Improve probiotic viability of probiotic yogurt in supermarket chain and consumer chain

Peng, Jingyu
MFQ 890208-647-120

Supervisors: Bea Steenbekkers
Catriona Lakemond

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Chapter 1. Introduction

1.1 Background

Probiotic is a word originated from two Greek words that mean *for life*. It is defined as "live microorganisms, administrated in certain quantities that confer health benefits to the host" (FAO/WHO, 2001). The suggested benefits include such as promoting gut health (Beniwal et al., 2003; Beausolei et al., 2007; Sanders, 1999), improving immunity (Sanders, 1999; van der Water et al., 1999; Zubillaga et al., 2001), lowering the risk of colon cancer (Boutron et al., 1996), etc., which are appreciated by consumers due to the increasing public concern on health. Therefore, probiotics are frequently introduced to food products to give them added value, creating a category called functional food with other product which also aims to enhance human well-being by adding the health-promoting ingredient. The market potential is huge: analysis of the North American probiotics markets for human nutrition found that the probiotics sector earned revenues of US$ 698 million in 2006. It is expected to reach US$ 1.70 billion in 2013, with a compound annual growth rate of 13.7%; in Europe, the market for functional foods has experienced growth rates of 15% to 20% over the past 8 years (Granato et al., 2010), wherein Germany, France, the United Kingdom are the leading markets with the market value in 2003 as US$ 4.46, US$ 4.67, and US$ 4.01 billion, respectively (Blandon et al., 2007).

Dairy is the main vehicle of probiotics, wherein yogurt is a frequent carrier. Probiotic yogurt is a large segment in the functional dairy category. Technologically, adding probiotics better fits the yogurt production process compared with processing of other dairy products; from the marketing view, since most consumers understand the production of normal yogurt with application of yogurt bacteria (*L. delbrueckii* ssp. *Bulgaricus* and *Streptococcus thermophilus*), it is easier to inform the consumers the new product properties and help them to perceive the healthiness. Currently, probiotic bacteria employed in the yogurt production are mostly species from *Lactobacillus* and *Bifidobacterium*, such as *Lactobacillus acidophilus*, *L. casei*, *L.*rhamnosus, *Bifidobacterium bifidum, B. longum* etc., which are the normal components of the human intestinal microbiota and have a long tradition of safe application within the food industry (L-Hattingh, 2001; Kociubinski & Salminen, 2006; Guyonnet et al., 2007; Cogan et al., 2007). Unlike yogurt bacteria, probiotics are capable to survive the digestion and adhere to the mucosal surfaces of the intestinal tract (del Campo, 2005; Lin et al., 2006), so that their colonization in intestine can limit growth of pathogens, and make the inside conditions less favorable for pathogenicity (Tamime & Robinson, 2007), performing various functional properties to benefit human health.

A prerequisite to realize the functionality of probiotic yogurt is enough amount of viable cells should be inside the product at consumption. The threshold concentration is generally accepted as 6 log cfu mL^{-1} or g^{-1} (Mattila-Sandholm et al., 2002) as the lowest level, although different recommendations vary up to 8 log cfu·mL^{-1} or g^{-1}. However, viability of probiotic bacteria in yogurts after storage throughout shelf life (about 30 d) is reported to be unsatisfactory, that is, under 6 log cfu mL^{-1} or g^{-1} (Rybka & Kailasapathy, 1995; Dave & Shah, 1997). The analysis on a number of commercial products in Europe and Australia of the presence of probiotics strains in *Lactobacillus* and *Bifidobacterium* were not encouraging (Iwana, et al., 1993; Shah, et al., 1995; Micanel, et al., 1997): these products contained variable, if not very low or none, content of claimed probiotics. Considering the huge sales mentioned before, it implies that a tremendous amount of consumers are purchasing the products at higher price than normal yogurt without actually benefiting from them.

The question is how to improve viability of probiotic bacteria in commercial probiotic products in order to reach or exceed 6 log cfu mL^{-1} or g^{-1} as consumed, since it is to be the threshold level for realizing the health functionality.

1.2 A diagnosis of the whole chain of probiotic yogurt

Because the problem that insufficient viable probiotic cells exist in the product is found at the end of shelf life, the possible causes can be traced back to any step before. An outlook on the whole chain helps to sophisticate the problem in a narrowed area, and generating intense solutions for improvement. The main activities throughout the chain from probiotic yogurt production to consumption are shown in figure 1. The last element “viable count of probiotics” is not an activity, nor does it belong to the chain, but it is depicted as an outcome of previous activities, and it is an indicator of the quality of the probiotic yogurt product – only when the viable probiotic cells in the product reach the recommended concentration (6 log cfu mL^{-1} or g^{-1}) at consumption, the suggested benefits on health are possible to be realized. In this project, the quality of probiotic yogurt refers to probiotic viability only, sensory and other attributes are not emphasized unless specified in the context.

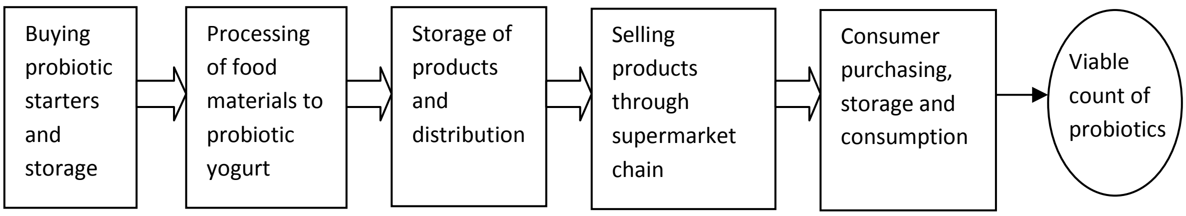


Figure 1: A whole chain of probiotic yogurt from production until consumption.

1.2.1 Buying probiotic starters and storage

The first step “buying probiotic cultures and storage” refers to purchasing probiotic starter cultures and storage before processing. Preferably high quality probiotic starters with undamaged, viable cells should be purchased, and stored properly until they are put into production, because the quality of probiotic starters is an important factor deciding if the probiotic cells can grow well in production and finally meets the required level; injured or dead cells cannot duplicate during the incubation phase, and if the portion of them is high in the starter culture, it could end in a failure in reaching the ideal amount after yogurt fermentation.

In the past, the probiotic cultures were generally present in a frozen or liquid way, in which oxygen exposure and temperature fluctuation damage the microorganism easily, mainly through damaging the membrane. It was also costly in transportation and storage. Fortunately, intense researches on production methods of starter cultures warranted the improvements on their quality. Freeze drying and spray drying methods are developed to produce starter cultures (Champagne et al., 1991; Mattila-Sandholm et al., 2002), which save costs and are more convenient. Particularly, freeze drying better improves starter viability than spray drying, because the cells are more tolerant to the cold pressure in freeze drying than the heat pressure in spay drying. Currently, the starter cultures to be employed in the food industry are generally available in freeze-dried or frozen form (Succi et al., 2007), they are ready-to-use in fermenter or in vat, without risks of contamination (Gilliland & Speck, 1974; Monnet et al., 2003).

Given that culture preparations with good quality are available on the market and are widely applied to commercial products (Krishnakumar & Gordon 2001; Holm, 2003; Playne et al., 2003), and the instructions of storage requirements on the label of probiotic preparation ensure the effectiveness in production, this step is therefore not emphasized in this project. If the producer purchases good quality starter cultures, and stores them properly, it is not the cause of low probiotic viability in yogurt product at consumption or at the end of shelf life.

1.2.2 Processing of food materials to probiotic yogurt

Generally the steps for yogurt production are: 1. homogenization of milk and other ingredients, 2. pasteurization, 3. adding starter cultures and incubation, 4. cooling down to end the incubation in previous step (Dave & Shah, 1997). It is in the incubation phase that the amount of probiotic cells would increase exponentially, so how well they grew in the incubation phase is one of the determinants of whether or not probiotic content would meet the standard in the latter phases.

However, the growth during incubation was reported to be problematic due to the nature of probiotics. Firstly, they are fastidious and need multiple nutrients to initiate their growth. The nutrients, e.g. the free peptide, in milk can be insufficient for probiotics though milk is considered to be one of the most notorious foods for human. Secondly, the optimum temperature for incubation of normal yogurt is 42-45°C because the yogurt bacteria are thermophilic or thermo-tolerant. But applying to probiotics, such as *Lactobacills acidophilus*, *Bifidobacterium* spp., optimal temperature is around 37°C. Thirdly, probiotics are stressed by the pH especially when it is under 5, but traditionally the fermentation is ended when pH dropped to around 4.5, which is not only for food safety considerations, but a desirable sour taste and a curd texture as well (pH at 4.5 is about the isoelectric point of casein). Fourthly, while *L. acidophilus* are mostly microaerophilic, *Bifidobacterium* are strictly anaerobic, hence, the presence of oxygen would have adverse effect on their growth and survival, an anaerobic environment is essential (Rasic, 1990). Further, there is antagonism between yogurt bacteria and probiotics. For example, the hydrogen peroxide produced by *L. delbrueckii* ssp. *Bulgaricus* would inhibit the growth of some probiotics. If not for their health benefits, probiotics are hardly possible to be considered to be introduced in the industrial production of yogurt or other food (Mark, 2004).

