, R. K. (1990). Quantitative determination of volatile compounds produced by *Lactococcus* spp. Using direct automatic headspace gas chromatography. *Brief communications and Abstracts of posters, Vol. II. XXII International Dairy Congress, Montreal, Canada*.

Nguyen, T. D., Kang, J. H., Lee, M. S. (2007). Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterol lowering effects. *International Journal of Food Microbiology 113:* 358-61.

Nighswonger, B. D., Branshears, M. M., Gilliland, S. E. (1996). Viability of *Lactobacillus acidophilus* and *Lactobacilus casei* in fermented milk products during refrigerated storage. *Journal of Dairy Science 79:* 212-219.

Okonkwo, P., Kinsella, J.E. (1969). Orotic acid in yoghurt. J*ournal of Dairy Science 52:* 1861– 1862.

O'Toole, P. W., Cooney, J. C. (2008). Probiotic bacteria influence the composition and function of the intestinal microbiota. *Interdisciplinary Perspectives on Infectious Diseases Article ID 175258:*9.

Ott, A., Fay, L. B., Chaintreau, A. (1997). Determination and origin of the aroma impact compounds of yogurt flavor. *Journal of Agriculture Food and Chemistry 45:* 850–858.

Ott, A., Germond, J., Baumgartner, M., Chaintreau, A. (1999). Aroma comparisons of traditional and mild yogurts: Headspace gas chromatography quantification of volatiles and origin of α-diketones. *Journal of Agriculture Food and Chemistry 47:* 2379–2385.

Ott, A., Hugi, A., Baumgartner, M., Chaintreau, A. (2000). Sensory investigation of yogurt flavor perception: Mutual influence of volatiles and acidity. *Journal of Agriculture and Food Chemistry 48:* 441–450.

Ouwehand, A. C., Salminen, S., Isolauri, E. (2002). Probiotics: an overview of beneficial effects. *Antonie Van leeuwenhoek 82:* 279-289.

Palframan, R. J., Gibson G. R., Rastall, R. A. (2003). Carbohydrate preferences of Bifidobacterium species isolated from the human gut. *Current Issues in Intestinal Microbiology 4*:71–75.

Panagiotidis, P., Tzia, C. (2001). Effect of milk composition and heating on flavor and aroma of yogurt. In: Food Flavors and Chemistry: A*dvances of the New Millennium*. pp. 160–167. Spanier, A. M., Shahidi, F., Parliment, T. H., and Ho, C.-T., Eds., Royal Society of Chemistry, Cambridge, UK.

Parvez, S., Malik, K. A., Ah Kang, K. S., Kim, H. Y. (2006). Probiotics and their fermented food products are beneficial. *Journal of Applied Microbiology 100 (6):* 1171-1185.

Playne, M. J. (1993). Probiotic Foods. In Dariy. *The Food Industry Conference Proceedings, Sydnye Convention and Exhibition Centre. Publ. FoodPro.93, Sydney :* 3-9

Pourahmad, R., Assadi, M. M. (2005). Yoghurt production by Iranian native starter cultures. *Nutrition & Food Science 35:* 410–415.

Quintans, N. G., Blancato V., Repizo, G. Magni C., López P. (2008). Citrate metabolism and aroma compound production in lactic acid bacteria. *Molecular Aspects of Lactic Acid Bacteria for Traditional and New Applications:*65-88.

Rash, K. (1990). Compositional elements affecting flavor of cultured dairy foods. *Journal of Dairy Science 73:* 3651–3656.

Rasic, J. L., Kurmann, J. A. (1978). Yoghurt: Scientific Grounds, Technology, Manufacture and Preparations. *Technical Dairy Publishing House,Copenhagen, Denmark.*

Raghuveer, C., Tandon, R., (2009). Consumption of functional food and our health. *Pakistan Journal of Physiology 5:* 76-83.

Renan, M., Guyomarc'h, F., Arnoult-Delest, V., Paˆquet, D., Brule, G., Famelart, M. H. (2008). The rebodying of stirred yoghurt: interactions between proteins. *Journal of Dairy Research 75:* 450-456.

Robinson, R. K. (1987). Survival of *Lactobacillus acidophilus* in fermented products, *Suid Afrikaarzse Tvclstrif Vir Suiwelkunde19:* 25-27.