However, intense studies have been done in this stage to enhance probiotic growth and viability. A lot of recommendations are given with promising results, including usage of a high level of inoculum at suitable proportion with yogurt bacteria (Anon, 1994), lowering the incubation temperature to 37°C to favour growth of bifidobacteria with increased incubation time (Kneifel et al., 1993), adding supplements to enhance growth ability (Donkor et al., 2007), adapting the heat treatment method to release more free amino acid to initiate growth (Mortazavian et al., 2006), or even excluding *L. delbrueckii* ssp. *bulgaricus* from the fermentation to eliminate antagonistic effects of substances such as hydrogen peroxide (Damin et al., 2008), etc. Many of these recommendations have been applied in the probiotic yogurt manufacturing and warranted probiotic growth during incubation. And the viability of many probiotics at the end of production were reported to meet the requirement of 6 log cfu mL^{-1} or g^{-1} (Shah et al., 1995; Jayamanne & Adams, 2006). Therefore, considering the commercial products on a whole level, this step is not considered as needing urgent improvement, though for specific producers improvements can be made in this step.

1.2.3 Storage of products and distribution

At the end of production, the temperature is dropped under 7°C to terminate the incubation. And the retail product is stored in the plant overnight under 4°C to gain a desirable texture. A low temperature around 4°C is considered to be optimal for yogurt quality as well as probiotic viability. Technologically speaking, although cold stress would have some negative effect on probiotic survival, the main concern is to suppress the microbiological activities in yogurt so to limit the death rate. Otherwise, under a high temperature, the stress factors such as pH, oxygen etc. are much more detrimental than under a low temperature. For example, Talwalkar et al. (2004) reported that oxygen is most deleterious to the cells at 37°C.

However, even under around 4°C, a constant loss in probiotic viability is observed (Shah et al., 1995; Damin et al., 2008). The producers only test probiotic viability at the end of production, but the viability at the end of shelf life is not always assured. Shah et al. (1995) tested several products obtained directly from the processor, results shown that some products contained 6 log cfu g^{-1} probiotics at the end of production, or even higher. More recently, Jayamanne and Adams (2006) tested 10 probiotic yogurts, 9 of them contained bifidobacteria at levels above 6 log cfu g^{-1} at the time of purchase, indicating that manufacturers are performing better. This might be correlated with the tremendous researches on improvements in the production phase. However, at the end of shelf life, which is around 30 days, both studies showed that the probiotics in many products did not survive well to meet the 6 log cfu g^{-1} requirement, and all the products showed a constant decline in the viable count probiotics during storage. It is deducted that the decline during storage might be the reason that the probiotic viability does not meet the required level. The loss in viability might be underestimated or even ignored by the producers. Currently, most studies on probiotic viability during storage were conducted under temperature around 4°C in laboratory (Rosburg, et al., 2010; Donkor, et al., 2007; Damin, et al., 2008; Hughes and Hoover, 1995), which is suggested to be optimal to keep the viability. However, in the real conditions during yogurt logistics, there are possible interruptions during the cold chain.

1.3 From supermarket chain to consumer chain: a blank

In the European market, which is the largest one for probiotic yogurt, the products are sold to consumers mainly through supermarket chains. Examples of supermarket chains are Albert heijn (Netherlands), Carrefour (France), and Tesco (UK), etc. A supermarket chain includes its own distribution centers and supermarkets. The distribution center is also called as warehouse where large quantities of goods are stored and distributed to its own supermarkets; a supermarket is a self-service store where various groceries are put together so consumers can usually find all the goods they need for daily life. With regard to probiotic yogurt, bought by the consumers in the supermarkets, they are usually taken home, put in the home refrigerator, and taken out for consumption. In this sense, the handling behaviour of the consumers also creates a ‘chain’ through which the products was transferred from place to place; and each single consumer creates a chain for the flow of a small amount of products he bought, the ‘consumer chain’ is a term used in this project to represent the flow of products from the purchase to consumption by the consumer.

Both the supermarket chain and the consumer chain might have an influence on probiotic viability due to the possible interruption of ideal storage temperature when the yogurt is out of the production plant, since there are continual changes in place of storage of the products: in the warehouse, on the shelves of supermarket, carried by consumers to home, in the home refrigerator, etc. The fluctuation of temperature is almost unavoidable, and can probably cause great loss in viable cells, especially when under extreme temperature conditions. For example, if the consumer forgets to put the probiotic yogurt in refrigerator and leave it under room temperature for hours or even a day, most probiotics would die (Scharl et al., 2010). The possible disadvantageous effect on probiotic viability from the supermarket chain to the consumer chain somehow supports the deduction that the low viability of might be caused by the supermarket chain and/or the consumer. However, from the supermarket chains to the consumer chains, little work has been done to understand their impact on probiotic viability. If the producers did not know the actual loss in real life, which is thus not taken into account into production, the viability at consumption are very possible to be below 6 log cfu mL^{-1} or g^{-1}, especially since the initial probiotic viability of commercial products are not likely to be very high due to technological difficulties. At best, if there are regulations, for example, National Yogurt Association (NYA) of the United States requires 8 log cfu g^{-1} of lactic acid bacteria at the time of manufacture (Kailasapathy & Rybka, 1997), the yogurts are only 2 log cfu g^{-1} higher than the threshold, not mentioning if legislations do not exist. Therefore, this project aims to investigate the supermarket chain and the consumer chain with regard to the handling activities on the yogurt product, and estimate the subsequent impacts on probiotic viability on a general level; the handling of supermarket chain and consumer chain serves as a reference to be imitated while a specific producer wants to assess the viability loss of its own products.

1.4 Food quality relationship guides quality improvement

Probiotic viability, as an important indicator of probiotic yogurt quality, is repeatedly lower than the threshold (6 log cfu mL^{-1} or g^{-1}) at expiry, resulting in a structural quality problem rather than a randomly happening one. Improvement of probiotic viability is needed in order to solve the problem on a continuous basis; finally the quality would meet or even exceed the standards. According to Luning & Marcelis (2009), quality improvement always includes an analysis step, and they proposed a food quality relationship to underpin the analysis of food quality issues: food quality is the function of food behaviour and human behaviour. Applied to this case, food quality is indicated by probiotic viability; food behaviour includes the probiotic death kinetics under certain stress factors such as time, temperature, etc.; human behaviour relates to the handling of supermarket chain and consumer chain. And in this case, human behaviour of the supermarket chain and consumer chain does not kill the probiotic cells in the product directly, but by influencing the stress factors that have an impact on probiotic death kinetics. The probiotic yogurt quality relationship is structured below (figure 2).

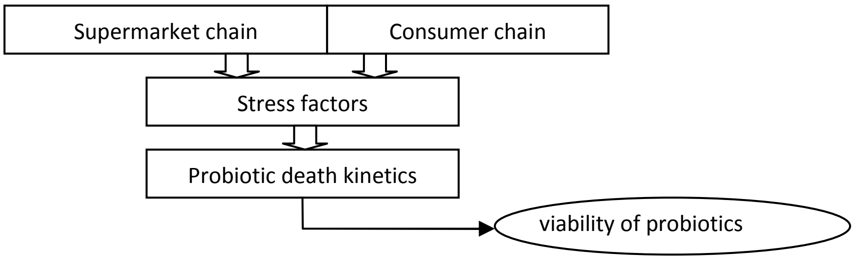


Figure 2: Probiotic yogurt quality relationship

Under the guidance of the model shown in figure 2, the analysis imbedded in quality improvement refers to understanding the probiotic death kinetics, the stress factors that influence death kinetics, and the behaviour of supermarket chain and consumer chain with respect to their influence on the stress factors and the impact on death kinetics and viability. In the following chapters, the model is elaborated form bottom to top, that is, the probiotic death kinetics under a low temperature are studied first, followed by the stress factors regarding how they influence the death kinetics; then the supermarket chain and the consumer chain are investigated to understand the condition of stress factors created in real life, and their impacts on probiotic viability are estimated. The aim is to find out the critical causes of the quality problem, which in this case is the low probiotic viability of commercial products, and generate solutions to solve the problem on a continuous basis. Worth mentioning, when thinking about possible solutions, the production phase should also be taken into concern. Because it is possible that the supermarket chains and the consumers are not very motivated to improve their behaviour, supplementary solutions, if not the only solution, might go back to the production phase, such as producing yogurt with a very high amount of viable probiotic cells, so even after the maleficent practice of supermarket chains and consumers the final quality could be met.

1.5 Problem definition

Neither is it clear what probiotic behaviour (death kinetics and stress factors) is in the product, nor the scenario[1] in the supermarket chain and the consumer chain and the consequent impact on probiotic viability as consumed.

[1] Scenario: in this project, the scenario refers to the handling behaviour, then environment of handling and the characteristics of the handlers. The scenario is elaborated as a model (figure 3) and explained in detail in 3.1

1.6 Hypothesis

The supermarket chains and the consumer chains create conditions adversely influencing probiotic death kinetics and probiotic viability, causing that the probiotic viability in probiotic yogurt is frequently lower than 6 log cfu mL^{-1} or g^{-1}.

1.7 Research objective

To understand the probiotic death kinetics and the stress factors influencing the kinetics.

To investigate the scenario from the supermarket chain to the consumer and the impact on probiotic viability.

To generate various solutions to improve probiotic viability.

1.8 Research questions

- What is the probiotic behaviour with respect to probiotic death kinetics and the stress factors during the prolonged storage?
  - What are the probiotic death kinetics under 4°C or around during the prolonged cold storage?
  - What are the stress factors influencing the probiotic death kinetics and probiotic viability, and how?
  - Which are the most important stress factors with respect to the supermarket chain and the consumer chain?

- What is the scenario from the supermarket chain to the consumer with respect to their handling of probiotic yogurt?
  - What are the basic characteristics of the supermarket chain?
  - What are the handling conditions with regard to selling probiotic yogurt and the subsequent conditions of stress factors created by action of supermarket chain?
  - What are the basis characteristics of the consumers?
  - What are the handling conditions with regard to handling probiotic yogurt and the subsequent conditions of stress factors created by the consumers?

- What are the impacts of the supermarket chain and the consumer chain on probiotic viability in the yogurt as consumed?
  - What are the impacts of supermarket chain on probiotic viability in the yogurt product as consumed?
  - What are the impacts of consumer on probiotic viability in the yogurt products as consumed?

Chapter 2. Probiotic behaviour in the product

As stated before, the food behaviour, including probiotic death kinetics and stress factors, which are the lower part in the quality relationship model (figure 2, 1.4), will be studied first to guide the study on the supermarket chain and the consumer chain.

2.1 Probiotic death kinetics

2.1.1 Probiotic strains used in the industry

Currently, the cultures added into the probiotic yogurt are generally strains from *Lactobacillus* and *Bifidobacterium*, including some leading commercial cultures (table 1). Strains such as *L. casei* Shirota, *Lactobacillus* GG, *B. lactis* Bb12, and *L. reuteri* have already been documented with desirable clinical effects and properties (Salminen et al., 1998).

Table 1: leading commercial probiotic Lactobacilli and Bifidobacteria (adapted from Krishnakumar & Gordon 2001; Holm, 2003; Playne, et al., 2003)

Lactobacillius	strain	manufacturer
L. acidophilus	La-5	Chr. Hansen, Denmark
L. acidophilus	NCFM	Rhodia, USA
L. acidophilus	Johsonii La1	Nestle, Switzerland
L. rhamnosus	GG	Valio, Finland
L. casei	Shirota	Yakult, Japan
L. reuteri	MM2	Biogia, Sweden and USA
Bifidobacterium	strain	manufacturer
B. animalis spp. *lactis*	Bb-12	Chr. Hansen, Denmark
B. longum	BB536	Morinaga Milk Industry, Japan
B. longum	SBT-2928	Snow Brand Milk Products, Japan
B. breve	-	Yacult, Japan
B. lactis Lafti^{TM}	B94	DSM, Australia
B. longum	UCC 35624	UCCork, Ireland
B. lactis	DR10/HOWARU	Danisco, Denmark

2.1.2 Genus Lactobacillus and Bifidobacterium

The genus *Lactobacillus* is comprised of Gram-positive, catalase-negative, non-sporulating, microaerophilic rods, which produce lactic acid as the major end product during the fermentation of carbohydrates (Axelsson, 1998). At present more than a hundred species are identified in this genus. Table 1 shows the examples of species and strains from Lactobacillus that often added as probiotics. *L. acidophilus* has long been known to be therapeutic, and helpful in maintaining intestinal health. Strains from this species are most studied, and its major characteristics are quite representative, so it will be discussed here. *L. acidophilus* requires carbohydrates as energy and carbon sources as well as nucleotides, amino acids, and vitamins. Most strains in *L. acidophilus* are microaerophilic, while the others are aerotolerant or anaerobic. The optimum growth temperature is around 37°C, and the maximum growth temperature ranges as high to 45°C. The suitable growth pH is 5.5-6.0, while it can grow in pH from 4.5-6.4. (Shah, 2006)

Bifidobacteria are Y-shaped, Gram-positive bacteria, but not all cells in a culture exhibit the split, Y-shaped morphology. Diverse species and strains in this genus are recognized as probiotics, as partly shown in table 1. *Bifidobacterium* spp. are obliged anaerobic, although some strains may tolerate limited amount of air slightly better than others, the presence of oxygen has adverse effect on their growth and survival. The optimum growth temperature is also around 37°C, while the maximum growth temperature ranges around 40-45°C. The optimum pH for is 6.0-7.0, and at pH 4.5-5.0 there is no growth.

2.1.3 Probiotic death kinetics during refrigerated storage of retail product

Empirically, 1-3 log cfu mL^{-1} or g^{-1} of probiotic viability is often lost during the prolonged storage even at around 4°C (Damin et al., 2008; Vasiljevic et al., 2007; Jayamanne & Adams, 2006; Antunes et al., 2005; Lourens-Hattingh & Viljoen, 2002), which is suggested to be the optimal temperature. Taking the bacteria growth curve into account, the death during storage is reasonable because the probiotics had already experienced the lag phase and the exponential phase, and had often reached the stationary phase after the fermentation, the death phase is as followed during the storage. A constant decline during prolonged storage is therefore unavoidable, though suitable environment slows down the death rate.

It was found that under a low temperature, the relationship between probiotic viability (log cfu mL^{-1} or log cfu g^{-1}) and time (day) is nearly linear (Allgeyer et al., 2010; Ramchandran & Shah, 2010). Hence, the probiotic death kinetics is formulated as $y = ax + b$; y is the probiotic viability at time x, b is the initial viability, and absolute value of a is the death rate of probiotics per day; R^2 evaluates the linear relationship. In table 2, death kinetics of different probiotics in studies of the recent decade are reviewed. The death rate is shown to be diverse, indicating that it might be strain specific. However, there is a general trend: 10 out of 25 are as high to 0.08-0.13 log cfu mL^{-1} (or g^{-1}) per day; 5 are as low to 0.02-0.04 log cfu mL^{-1} (or g^{-1}) per day, and the rest are in between. Usually, the lower death rate is accompanied by some improvement when making the yogurt, such as adding β-glucan (Allgeyer et al., 2010), and using genetically modified yogurt bacteira (Ongol et al., 2007), etc. Since these improvements are not universally applied by the producers, the higher death rates will be considered in the later chapters when estimating the viability loss.

Table 2: A review on probiotic death kinetics and calculation of death rate

Study	Probiotics	Storage	Probiotic death kinetics	Remarks
Allgeyer et al., 2010	*L.acidophilus* La-5	4°C, 30d	$y = -0.089x + 8.4; R^2 = 0.9888$	
	B.animalis Bb-12	4°C, 30d	$y = -0.081x + 8.2; R^2 = 0.9801$	
Rosburg et al., 2010	*B. longum*	4°C, 21d	$y = -0.089x + 9.8; R^2 = 0.9116$	β-glucan treatment in yogurt production
Ongol et al., 2007	*B. breve* JCM 1192™	10°C, 21d	$y = -0.13x + 8.3; R^2 = 0.8731$	
		10°C, 21d	$y = -0.039x + 8.9; R^2 = 0.9905$	*L. delbrueckii* subsp. *bulgaricus* SBT0164 No. 55-1 was used to inhibit post-acidification
	B. breve JCM 7017	10°C, 21d	$y = -0.063x + 8.4; R^2 = 0.8365$	
		10°C, 21d	$y = -0.020x + 8.4; R^2 = 0.9512$	*L. delbrueckii* subsp. *bulgaricus* SBT0164 No. 55-1 was used to inhibit post-acidification
Mortazavia	*L. acidophilus*	2 °C, 20d	$y = -0.051x + 7.5; R^2 = 0.9623$	

n et al., 2006		5 °C, 20d	y = -0.096x + 7.6; R^2 = 0.9001	
		8 °C, 20d	y = -0.084x + 7.5; R^2 = 0.9858	
	Bifidobacteria	2 °C, 20d	y = -0.090x + 7.7; R^2 = 0.9663	
		5 °C, 20d	y = -0.090x + 7.7; R^2 = 0.9663	
		8 °C, 20d	y = -0.072x + 7.7; R^2 = 0.9364	
Varga et al., 2002	*L. acidophilus*	4 °C, 42d	y = -0.011x + 7.5; R^2 = 0.8954	excluded the 0d data
		15°C, 18d	y = -0.010x + 7.3; R^2 = 0.9120	excluded the 0d data
	Bifidobacterium spp.	4 °C, 42d	y = -0.047x + 6.8; R^2 = 0.9424	excluded the 0d data
		15°C, 18d	y = -0.051x + 5.9; R^2 = 0.7309	
Adhikari et al., 2000	*B. longum* B6	4.4°C, 30d	y = -0.019x + 9.1; R^2 = 0.8492	
	B. longum ATCC 15708	4.4°C, 30d	y = -0.018x + 9.2; R^2 = 0.9562	
Dave and Shah, 1997	*L. acidophilus*	4 °C, 35d	y = -0.050x + 7.3; R^2 = 0.9666	glass bottles
		4 °C, 35d	y = -0.12x + 7.4; R^2 = 0.9369	plastic cups
		10 °C, 35d	y = -0.11x + 7.6; R^2 = 0.8431	plastic cups
	Bifidobacteria	4 °C, 35d	y = -0.025x + 7.9; R^2 = 0.8987	glass bottles
		4 °C, 35d	y = -0.025x + 7.9; R^2 = 0.8987	plastic cups; without 0d data
		10 °C, 35d	y = -0.048x + 7.7; R^2 = 0.8300	plastic cups; without 0d data

2.2 Stress factors and their impacts on probiotic viability

Generally, viability of probiotics is stressed by factors such as pH, temperature, oxygen, water activity, antagonism of yogurt bacteria etc. The factors behind them, for instance, the refrigerator that influence the temperature or the package that prevents oxygen, are not considered here.