Routray, W., Mishra, H. N., Scientific and Technical Aspects of Yogurt Aroma and Taste: A Review. Comprehensive reviews in food science and food safty.

Rybka, S., Kailasapathy, K. (1995). The survival of culture bacteria in fresh and freeze-dried AB yoghurts. *Journal of Dairy Technology:* 50, 51-57.

Rybka, S. (1994). The enumeration of *Lactobacillus*, *Streptococcus* and *Bifidobacterium* species in yogurt. B.Sc. dissertation, University of New South Wales, Sydney.

Rysstad, G., Abrahamsen, R. K. (1987). Formation of volatile aroma compounds and carbon dioxide in yoghurt starter grown in cow's and goats' milk. *Journal of Dairy Research 54:* 257–266.

Sander, M. E. (2000). Considerations for use of probiotic bacteria to modulate human health. Symposium: Probiotic Bacteria: implications for human health. *Dairy and food Culture Technologies, Littleton, Co 80122.2526*.

Sanders, M. E., Klaenhammer T. R. (2001) Invited review: the scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. *Journal of Dairy Science 34:* 319-334.

Sandine, W. E., Daly C, Elliker, P. R., Vedamuthu, E. R. (1972). Causes and control of culture related flavor defects on cultured dairy products. *Journal of Dairy Science 55(7):*1031–9.

Scardovi, V. (1986). Genus Bifidobacterium Orla-Jensen 1974, 472 AL. In: Sneath, P. H. A., Mair, N. S., Sharp, M. E., Holt, J.G., (eds): *Bergey's Manual of Systematic Bacteriology Vol. 1. Williams & Wilkins, Baltimore.* 1418-1434

Schillinger, U. (1999). Isolation and identification of lactobacilli from novel-type protiobic and mild yoghurts and their stability during refrigerated storage. *International Journal of Food Microbiology 47:* 79-87.

Schlichtherle-Cerny, H., Oberhoizer, D. (2007). Comparision of aroma compounds of mild and traditional acidic yoghurts using SPME-GC-O-MS. *5th NIZO Dairy Conference- Prospects for flavore formation and perception Papendal.*

Schlichtherle-Cerny, H., Oberhoizer, D., Zehntner, U. (2008). Odorants in mild and traditional acidic yoghurts as determined by SPME-GC/O/MS, *Expression of Multidisciplinary flavor science, Proceedings of the 12th Weurman Symposium Interlaken, Switzerland*.

Shah, N. P., jelen, P. (1990). Survival of lactic acid bacteria and their lactases under acidic conditions. *Journal of Food science 55:* 506-509.

Shah, N. P., Lankaputhra, W. E. V., Britz, M. L., Kyle, W. S. A. (1994). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in Commercial yoghurt during refrigerated storage. *International Dairy Journal 5:* 515-521.

Shah, N. P. (1999). Symposium: probiotic bacteria. Probiotic bacteria: selective enumeration and survival in dairy foods. *Journal of Dairy Science 78 (7):* 894-907.

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

Shah, N. P., Lankaputhra, W. E. V., Britz, M. L., Kyle, W. S. A. (1995). Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in commercial yoghurt during refrigerated storage. *International Dairy Journal 5:*515.

Shah, N. P., Tharmaraj, N. (2003). Selective enumeration of *Lactobacillus delbrueckii* ssp. *bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Bifidobacteria, Lactobacillus Casei, Lactobacillus rhamnosus,* and *Propionibacteria*. *Journal of Dairy Science 86(7):* 2288-2298.

Stenby, E. (1998). In II International Fermented Milks Seminar, 7–8 May, *Chr. Hansen Ind., Rio de Jane*rio.

Soccol, C. R., de Souza Vandenberghe, L. P., Spier, M. R., Medeiros, A. B. P., Yamaguishi, C. T., Lindner, J. D. D., Pandey A., Thomaz-soccol, V. (2010). The potential of probiotics: a review. *Food Technology and Biotechnology 48 (4):* 413–434.

Srinivas, D., Mital, B. K., Garg, S. K. (1990). Utilization of sugars by *Lactobadillus acidophilus* strains. *International Journal of Food Microbiology 10:* 51-58.