2.2.1 pH

Low medium pH has been identified as an important factor hindering viability of lactic acid bacteria (Shah & Jellen, 1990). Medina and Jordano (1995) reported a highly correlation coefficient ($P<0.001$) between the pH and probiotic viability of *L. acidophilus* and *Bifidobacterium* spp. Stored under around 4°C, a pH at 4.5 or lower jeopardises the cell viability of the probiotic organisms in yogurt (Vinderola et al., 2000), meanwhile, a constant decline in pH during storage is observed. At the end of shelf life, a pH decline of 0.2-0.3 is often reported (Medina and Jordano, 1995; Varga 2006, Damin et al. 2008). Especially, when the temperature is higher, e.g. > 10°C, the decline in pH is larger (Lourens-Hattingh & Viljoen, 2002). The decline in pH is due to the metabolism of yogurt bacteria and probiotics during storage, which is called post-acidification. Hence, when the temperature is high, which causes the accelerated metabolism of yogurt bacteria and probiotics, not only the pH decreases faster, but its deleterious effect is strengthened due to the activated metabolism. Therefore, the pH is an important factor that influences probiotic viability, and the temperature moderates the extent of its influence.

2.2.2 Antagonistic effect of yogurt bacteria

Antagonism is reported between the yogurt bacteria *L. delbrueckii* ssp. *Bulgaricus* and probiotic organisms (Heller, 2001). The antagonistic effect is often based on the production of substances that harms the growth and survival of other related organisms. Though the lactic acid produced during storage can be considered as antagonism in this sense, it is considered as the same factor as the pH.

Rather, hydrogen peroxide produced by *L. delbrueckii* ssp. *Bulgaricus* is considered as the main source of antagonism, which is often reported to harm the viability of *L. acidophilus* and *Bifidobacterium* spp. (Lankaputhra et al., 1996b; Damin et al., 2008). The hydrogen peroxide transferred into the probiotic cells stimulates the oxidative reactions and result in the accumulation of toxic metabolites, harming the probiotic viability. The antagonism is species depending due to different oxidative reactions (de Vries & Stouthamer, 1969; Uesugi and Yajima, 1978; Shimamura et al., 1992; Shin & Park; 1997). Damin et al. (2008) reported that the viability of *L. acidophilus* was affected by the presence of *L. deibrueckii* ssp. *bulgaricus,* whereas biiidobacteria seems more tolerant; but viability of both probiotic organisms was improved when *L. deibrueckii* ssp. *Bulgaricus* is excluded from production. In general, the hydrogen peroxide produced by yogurt bacteria is considered as a stress factor influence probiotic viability. The temperature again moderates the extent to which that antagonism is influential, for higher temperature means faster metabolism and therefore a more harmful effect of hydrogen peroxide.

2.2.3 Oxygen

Oxygen exposure is considered to be deleterious for the cellular machinery of probiotic bacteria, especially bifidobacteria (Condon, 1987), since they are categorized as strict anaerobes (2.1.2). The oxygen toxicity is realized when it is transferred as hydrogen peroxide inside the cell. The oxygen content in the product at the end of production is low because it was consumed by the yogurt bacteria during the fermentation. During storage, the oxygen content in the products is increasing, given the commonly used package materials (Ishibashi and Shimamura, 1993). Curiously, the effect of the oxygen, albeit harmful to probiotic cells theoretically, is not as obvious as it was thought to be. Talwalkar et al. (2004) compared the probiotic viability in yogurts packaged commonly and in the oxygen-preventing material, no significant difference in viability loss was found in between, though the increase in oxygen is from less than 10ppm on the first day to about 40ppm on day 28 in the common packaging and is negligible in the oxygen-preventing package. On the one hand, the low temperature (4-6°C) used in this study inhibited the metabolism of probiotics and thus suppressed the harmful effect of oxygen; on the other hand, as discussed by Talwalkar et al. (2004), the probiotics were possibly allowed to develop some resistence toxygen due to the slow diffusion rate of oxygen (Ishibashi and Shimamura, 1993) and the non-uniform distribution of oxygen in the package (Miller, et al., 2002). Thus, oxygen is one of the stress factors but its effect largely depends on the temperature.

2.2.4 Water activity

Water activity is a measurement of water content. It is defined as the vapour pressure of a liquid divided by that of pure water at the same temperature; pure distilled water has a water activity of 1 exactly. The viability of probiotic cultures for production purpose is often reported to be influence by water activity (Weinbreck et al., 2010; Ying et al., 2010), however, it seems not a significant factor influencing probiotic viability in yogurt product during storage. Shah and Ravula (2000) compared five one-litre batches of probiotic yogurts made with reconstituted skim milk supplemented with 0, 4, 8, 12 or 16% sucrose, the counts of probiotic organisms declined during storage, in particular in the batches containing 12% and 16% sugar. However, the significance of the impact of sugar content (a_w) on probiotic viability was not confirmed in their study. And sugar content as high to 16% is not very common in yogurts but dairy desserts. Therefore, the water activity is not considered as an important factor for probiotic viability during storage.

2.2.5 Temperature

The effect of low temperature on probiotic viability seems paradoxical. On the one hand, low temperature such as 4°C puts cold stress on the probiotic cells. The probiotic tolerance to coldness varies between the species of these microorganisms, for instance, bifidobacteria are significantly less tolerant of low temperature storage than was *L. acidophilus*. (Hughes and Hoover, 1995; Dave and Shah, 1996). On the other hand, low temperature supresses the deleterious effect of pH and oxygen etc. by inhibiting the physiological activities of the probiotics. If the temperature is high, particularly as high to be suitable for microbial metabolism, post-acidification is quickly taken place to lower the pH, *L. delbrueckii* ssp. *Bulgaricus* emits more hydrogen peroxide, making the environment even more tough, so the death rate is much higher. In comparison, the death caused by cold stress is ignorable.

However, a small increase in temperature seems not very influential on probiotic viability. For example, it was reported that compared with storage at 4°C, storage at 10°C had little effect on the viability of all organisms inside the product despite the changes in pH (Micanel et al., 1997; Lourens-Hattingh and Viljoen, 2002); in another study, the variations in titratable acidity, pH, dissolved oxygen and hydrogen peroxide were almost identical at storage temperatures of 4 and 10°C (Dave & Shah, 1997). Other tests under temperature as 2°C, 5°C, 7°C and 8°C, etc. (Mortazavian et al., 2006; Medina & Jordano, 1995) showed that the death rate on general do not vary too much. Therefore, temperature under 10°C is considered as favourable for probiotic viability. Varga et al. (2000) compared the storage at 15°C and 4°C, a there was a sharp decrease in viability in the first three days, indicating a higher death rate. Scharl et al. (2010) reported a death of one third to half viable cells after expose under 20°C for only 6 hours. Death rate of probiotics under 30°C is currently lacking, but it can be deducted theoretically that probiotics die faster because 30°C is much more suitable for cell activity than 20°C. Furthermore, in the logistics of probiotic yogurt, the ideal temperature condition can be interrupted, causing more death of probiotics. Temperature is considered as one of the most important factors that influence probiotic viability.

2.2.6 Time is an implicit factor

Time is an implicit factor because the probiotics are dying constantly during storage. Rosburg et al. (2010) mentioned that culture counts of probiotics during cold storage were time dependent ($P < 0.01$). What is more, as time passes by, the pH of the products drops and the antagonistic effect of yogurt bacteria accumulates. But the change is not significant under low temperature, which is again a moderator in between.

2.3 Probiotic behaviour

2.3.1 Probiotic behaviour in the product

According to 2.1 and 2.2, the probiotic behaviour with respect to the death kinetics during storage and the stress factors is drawn in figure 3, which can be considered as the elaboration of the lower part of the quality relationship model (figure 2, chapter 1). Probiotic viability as consumed is the outcome of initial probiotic viability of the product minus the probiotic death rate plus time. It indicates that the initial probiotic viability should at least be higher than 6 log cfu mL^{-1} (or g^{-1}) due to the loss through storage time. The death rate is different according to different probiotic strains, but many are as high to 0.08-0.13 log cfu mL^{-1} (or g^{-1}) per day as reviewed in 2.1.3. Regardless of the strains, low pH is the main reason of the probiotic death, which is about 4.5 in most products at the end of production. Antagonism of yogurt bacteria mainly refers to the production of hydrogen peroxide, which also increase the death rate. Temperature moderates the impact of pH and antagonism on probiotic death rate. Theoretically, the higher is the temperature, the more harmful is the impact. However, it was found empirically that if the the temperature is under 10°C, it is not influential enough to raise the death rate. When the temperature is 20°C or even higher, e.g. optimal for probiotic metabolism, a significant effect is observed to accelerate probiotic death. As time passes by, the pH drops and the antagonistic metabolites of yogurt bacteria accumulates, however, the speed is again moderate by the temperature, the higher the temperature, the faster the change. But when the temperature is below 10°C, despite the change in pH, the probiotic death rate is not influenced significantly.