Suomalainen, T. H., Mayra-Makinen, A. M. (1999). Propionic acid bacteria as protective cultures in fermented milks and breads. *Lait's Journal 79:*165–174.

Tamime, A., Deeth, H. (1980). Yogurt: Technology and biochemistry. *Journal of Food Protection 43:* 939–977.

Tamime, A.Y., Robinson, A. K. (2000). Yoghurt: science and technology. 2nd ed. *Woodhead Publishing Limited*.

Tamime, A.Y. (2005). Probiotic dairy products. 1nd ed. backwell publishing *Limited*.

Thompson, J., Chassy, B. M. (1981). Uptake and metabolism of sucrose by *Streptococcus lactis*. *Journal of Bacteriology 147:* 543-551.

Tunick, M.H. (2009). Dairy innovations over the past 100 years. *Agricultural and Food Chemistry (57):* 8093-8097.

Ulberth, F. (1991). Headspace gas chromatographic estimation of some yogurt volatiles. *Journal of Association Offcial Analytical Chemistry 74:* 630–643.

Ulberth, F., Kneifel, W. (1992). Aroma profiles and sensory properties of yogurt and yogurt-related products. II. Classification of starter cultures by means of cluster analysis. Milchwissenschaft 47: 432–434.

Urbach, G. (1995). Contribution of lactic acid bacteria to flavor compound formation of dairy products. *International Dairy Journal 5:* 877–903.

Valio (2009). TINE dairies process pure, natural raw materials into good, healthy food products, *Foods and Functionals 2:* 10.

Vescovo, M. (1970). Effect of milk composition and treatment on the formation of carbonyl compounds in yoghurt. *Sei Tee Latt-Casearia. 21:* 171–174.

Vinderola, C. G., Reinheimer, J. A. (1999). Culture media for the enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in the presence of yoghurt bacteria. *International dairy journal 9:* 497-505.

De Vries, W., Stouthamer, A. H. (1967). Pathway of glucose fermentation in relation to the taxonomy of Bifidobacteria. *Journal of Bacteriology 136:* 415-425.

Walstra, P., Wouters, J. T. M., Geurts, T. J. (2006). Dairy science and technology. 2nd ed. *CRC Press, Taylor & Francis Group.*

Welman, A. D., Maddox, I. S., (2003). Exopolysaccharides from lactic acid bacteria: perspectives and challenges. T*rends in Biotechnology 21(6):*269-274.

http://en.wikipedia.org/wiki/Yoghurt, 2011.

Yuguchi, H., Hiramatsu, A., Doi, K., Ida, Ch., Okonogi, Sh. (1989). Studies on the flavor of yoghurt fermented Bifidobacteria- significance of volatile *components* and organic acids in the sensory acceptance of yoghurt. *Japanese Journal of Zootechnical Science 60:* 734-741.

Yu, J. H., Nakanishi, T. (1975). Studies on production of flavour constituents by various lactic acid bacteria. II. Effect of milk fat on formation of volatile carbonyl compounds by various lactic acid bacteria. *Japanese Journal of Dairy Science 24:*A27–A31.

Xu, Z. M., Emmanouelidou, D. G., Raphaelides,S. N.,Antonius, Antoniou, K. D. (2008). Effects of heating temperature and fat content on the structure development of set yogurt. *Journal of Food Engineering 85(4):*590-597.

Østlie, H. M., Helland, M. H., Narvhus, J. A. (2003). Growth and metabolism of selected strains of probiotic bacteria in milk. *International Journal of Food Microbiology 87:* 17-27

TINE Cultura range, http://www.handelsbladetfk.no/id/21762

TINE Biola range, http://www.facebook.com/note.php?note_id=385259901891

Molecular structures of lactose and sucrose, http://www.hcc.mnscu.edu/chem/V.25/page_id_32265.html)

http://www2.unibas.it/parente/Starter/gruppi.html

http://microbewiki.kenyon.edu/index.php/Lactobacillus

http://www.musee-afrappier.qc.ca/fr/index.php?pageid=3114c&image=3114c_lactobacillus

http://www.geneferm.com/b5/Lactic_Acid_Bacteria.htm

http://oncologiaesalute.wordpress.com/2008/02/17/i-probiotici-sono-letali-in-caso-di-pancreatite-acuta/

http://www.biotechnology4u.com/industrial_microbia_food_beverage.html

8.0 APPENDICES

APPENDIX 1 INGREDIENTS OF PROBIOTIC FERMENTED MILK PRODUCTS.

Table 1. Ingredients of tine probiotic fermented milk products

Products	Ingredeints
Cutlura	low fat milk (1.5%), Lactobacillus acidophilus La-5 and Bifidobacterium Bb-12
Biola Syrnet Lettmelk Naturell	low fat milk (1.5 %), Lactobacillus acidophilus La-5, Bifidobacterium Bb-12 and Lactobacillus rhamnosus GG
Biola Pluss Yoghurt Mild Naturell	low fat milk (1.5 %), whole milk, skim milk powder (about 1.2%), inulin(fiber), sugar (2%), yoghurt culture, Lactobacillus acidophilus La-5, Bifidobacterium Bb-12 and Lactobacillus rhamnosus GG

APPENDIX 2 NUTRITION CONTENT OF PROBIOTIC FERMENTED MILK PRODUCTS

Table 2. Nutrition of content ‘Cultura’ (nutrition per 100 g.)

composition	amount per 100 g	composition	amount per 100 g
salt	0.09 g	Vitamin D	0 mcg
energy	186 KJ (44 kcal)	Vitamin E	0 mg alfa TE
water	90 g	Vitamin B1	0.05 mg
protein	3.3 g	Vitamin B2	0.15 mg
carbohydrate	4.4 g	Niacin	0.1
fat	1.5 g	NiaEkv	0.9 NE
saturated fat	0.9 g	Vitamin B6	0.04 mg
trans unsaturated fat	0.1 g	folat	3 mcg
monounsaturated fat	0.4 g	Vitamin B12	0.2 mcg
polyunsaturated fat	0 g	Vitamin C	0 mg
cholesterol	7 g	calcium	100 mg
starch	0 g	iron	0 mg
dietary fiber	0 g	sodium	37 mg
mono and di-saccharide	4.4 g	potassium	154 mg
sugar added	0 g	magnesium	10 mg
alchohol	0	zinc	0.4 mg
retinol	13 mcg	selenium	1 mcg
B carotene	13 mcg	copper	0.01 mg
Vitamin A	14 RAE	phosphorus	85 mg

(www. Næringsinnhold.no)

Table 3. Nutrition content of ‘Biola Syrnet Lettmelk Naturell’

composition	Amount per 100 g
salt	0.1 g
energy	189 KJ (45.17 kcal)
water	90 g
protein	3.4 g
carbohydrate	4.5 g
fat	1.5 g
saturated fat	0.9 g
dietary fiber	0 g
mono and di-saccharide	4.5 g
Vitamin B2	0.15 mg
calcium	115 mg
sodium	40 mg
phosphorus	90 mg
idine	16 mcg

(www. Næringsinnhold.no)

Table 4. Nutrition content of ‘Biola Pluss Yoghurt Mild Naturell’

composition	Amount per 100 g
salt	0.13 g
energy	307 KJ (73.37kcal)
water	90 g
protein	4.5 g
carbohydrate	8.1 g
fat	1.9 g
saturated fat	1.2 g
dietary fiber	2.9 g
mono and di-saccharide	8.4 g
Vitamin B2	0.15 mg
calcium	145 mg
sodium	50 mg

(www. Næringsinnhold.no)

APPENDIX 3 RESULTS

Table 5. Viable cell counts of probiotic bacteria in ‘Cultura’ (log cfu/g)

Cutlura			
	1	2	3
L. acidophilus La-5			
Production A	9.38	9.18	7.11
Production B	9.08	8.94	6.94
Production C	9.69	9.23	7.49
B. lactis Bb-12			
Production A	9.76	9.20	7.32
Production B	9.11	8.90	8.89
Production C	9.71	9.04	7.91

Table 6. Viable cell counts of probiotic bacteria ‘Biola Syrnet Lettmelk Naturell’ (log cfu/g)

Biola Syrnet Lettmelk Naturell			
	1	2	3
L. acidophilus La-5			
Production A	10.20	8.82	7.96
Production B	9.08	8.82	8.15
Production C	9.94	9.15	7.53
B. lactis Bb-12			
Production A	9.15	8.26	7.53
Production B	8.91	8.18	6.95
Production C	9.74	8.36	7.20
L. rhamnosus GG			
Production A	9.30	8.51	7.85
Production B	8.80	8.08	7.80
Production C	9.66	8.36	8.28