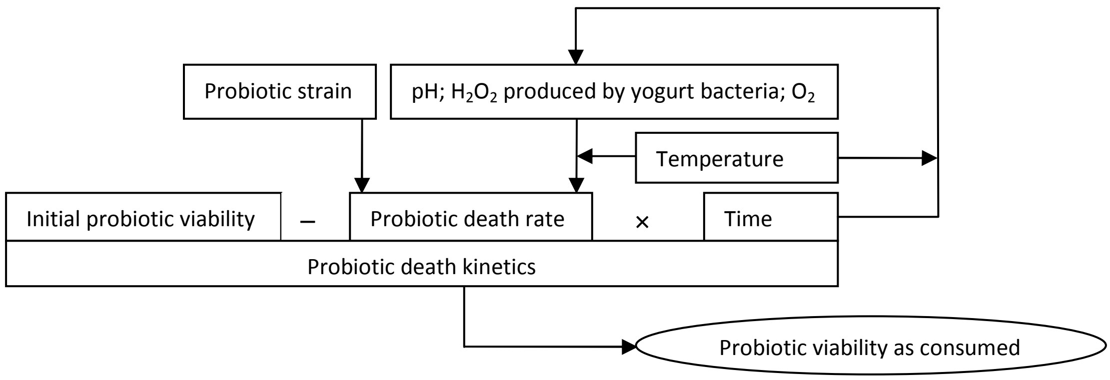


Figure 3: probiotic behaviour in the product with respect to death kinetics under different stress factors

2.3.2 Simplified probiotic behaviour regarding the supermarket chain and consumer chain

Though it is shown in figure 3 that many factors influences the probiotic viability of the yogurt product, not all of them will be investigated in the following study from the supermarket chain to the consumer chain. Inspecting each element in the model above, the probiotic strain is decided by the manufacturer and is not influenced by the supermarket chain or the consumer chain, so is the initial probiotic viability; both are not investigated in the following study. The pH, though an important factor influencing viability, is valued about 4.5 in most commercial yogurts, which is actually used an indicator to decide whether to end the fermentation or not. When the pH drops to around 4.5, the taste and texture, etc. of the yogurt are considered ideal. It is legislated in some countries that the pH of the yogurt at the end of fermentation should reach 4.5 for the consideration of product safety (inhibit the pathogen growth). The antagonistic effect of yogurt bacteria and the oxygen are not factors directly influenced by supermarket chain or the consumer chain. they are not investigated as well. Temperature and time, however, are factors directly related to the supermarket chain and the consumer chain. Temperature is a moderator between pH, hydrogen peroxide and oxygen and death rate; time influences probiotic viability directly, and also the change of pH, hydrogen peroxide and oxygen. Given the aim of this project is to understand the impact of the supermarket chain and the consumer chain on probiotic viability regardless of the brand of the products, the directly changeable factors in the process, the temperature-time conditions, are considered as the most important factors influencing probiotic viability in the process. The complete probiotic behaviour model is simplified as below figure 4 for further investigation in the supermarket chain and consumer chain.

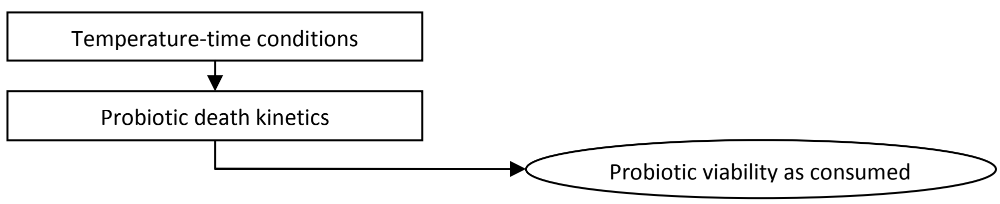


Figure 4: simplified model of probiotic behaviour to be studied from the supermarket chain to the consumer

Chapter 3. Scenario of supermarket chain and consumer chain

After studying probiotic behaviour inside the yogurt with respect to probiotic death kinetics and related stress factors, the next is to investigate the stress factors in real life and their impact on probiotic death kinetics and viability. As concluded in 2.3.2, only the temperature and the time during storage will be focused in the scenario from the supermarket chain to the consumer chain.

3.1 The scenario method

Currently it is not clear what happens from the supermarket chain to the consumer chain, especially, what temperature-time conditions are created accordingly. Therefore, a lot of information is needed to describe both chains. Wolters and Steenbekkers (2006) proposed a scenario method to cover as much information of the users/handlers as needed for further improvement of a product (figure 5), which is considered to fit the purpose of this project to improve quality of proibiotic yogurts.

SCENARIO

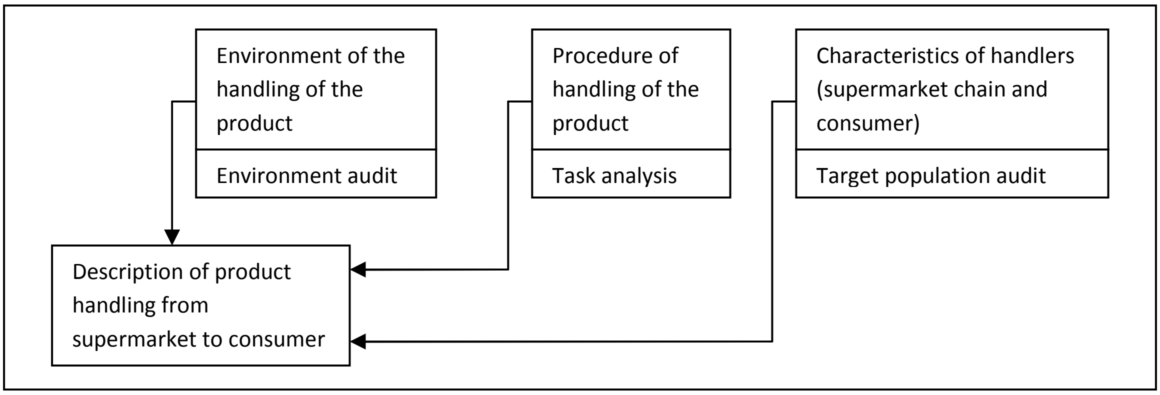


Figure 5: scenario method (adapted from Wolters and Steenbekkers, 2006)

In the scenario model above, the target population audit refers to describing the basic characteristics of the handlers. In this project, the consumer is undoubtedly the handler, while the supermarket chain overall is considered as a "handler". The purpose is to give extra information to complete the scenario and to shed light on further research. The task analysis refers to understanding the procedure of handling of probiotic yogurt products, which is the transportation of the products, resulting in the change of storage places. For example, in the supermarket chain, the procedure is: receiving products at the warehouse, storing, transporting to the supermarket stores and selling to the consumers; and the procedure of the consumer chains are buying the products and taking home, storing in the refrigerator and consuming. The environment audit is to investigate the environment of each place where the products stayed, with emphasis on the temperature-time conditions. Finally, the impact on probiotic viability can be estimated on a general level.

Although some characteristics of the handlers and their relationship with the handling condition will be studied, it is not the primary concern. The causes behind the handling behaviour will not be studied, because currently it is urgent to know the scenario from the supermarket chain to the consumer and the impact on probiotic viability. However, after drawing the scenario and estimating its impact on viability which sophisticate the problem to a bottleneck, the causes behind the handling conditions is in need of research in order to generate wise solution.

3.2 Supermarket chain scenario

Europe is the main market for functional dairy products, wherein Germany, France, UK and the Netherlands together occupied two thirds of all sales (Hilliam, 2000). The products are preferred to be sent via a distribution center to the supermarkets (Bourlakis and Bourlakis, 2001), which together compose a supermarket chain.

3.2.1 Characteristics of supermarket chain

A supermarket chain includes its own distribution centers and the local supermarket stores; a distribution center is a warehouse stocked with various, large quantity of products to be re-distributed to the local supermarkets, and a supermarket is a self-service store offering a wide variety of food and household merchandise. The supermarket chains differentiate in their size, performance and operational conditions, etc.

Inventory control is an important part of supermarket chain management. Higher inventory than demand is kept to prevent out-of-stock, paradoxically the chain also struggles to lower the inventory because the exceeded part is actually a waste. The distribution center management is supported by software providing suite. What is more, in the last decade large supermarket chains have started developing and implementing automated store ordering (ASO) systems in order to improve both the quality and efficiency of the inventory replenishment decisions in their stores (van Donselaar et al., 2006), but the store managers may not follow order advices generated by an automated inventory replenishment system if their incentives differ from the cost-minimization objective of the system or if they perceive the system to be suboptimal, it was found that the managers improved upon the automated replenishment system by incorporating two ignored factors: in-store handling costs and sales improvement potential through better in-stock (van Donselaar et al., 2010).

Temperature control in the chain is of great importance for the perishable products. The building of a distribution center is often with refrigeration or air conditioning for perishable products that need cold storage; the refrigerated trucks are used to deliver those products; and in the supermarkets, cold cabinets were used in the store to display most of the perishables. The expense on electricity is one of the major costs (Tahir and Bansal, 2005).

3.2.2 Handling of probiotic yogurt in supermarket chain

The procedure of the handling of the supermarket chain on probiotic products is drawn as the major part in figure 6. Firstly, the products are unloaded to the distribution center through the carousel (conveyor belt); after storing in the ware house for a certain time, they are re-loaded to the trucks (belonged to the supermarket chain) and distributed to the local supermarket stores. Once arrived at the local stores, they are again unloaded and stored in the small storeroom of the supermarket, and then they are put on the shelves, where they stayed until they are taken by consumers. The temperature-time conditions imbedded in each step are researched firstly in literature.

Unload and Store in warehouse (distribution center)	Distribution to the supermarket store	Unload at the backdoor and stay in supermarket storeroom	Move and stay on the cold shelf of supermarket	Taken by consumers and …
t 1	t 2	t 3	t 4	time
T 1	T 2	T 3	T 4	

Figure 6: supermarket scenario research model (t: time; T: Temperature)

3.2.3 Temperature-time conditions and their impacts on probiotic viability

T-t 1 The unloading of cargo can be done outside or inside the warehouse. When unloaded outside, each large bulk are transported into the warehouse quickly, e.g. through a carousel. When done inside, a small loading-unloading door is opened to inlet the chamber of the truck only, so the process of unloading is done under environment temperature of the warehouse. The whole process can take about 2 hours due to the huge quantity, but in advanced cases, shortest to 15min is possible to finish unloading. Because the products are stacked neatly in a large bulk, they are not very sensitive to the heat input (James and James, 2007). Though they are exposed to the ambient temperature, the time duration is too short to cause quality download.

After unloading, the bulk are put inside the refrigerated warehouse, where the temperature set is often 0°C but usually varies in different spaces. The typical arrangement of refrigerated warehouse arrangement and the temperature distribution inside is shown in figure 7a (Ho et al., 2010). A set of cooling units is installed along the front wall, in front of the arrays of product packages, to provide cold airflow to maintain low temperature in the space. The products are stacked on pallets into many parallel arrays with aisles between one another and clearances from the walls and from the floor. Each array consists of two back-to-back rows of four piles of three stacks of packages with both horizontal and vertical clearances between each other, as suggested by applicable guidelines (Ho, et al., 2010).

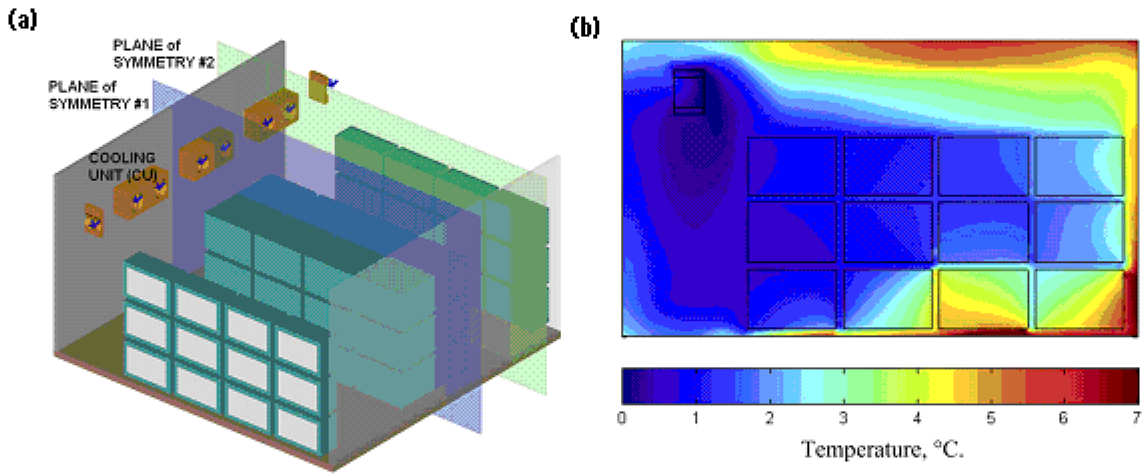


Figure 7a: the arrangement of a typical refrigerated house; 7b: the temperature distribution in a warehouse (Ho, et al. 2010).

Despite the temperature is set as zero, the distribution of temperature is uneven (figure 7b). The temperature of the area near the cooling unit is lower, which is coloured as blue, indicating the temperature is below 3°C, in the stratum near the celling, the temperature is higher than 4°C, but it is not a problem since the products usually does not reach there. On the ground corner that is far away from the cooling unit, the temperature ranges from 5 to 7°C, and Ho et al. (2010) reported a maximum temperature of 9°C and an average about 2°C. Given that death rate is generally the same when the temperature is below 10°C, in the phase 'unloading and storage in warehouse', only time is the stress factor. Compared with storage time, which is counted by day, the unloading time can be integrated with storage time.

T-t 2 After storage in the distribution center for certain days, the products are re-loaded to the refrigerated truck of the supermarket chain, the reloading process is comparable with the unloading discussed above, so there is no change on death rate. During the transportation, the temperature inside the truck and the time on the way is important. Refrigerated trucks are usually used to deliver dairy products, and the arrangement of goods inside the cargo looks like a slice of warehouse (figure

8). It is deducted that the temperature is not evenly distributed. Besides, the temperature might fluctuate over time, Ruiz-Garcia et al. (2010) put 4 sensors in different places of the cargo, though only mote 3 and 4 sent back the signals for temperature information, the data recorded during the transportation reflected a maximum temperature of 8.52°C and a minimum of −3.0°C. The average temperature registered in a certain interval during the shipment was 5.33°C, and the set temperature of the shipment was 0°C. Assuming that the temperature distribution in the cargo is similar with that in the warehouse (figure 7b), mote 3 should be the area with higher temperatures. However, since the maximum temperature did not exceed 10°C, the impact on death rate of probiotics can be neglected; the temperature is thus not the emphasis. Under the controlled temperature, death of probiotics is still kept at a normal rate. Time (days) is the factor needs investigation. Moreover, in T-t 1, Temperature is neither considered, the two steps can be merged and only the time will be focused.

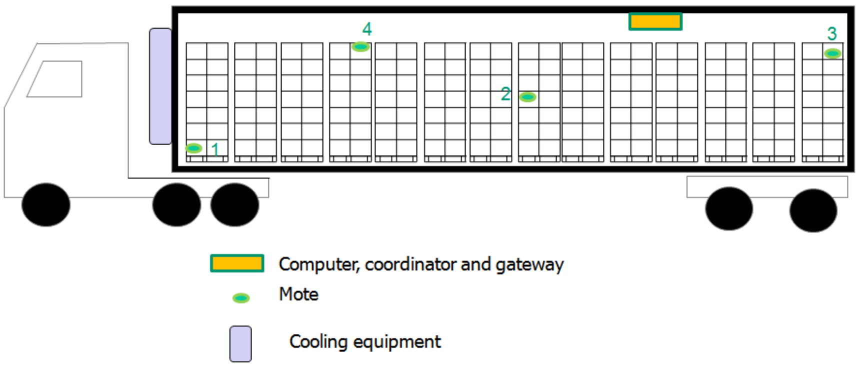


Figure 8: a refrigerated truck, side view (Ruiz-Garcia, et al., 2010)

T-t 3 When the truck reaches the supermarket store, the backdoor is opened and the products are unloaded and put in the storeroom of supermarket. The storeroom is air-conditioned to keep the temperature relatively low. There are usually several small rooms with better-controlled temperature conditions for different products separately, such as dairy products, meat, etc. If the products are not put immediately to the shelf, they are stored in that room. The dairy products, for instance, are stacked neatly in bulks, e.g. 6 or 12 packages per bulk, and the bulks are stacked neatly in the trolley. Compared to a single package, the trolley as a whole is less sensitive to heat input due to the size (James and James, 2007). The unloading and movement of these bulk pack are usually done within a time not longer than half an hour in total, and the time to move each set of trolley is much shorter, hence, the temperature fluctuation in the product can be neglected, so is the influence on probiotic viability.

After unloading, the yogurt products will be put on the shelf directly or probably stays in the storeroom for a certain time. It depends on the operational conditions of the supermarket. For example, if the truck with dairy product arrives in evening, but the workers stack up the shelf in the afternoon before the peak time (usually around half past five when people is back from work), it will stay in storeroom for one day. Furthermore, when supply exceeds the demand, the shelf will not have enough space for all the products arrived, so it needs to be stored in the storeroom for one or more days. If the temperature of the small closed storeroom is controlled well, only time will be an indicator. However, empirical evidence of temperature conditions in the small storage room of dairy product is lacking, if the temperature is higher than 10°C and the products are stored there for a one or more days, probiotic death kinetics might be adversely affected, consequently the product might contain viable probiotics less than the requirement when it is finally consumed by consumers. In this phase, both time and temperature are considered as factors. And if temperature is higher than 10°C in the storeroom for dairy products, it is out-of-tolerance.

T-t 4 After staying in the storeroom, the products are moved to fill in the shelf. Probiotic yogurt is stacked in the open refrigerated cold cabinet, and the stacking work is done beside the cabinet. The environmental temperature is lower near the open cold cabinet than the room temperature, furthermore, the duration of putting all the products on shelf is no more than two hours. And all the dairy products are taken out from the small storeroom by batches. So considering probiotic yogurt singly, the process is within ten to fifteen minutes. Therefore, the influence on the death rate is negligible since the time duration is too short.

After, the products stay on the open refrigerated cabinet before they are purchased. The temperature is controlled, supplemented by the thermometer on the top corner of the cabinet. The temperature displayed is often around 0°C, however, it does not always represents the actual temperature of every part of the shelf or of the products. In a study the differences between the displayed temperature and the tested temperature on various parts of the shelf can be up to 10°C, in the same place, the temperature can increase towards the end of the day by 4°C and towards the end of the week by almost 7°C (Willocx et al., 1994). This increase might be due to the flow of customers inside the supermarket. In another study, Warton and Wills (2002) carried out a study on the cabinet temperature where lettuce is hold, which is recommended to be stored at 5°C or lower, like the yogurt. They reported the temperature of refrigerated cabinets ranged from 0.6-11.0°C with only 20% of cabinets operating at the recommended temperature.