Table 7. Viable cell counts of probiotic bacteria ‘Biola Pluss Yoghurt Mild Naturell’ (log cfu/g)

Biola Pluss Yoghurt Mild Naturell			
	1	2	3
L. acidophilus La-5			
Production A	9.53	9.11	9.08
Production B	9.23	9.18	9.00
Production C	9.70	9.34	7.28
B. lactis Bb-12			
Production A	9.74	8.23	6.99
Production B	8.99	8.89	8.58
Production C	9.23	8.53	7.84
L. rhamnosus GG			
Production A	8.38	8.36	7.98
Production B	8.54	8.46	8.00
Production C	8.08	7.94	7.91
S. thermophilus			
Production A	10.67	9.34	9.11
Production B	9.74	9.30	9.15
Production C	9.20	8.96	8.87
L. delbrueckii subsp. *bulgaricus*			
Production A	8.79	8.38	8.34
Production B	8.94	8.45	8.26
Production C	8.34	7.88	7.34

Table 8. HSGC result of ‘Cultura’

	Acetaldehyde	Ethanol	Acetone	Diacetyl	2.3-pentadione	Acetoin
AC-1	16.269	34.681	3.315	5.286	0.108	153.324
AC-2	11.992	35.439	3.523	4.921	0.093	184.814
AC-3	10.814	37.594	4.002	3.861	0.069	201.925
BC-1	20.678	26.624	3.203	3.57	0.096	64.762
BC-2	17.93	27.458	3.361	4.705	0.119	79.041
BC-3	16.173	27.124	3.274	2.515	0.079	79.348
CC-1	16.629	26.629	4.108	5.214	0.136	118.089
CC-2	15.165	27.973	4.231	3.372	0.088	139.864
CC-3	9.58	28.82	3.99	2.85	0.06	173.31

Table 9. HSGC result of ‘Biola Syrnet Lettmelk Naturell’

	Acetaldehyde	Ethanol	Acetone	Diacetyl	2.3-pentadione	Acetoin
AS-1	0.294	15.214	2.814	2.069	0.147	18.137
AS-2	0.303	14.565	2.668	3.508	0.166	23.389
AS-3	0.276	15.219	2.8	4.556	0.194	29.885
BS-1	0.254	22.389	2.611	0.995	0.112	10.258
BS-2	0.479	22.025	2.796	3.703	0.166	23.749
BS-3	0.26	23.867	2.72	1.733	0.151	12.37
CS-1	0.415	27.165	3.069	1.538	0.094	13.496
CS-2	0.514	27.688	3.157	1.489	0.117	10.739
CS-3	0.2	26.48	3.07	1.11	0.14	9.08

Table 10. HSGC result of ‘Biola Pluss Yoghurt Naturell’

	Acetaldehyde	Ethanol	Acetone	Diacetyl	2.3-pentadione	Acetoin
AY-1	6.389	7.312	2.447	11.144	0.098	88.19
AY-2	3.763	7.172	2.429	10.03	0.087	86.207
AY-3	2.023	7.152	2.38	14.489	0.09	99.14
BY-1	6.963	8.418	4.021	6.814	0.129	87.957
BY-2	5.006	7.443	3.941	9.881	0.136	90.848
BY-3	3.86	7.33	3.88	10.05	0.1	98.8
CY-1	8.648	7.261	3.533	14.363	0.094	94.339
CY-2	4.74	7.41	3.61	10.31	0.12	89.91
CY-3	4.22	8.01	3.42	19.08	0.12	102.4

Table 11. HPLC results of ‘Cultura’

	Citric acid	a-ketogluta ric acid	Oroti c acid	Pyruvi c acid	Succini c acid	Lactic acid	Formic acid	Uric acid	DL-Pyroglutami c acid	Acetic acid
A										
AC-1	1158.67	153.21	69.44	30.78	57.51	5615.48	47.15	17.71	7.85	2662.18
AC-2	944.27	142.13	66.68	24.77	84.27	6004.79	43.95	19.46	11.22	2663.87
AC-3	1043.48	190.03	67.96	19.68	112.26	6406.33	51.65	8.38	30.08	2789.53
B										
BC-1	1481.26	142.87	61.79	94.71	32.33	5434.09	23.62	17.68	8.98	2360.83
BC-2	1508	136.72	61.96	110.69	41.78	5807.63	24.51	16.35	12.55	2459.56
BC-3	1337.18	128.37	62.25	41.99	58.03	5816.3	26.21	17.42	11.75	2362.65
C										
CC-1	1184.86	105.37	55.04	32.92	38.13	4014.05	22.79	16.9	9.83	1903.43
CC-2	661.49	101.86	64.05	8.87	51.91	4752.88	20.69	19.23	8.01	2329.46
CC-3	948.55	106.34	61.96	6.47	63.17	4856.91	21.81	18.34	10.1	2284.68

Table 12. HPLC results of ‘Biola Syrnet Lettmelk Naturell’

	Citric acid	a-ketoglutaric acid	Orotic acid	Pyruvic acid	Succinic acid	Lactic acid	Formic acid	Uric acid	DL-Pyroglutamic acid	Acetic acid
A										
AS-1	1724.83	38.07	66.25	12.66	69.23	5121.86	45.76	17.9	5.66	1079.02
AS-2	1579.06	22.64	60.89	12.78	67.93	6036.18	33.44	16.87	8.39	1053.59
AS-3	1727.22	73.79	60.91	10.78	71.71	7371.78	43.37	11.52	20.07	1083.54
B										
BS-1	1678.57	44.2	67.72	14.92	36.51	5377.88	59.34	17.1	5.02	1135.92
BS-2	1678.52	38.7	65.14	10.84	51.1	6345.76	54.4	16.87	10.18	1137.89
BS-3	1642.96	30.9	63.31	8.38	35.55	5876.86	57.08	18.06	8.77	1154.26
C										
CS-1	1642.81	70.36	64.31	21.12	21.67	4137.93	58.25	17.94	4.78	1148.85
CS-2	1471.54	51.91	60.15	18.31	23.63	4741.05	54.75	16.36	4.41	1078.52
CS-3	1642.96	30.9	63.31	8.38	35.55	5876.86	57.08	18.06	8.77	1154.26

Table 13. HPLC results of ‘Biola Pluss Yoghurt Naturell’

	Citric acid	a-ketoglutaric acid	Orotic acid	Pyruvic acid	Succinic acid	Lactic acid	Formic acid	Uric acid	DL-Pyroglutamic acid	Acetic acid
A										
AY-1	2548.74	48.55	94.51	27.42	40.27	9816.06	55.01	23.57	5.09	471.32
AY-2	2496.55	49.65	91.93	22.03	46.37	9867.89	56.56	21.96	5.76	447.78
AY-3	2355.84	42.89	88.47	19.77	46.49	9437.62	53.09	22.8	4.51	435.54
B										
BY-1	2647.71	56.24	94.8	20.93	42.29	10063.21	56	21	6.93	504.26
BY-2	2922.03	67.01	104.41	29.7	51.19	11154.08	61.25	21.73	6.56	542.91
BY-3	2877.45	57.38	105.26	35.23	56.96	11187.69	61.51	24.95	5.93	537.43
C										
CY-1	2598.08	60.88	95.94	38.96	38.54	9733.54	51.91	19.64	6.11	461.36
CY-2	2576.95	58.62	93.85	34.88	44.23	9784.17	52.05	21.72	8.82	457.25
CY-3	2502.28	49.15	92.43	21.54	46.86	9886.05	52.53	21.45	10.18	439.86

Table 14. Carbohydrate result of ‘Cultura’

	Lactose	Glucose	Galactose	Fructose
A				
AC-1	40801.53	432.37	515.56	-----
AC-2	40184.53	280.73	796.28	-----
AC-3	43928.87	229.81	1048.39	-----
B				
BC-1	42513.31	291.89	453.61	-----
BC-2	42541.92	260.78	756.26	-----
BC-3	41874.85	274.92	792.23	-----
C				
CC-1	44199.03	729.75	709.69	-----
CC-2	43630.59	620.45	813.12	-----
CC-3	42390.19	1010.79	1233.35	-----