However, in spite the fact that recommended temperature (around 4°C) is not always obeyed in the supermarket cabinet, the analysis in laboratory did not show obvious difference between the death rate of probiotics under 4°C and 10°C. So it is not a problem of temperature which is still kept under 10°C. However, sometimes the temperature of part of the cabinet could reach 15°C, which was observed in the uppermost part of the cabinet (Willocx et al., 1994). Vinderola et al. (2000) reported that the temperature of cabinet for cheese is often found to be around 12°C. Therefore, in this stage, shelf temperature higher than 10°C is suggested to be out-of-tolerance. In addition, the longer is the time on shelf, the larger is the adverse effect.

3.2.4 Supermarket chain scenario to be investigated

Basing on the information gathered above, the scenario of the supermarket chain to be investigated is drawn in figure 9, including the basic characteristics of the supermarket chain, the handling of the products and the temperature-time conditions created. In the distribution centre, only time needs investigation due to the well-controlled temperature. In the storeroom and the cabinet, the temperature is in need of investigation due to lack of information or the high temperature condition reported.

Characteristics of the supermarket chain (size, performance, operational condition)		
In the warehouse, until reaching the supermarket	In the storeroom of the supermarket	In the open refrigerated supermarket display cabinet
t	T-t	T-t

Figure 9: supermarket chain scenario to be investigated.

3.3 Consumer chain scenario

The handling of consumers is more diverse compared to that of the supermarket chain, because the consumers are not regulated when handling and consuming probiotic yogurt or other food stuff. But the basic steps from buying to consuming are more or less the same, the differences in between are the temperature-time conditions created.

3.3.1 Characteristics of consumers

The appearance of functional foods in the market is driven by increasing concern of consumers on health (Foster & Lunn, 2007; Grunert, 2010). Consumers of functional food are more likely to be female, better educated, affluent, non-smokers, light drinkers, and having a diet-related problem personally or in the family, but also tend to have an adequate nutritional intake (Radimer et al., 2000; Greger, 2001; Landström et al., 2007). The consumers of probiotic yogurt particularly tend to be female, not older than 65, consuming moderate to high vegetables, with low fat intake, reported in a survey involving more than a thousand Dutch respondents (de Jong et al., 2003).

Usually, most consumers buy the items lasts for one week's consumption (van Donselaar et al., 2006). But it is not necessary the consumption pattern of the consumers of probiotic yogurts. It is reported that only 3% of the Dutch consumers would consume probiotic yogurt daily, 5% once or several times per week, 5% once or several times per month, 19% less than monthly, 49% not at all, and 19% unknown (de Jong et al., 2003), therefore, the consumers, especially the frequent consumers of probiotic yogurts might share unique demographic characteristics and consumption pattern. Two commonly investigated characteristics, age and gender, will be explored as well as their relation with the temperature-time conditions created in the chain. In addition, knowledge on probiotic yogurt of the consumers is also considered as an important characteristic because knowledgeable consumers might have different handling behaviour. Hence, age, gender, consuming frequency and knowledge of consumers are characteristics to be investigated in this project.

3.3.2 Handling of consumers on probiotic yogurt

The handling of the consumers includes buying, taking home, putting and storage in the home refrigerator and taking out for consumption (figure 10), wherein different temperature-time conditions are created.

Take from shelf to the cart, until cash out	On the way home, put probiotic yogurt into refrigerator	Storage of the probiotic yogurt in home refrigerator	Take out from refrigerator, until consumption	
t 5	t 6	t 7	t 8	time
T 5	T 6	T 7	T 8	

Figure 10: consumer scenario research model (t: time; T: Temperature)

3.3.3 Temperature-time conditions and their impacts on probiotic viability

T-t 5 When the consumer take the probiotic yogurt from shelf to the cart, the product is exposed to the ambient temperature of supermarket. According to the guidance set out by the Chartered Institution of Building Services Engineers, supermarkets maintain a temperature of 19-21°C in winter and 21-23°C in summer. The products taken away by consumers are single sets, which are more sensitive to the heat transfer compared to the closely stacked carton, indicating a risk on probiotic viability. For example, an exposure of 6 hours under 20°C causes a significant decrease on probiotic viability, about one third to a half probiotics die (Scharl et al., 2010). However, the shopping time inside the store solely is hardly possible to be 6 hours.

T-t 6 After the consumers cash out, the products are taken home under the environmental temperature. Taking the Netherlands as an example, from November to the next March, the average maximum temperature is below 10°C, and from May to September, the average maximum temperature is around 20°C (resource: http://www.climatetemp.info/netherlands/ Access date: 18-01-2010). Therefore, if it is not in winter, environmental temperature can be hazardous to probiotic viability if the time is long, e.g. 6 hours (Scharl et al., 2010). But usually the duration on the way hardly reaches 6 hours. Farber et al. (2010) studied the time-use pattern of shopping participants in three Canadian cities, the mean time spend on the way is around 40min, while on the way home, it takes half time as 20min. However, it is not known when the products are finally put into the home refrigerator. Taking into account the time in supermarket (t5), it is possible that the time exceeds 6 hours in total. Therefore, the T-t 5 & 6 are merged as "buying and taking home until the products are put in the refrigerator", basing on the condition that is not in winter.

T-t 7 Once put in the home refrigerator, the probiotic yogurts are stored there until taken out for consumption. The temperature inside the home refrigerator is diverse, a study on refrigerator temperature of more than thirty Dutch household reported a range from 3.8-11.5°C (Terpstra et al., 2005). A larger sample size of 102 Sweden household was studied on the temperature of different food items in their refrigerator, such as meat, milk, cheese, ready-to-eat salad etc. Mean storage temperature was around 7°C, maximum temperature of milk reached higher than 12°C (Marklinder et al., 2004). However, in rare cases the temperature is higher than 10°C, and only by a little bit, so generally temperature in this phase is not considered as an factor influencing probiotic viability. Time is the only factor because the probiotics are dying over time. The storage time is various between consumers, and might be disadvantageous for probiotic viability. For example, in the study of Terpstra et al. (2005), 16 out of 32 households mentioned a maximum storage time of dairy products that was longer than the expiration date printed on the package. While with probiotic yogurt, enough probiotic viability is not the responsibility of the manufacturer and will not be guaranteed.

T-t 8 If the probiotic yogurt is consumed immediately after taken out, the temperature-time condition is not influential on probiotic viability. However, it is observed by the author that some consumers would take the probiotic yogurt or conventional yogurt with them as a snack between lectures or as lunch. In this case, the product is exposed to the ambient temperature for hours. A significant decrease in probiotic viability is reported if the products put under 20°C for 6 hours; only about a half or two thirds of the probiotics exists (Scharl et al., 2010), equals to a loss of viability of 0.2-0.3 log cfu mL^{-1} or g^{-1}. Theoretically, the quick death is mainly attributed to the toxicity of the low pH, and probably the oxygen also, which is in accordance with the analysis in chapter 2. Therefore, if the consumers keep the yogurt at room temperature for hours before consumption, the effect on probiotic viability is considered to be unfavourable.

3.3.4 Consumer chain scenario to be investigated

With the analysis above, the consumer chain scenario to be investigated is drawn in figure 11, including the characteristics of the consumer, their handling of the products and the temperature-time conditions created. The characteristics of consumers to be investigated include age, gender, consuming frequency and knowledge on the probiotic yogurt product.

<table>
<tr><td colspan="3">Characteristics of consumer (age, gender, consuming frequency and knowledge)</td></tr>
<tr><td>Taken home until put in refrigerator</td><td>In the home refrigerator</td><td>Out of refrigerator before consumption</td></tr>
<tr><td>T-t</td><td>t</td><td>T-t</td></tr>
</table>

Figure 11: consumer chain scenario with temperature-time conditions to be investigated

Chapter 4. Conceptual research model

The general quality relationship model in chapter 1 guides the following study. In chapter 2, the death kinetics of probiotic viability and the stress factors were studied, resulting in a complete model describing probiotic behaviour and a simplified model (figure 4) that fits the study from the supermarket chain to the consumer chain. Subsequently, in chapter 3, the scenarios of the supermarket chain and the consumer chains were depicted and analyzed, resulting in two scenarios to be studied in real life condition. Therefore, the models in figure 4, 9 & 11 in chapter 2 and 3, which are the elaboration on different parts of the general quality relationship model (figure 2) in chapter 1, are merged together according to the structure of the general model.

4.1 Conceptual research model from supermarket chain to consumer chain

The conceptual research model show below (figure 12) is an elaboration of the quality relationship model (figure 2) in chapter 1. It consists of the scenario from the supermarket chain to the consumer chain, the impact of both chains on probiotic death kinetics and probiotic viability as consumed. The scenario includes the characteristics of supermarket chains and consumer chains, the handling procedure and the temperature-time conditions; each step in the handling procedure is an indicator, and the level of the indicator will be constructed based on the temperature-time conditions. The characteristics of the supermarket chain to be investigated are size, performance, and the operational conditions related to handling probiotic yogurt products; and the characteristics of the consumers to be investigated are the age, gender, consuming frequency and the knowledge on probiotic yogurt. The characteristics of the supermarket chain and consumer will be studied to give an informative base of the scenario, and shed light on the direction of future researches.