15. Carbohydrate result of ‘Biola Syrnet Lettmelk Naturell’

	Lactose	Glucose	Galactose	Fructose
A				
AS-1	798.81	47727.02	-----	74.54
AS-2	684.29	47734.57	-----	75.07
AS-3	635.43	47914.77	-----	76.82
B	-----	45820.31	-----	84.38
BS-1	-----	45467.27	-----	77.63
BS-2	-----	46491.7	-----	88.62
BS-3	-----	47485.89	-----	194.2
C	-----	46583.37	-----	194.93
CS-1	-----	49525.98	-----	187.65
CS-2	798.81	47727.02	-----	74.54
CS-3	684.29	47734.57	-----	75.07

Table 16. Carbohydrate result of ‘Biola Pluss Yoghurt Naturell’

	Sucrose	Lactose	Glucose	Galactose
A				
AY-1	16137.48	54702.97	184.89	5863.39
AY-2	16726.54	54227.13	-----	6066.94
AY-3	15338.66	51907.55	-----	5789.87
B				
BY-1	15953.89	52036.19	-----	5424.27
BY-2	16566.43	53440.57	-----	5745.63
BY-3	16892.62	53854.21	-----	6065.19
C				
CY-1	16440.61	52085.5	-----	5871.6
CY-2	18720.91	54350.25	-----	6239.61
CY-3	18274.81	52963.86	-----	6165.08

Table 17. pH of probiotic fermented milk

	1	2	3
Cultura Naturell			
Production A	4.49	4.48	4.35
Production B	4.39	4.34	4.33
Production C	4.50	4.5	4.53
Biola Syrnet Lettmelk Naturell			
Production A	4.4	4.38	4.36
Production B	4.52	4.37	4.36
Production C	4.6	4.5	4.39
Biola Pluss Yoghurt Mild Naturell			
Production A	4.34	4.34	4.31
Production B	4.34	4.34	4.31
Production C	4.29	4.28	4.28

Table 18. Viscosity of probiotic fermented milk

	1	2	3
Cultura			
Production A	17s	16s	10s
Production B	15s	11s	17s
Production C	10s	14s	13s
Biola Syrnet Lettmelk Naturell			
Production A	8s	7s	8s
Production B	8s	6s	7s
Production C	5s	7s	6s
Biola Pluss Yoghurt Mild Naturell			
Production A	257s	281s	471s
Production B	228s	194s	258s
Production C	357s	380s	381s

Table 19. Sensory results of ’Cultura Naturell’

	A1C	A2C	A3C	B1C	B2C	B3C	C1C	C2C	C3C
Sourness	5.2	7	5.8	6.6	5.2	6	4.8	4.8	4.4
Sweetness	1.2	1.4	1.2	1.2	1.4	1	1.6	1.4	1
Vinegar taste	2.8	3.8	2.6	3	1.8	2.8	1.4	2	2
Yoghurt taste	1.2	1.6	2	1.4	2	1.4	2	1.8	1.6
Cultured milk taste	3.6	1.4	3.4	1.8	3.6	2	3.8	2.2	2.4
Bitter taste (besk)	1	1	1	1.4	1	1	1	1	1.2
Bitter taste	1.2	1	1.4	1	1	1	2	1.4	1
Off-flavor	1.2	1	1.2	1	1	1.6	1.8	1	1
Viscosity	2.2	1.4	4.2	3.8	3.6	2.8	4.8	3.2	3.4
Graininess	1	1	1	1	1	1	1	1	1.8
Creaminess	8	7	7.2	7.2	7.4	7.4	7.2	7	6.2
Fresh taste	5.2	5.8	6.2	5	3.8	5.2	5	4.4	6

Table 20. Sensory results of ’Biola Syrnet Lettmelk Naturell’

	A1S	A2S	A3S	B1S	B2S	B3S	C1S	C2S	C3S
Sourness	3	4.6	5	3.6	3.4	5	3.2	2.6	5
Sweetness	2.2	1.4	1.2	1.6	1.2	1.2	2.8	2	1
Vinegar taste	1.8	2.6	2.4	1.2	2	1.4	1.6	1.4	2.2
Yoghurt taste	1	1	1.6	1	1.2	1.4	1.4	1.2	1.2
Cultured milk taste	3	2.6	2.2	2.8	2.4	2.8	2.4	1.6	2.8
Bitter taste (besk)	1	1	1	1	1	1	1	1	1
Bitter taste	1	1	1.4	1	1	1	1.2	1	1
Off-flavor	1	2.5	2.4	1	1.5	1	1.6	1.25	1
Viscosity	1.8	1.6	2.6	3.4	3.2	3.2	3	2.4	2.8
Graininess	1	1	1	1	1	1	1.2	1	1.2
Creaminess	7.2	7	6.8	7	7.4	7.2	7.2	7.2	7
Fresh taste	3.6	4.2	4.4	3.4	4	5	2.4	3.2	4.4

Table 21. Sensory results of ’Biola Pluss Yoghurt Mild Naturell’

	A1Y	A2Y	A3Y	B1Y	B2Y	B3Y	C1Y	C2Y	C3Y
Sourness	4.6	5	4.4	4.4	4	4.8	3.6	4.8	5.2
Sweetness	3.2	3.2	3	3.8	3.8	3.6	5	3.4	2.8
Vinegar taste	1.5	1.6	1.6	1.6	1	1.2	1	1.4	1.8
Yoghurt taste	3.8	3	3.2	3.4	3	3	3	3	3
Cultured milk taste	1.8	1.4	1.6	1.6	1.2	1.4	2.2	1.4	1.4
Bitter taste (besk)	1	1	1	1	1	1	1.2	1	1
Bitter taste	1	1	1	1	1	1	1.2	1	1
Off-flavor	1.6	1.2	1	1.2	1	1	1.4	1	1
Viscosity	6.4	7.2	6.6	7.6	7.4	7.4	7.8	7.2	6.6
Graininess	2.2	1	1.2	1	1	1.2	1.2	1.2	1.2
Creaminess	6.6	7.2	7	6.8	7	6.8	7	7	6.8
Fresh taste	5	4	4.6	4.6	4.8	5.4	4.6	5	4.6

APPENDIX 4 SAMPLING PLANS

Table 22. Sampling plan for products from production A

Dato (mar.)	**07 mon**	**08 tue**	**09 wes**	**10 thu**	**11 fri**	**12 sat**	**13 sun**	**14 mon**	**15 thu**	**16 wes**	**17 thu**	**18 fri**	**19 sat**	**20 sun**	**21 mon**	**22 tue**	**23 wes**	**24 thu**	**25 fre**	**26 sat**	**27 sun**	**28 mon**	**29 thu**	**30 wes**	**31 thu**
Storage time*	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
B-Yoghurt		X											X												X
Storage time*	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21							
Cultura		X								X								X							
Storage time	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21						
B-Soured melk		X								X									X						

(* = products obtained from Tine were not new and the storage time shows the days the products have been stored. The three sampling dates distributed evenly from the first day the products arrived to the expiry date.)

Table 23. Samplings plan for products from production B

Dato (mar.)	**15 tue**	**16 wes**	**17 thu**	**18 fri**	**19 sat**	**20 sun**	**21 mon**	**22 tue**	**23 wes**	**24 thu**	**25 fri**	**26 sat**	**27 sun**	**28 mon**	**29 tue**	**30 wes**	**31 thu**	**01 fri**	**02 sat**	**03 sun**	**04 mon**	**05 tue**	**06 wes**	**07 thu**
Storage time*	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
B-Yoghurt	X											X												X
Storage time*	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21							
Cultura	X									X							X							
Storage time	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21						
B-Soured melk	X									X								X						

(* = products obtained from Tine were not new and the storage time shows the days the products have been stored. The three sampling dates distributed evenly from the first day the products arrived to the expiry date.)

Table 24. Sampling plan for products from production C

Date (mar.-apr.)	23 wes	24 thu	25 fri	26 sat	27 sun	28 mon	29 tue	30 wes	31 thu	01 fri	02 sat	03 sun	04 mon	05 tue	06 wes	07 thu	08 fri	09 sat	10 sun	11 mon	12 tue	13 wes	14 thu	15 fri
Storage time*	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
B-Yoghurt	X											X											X	
Storage time*	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21								
Cultura	X							X								X								
Storage time	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21			
B-Soured melk	X										X										X			

(* = products obtained from Tine were not new and the storage time shows the days the products have been stored. The three sampling dates distributed evenly from the first day the products arrived to the expiry date.)