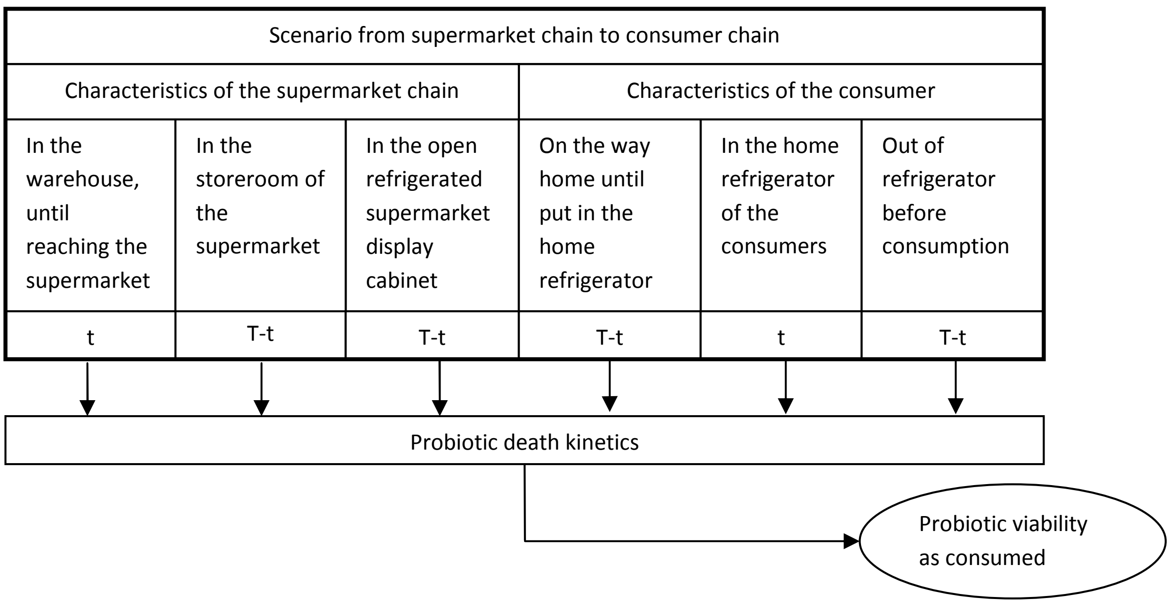


Figure 12: conceptual research model (t: time; T: temperature)

4.2 Indicators and level construction

4.2.1 Distribution center/warehouse (until reaching the supermarket)

From unloading, storage, reloading and transporting until reaching the supermarket, only time is an factor that influence probiotic viability. Storage time in the distribution center can be as short to zero if direct delivery from the manufacturer or cross-docking happens. Under this circumstance, no influence is on probiotic viability in this step and it is constructed as level 0. However, it is not the preferred logistic method by the supermarket chains in the Netherlands (van Donselaar et al., 2006). Probiotic yogurts, with a shelf life around 30 days, are not as perishable as the fresh fruit and vegetables; they are preferred to be sent from the distribution center with other dairy, which facilitates the inventory control and transportation efficiency. Therefore, usually the products are stored in the distribution center for days.

The levels of this indicator are constructed taking into account the shelf life and particularly the impact on loss of probiotic viability. The shelf life of probiotic yogurt products is about 30 days, if the products are stored for too long, the remaining shelf life will be short when they are displayed on the shelf, resulting in both a low quality image and a large loss on viability. The death rate of many probiotics are 0.08-0.13 log cfu g^{-1} (or mL^{-1}) per day; though better viability (lower death rate) was also present in some yogurts, the higher death rate in most products is taken into account. In one week, up to 1 log cfu g^{-1} or mL^{-1} viability is lost, the levels are constructed as below.

Level 0: storage 0 day, direct delivery or cross-docking (no influence on probiotic viability).

Level 1: storage for 1-7 days (up to 1 log cfu g^{-1} or mL^{-1} viability is lost).

Level 2: storage for 8-14 days (up to 2 log cfu g^{-1} or mL^{-1} viability is lost).

Level 3: storage for more than 15 days (a loss more than 2 log cfu g^{-1} or mL^{-1} maximally).

4.2.2 Storeroom of dairy products in supermarket

In the storeroom, a temperature higher than 10°C is considered as out of tolerance. On the one hand, the cold chain is not respected as it should be in the supermarket; on the other hand, the death rate is increased, giving rise to faster loss in viability thereafter. When the temperature is lower than 10°C, no significant influence is on the death rate, time (days) is the only factor influencing probiotic viability. The main purpose of the storeroom is for temporal storage, ideally the storage time should not exceed 1 day, so the interval of time used to construct the level is smaller.

Level 0: Maximum temperature under 10°C, storage for 1 day or less (no influence on viability).

Level 1: Maximum temperature under 10°C, storage for 2-3 days (a loss up to 0.4 log cfu g^{-1} or mL^{-1}).

Level 2: Maximum temperature under 10°C, storage for 4 days or more (a loss maximally more than 0.4 log cfu g^{-1} or mL^{-1}).

Level 3: Maximum temperature above 10°C (the cold chain is not respected, the death rate might be higher and result in faster loss of viability during the subsequent storage, in case that the products are stored for days, the loss of viability can be very large even that no probiotics are alive).

4.2.3 Open refrigerated supermarket display cabinet

The shelves are replenished every day, but not all the products are replenished due to the unstable consumption of probiotic yogurt, which might be attributed to the product varieties and the small population of consumer group. So the time of storage on shelf can vary largely. Besides time, temperature of 10°C is used as threshold to construct the levels considering the influence on death rate, and that high temperature of the open refrigerated display cabinet is often reported (Willocx et al., 1994; Vinderola et al., 2000). The level construction is shown below:

Level 0: Maximum temperature under 10°C, storage of products for less than 1 day (no influence on probiotic viability).

Level 1: Maximum temperature under 10°C, storage of products for 1-3 days (up to 0.4 log cfu g^{-1} or mL^{-1} viability is lost, considering the highest death rate reviewed in table 2).

Level 2: Maximum temperature under 10°C, storage of products for 4-7 days (up to 1 log cfu g^{-1} or mL^{-1} viability is lost, considering the highest death rate reviewed in table 2).

Level 3: Maximum temperature above 10°C, or storage of products for more than 7 days (more than 1 log cfu g^{-1} or mL^{-1} viability is lost, considering the highest death rate reviewed in table 2).

4.2.4 On the way home until put in home refrigerator

When counting the time used by the consumers to take the product from shelf to home and put them in the refrigerator, hours are used instead of days. In this process, temperature fluctuation is almost inevitable if the season is not in winter. Unlike the large bulk stacked neatly in the trolley, the package bought by the consumers are much smaller and thus more sensitive to the heat input, indicating that a short exposure to high temperature might result in an apparent death of probiotics. Scharl et al. (2010) reported a significant decrease in probiotic viability by storage for 6 hours under 20°C, which is commonly the room temperature, and the temperature out door from late spring to early autumn (in the Netherlands). If the duration from supermarket to the point the products are put in home refrigerator exceeds 6 hours, it would be considered as intolerable. Besides, the duration of 2 hours is used to construct the level in between, because it might be the time duration for most consumers (3.3.3). And it can cause a problem on viability in some cases when the temperature is higher than 30°C. However, in winter, when the temperature is generally below 10°C, the death rate is not increased significantly, the time on the way home is too short to cause an obvious loss in viability, this condition is considered to be level 0.

Level 0: exposure to low environment temperature e.g. in winter (no influence on probiotic viability).

if not in winter, when temperature is high, e.g. around 20°C,

Level 1: time duration within 2 hours before putting the product into home refrigerator (the influence on viability is not obvious at 20°C, but is possibly a problem when temperature is higher).

Level 2: duration 2-6 hours (up to one third or a half of the probiotics die given the temperature at 20°C, equals to 0.2-0.3 log cfu mL^{-1} or g^{-1}).

Level 3: duration more than 6 hours (more than 0.2-0.3 log cfu mL^{-1} or g^{-1} the probiotics die given the temperature at 20°C).

4.2.5 Home refrigerator

Because the consumers tend to buy the products they need to consume for the following days, it implies that the probiotic yogurt they bought might last for consumption of several days, indicating days of storage in the home refrigerator. Considering the size of package of several commercial products, it is possible that the products are consumed within 1-4 days or 5-10 days if 1-2 units of products are bought, hence, they are considered as level 0 and 1 respectively. It is also possible that the consumers finish the product within more than 10 days, or even after expiration (Terpstra et al., 2005). Temperature, as analyzed before, is not a factor influencing probiotic viability and not considered to construct the level. The levels of time in the home refrigerator are shown below:

Level 0: consumed within 1-4 days (up to 0.5 log cfu g^{-1} or mL^{-1} viability is lost).

Level 1: consumed within 5-10 days (up to 1 log cfu g^{-1} or mL^{-1} viability is lost).

Level 2: consumed more than 10 days, but before expiration date (more than 1 log cfu g^{-1} or mL^{-1} viability is lost maximally).

Level 3: consumed after the expiration date (viability is not a responsibility of the producers and not guaranteed).

4.2.6 Out of refrigerator before consumption

Most consumers would consume the probiotic yogurt immediately after taking it out of the refrigerator. This process is too short to have an impact on probiotic viability, so it is considered as level 0. However, consumers might take the yogurt out of refrigerator and carry it with them to elsewhere, e.g. working place, school, until consumption. If the environmental temperature is lower than 10°C and the consumer intensely put the product under that environment, the effect is also neglected. Otherwise, the products are considered to be stored under room temperature where 6 hours is considered as a threshold to construct the level, according to Scharl et al. (2010). However, it is observed by author that refrigerators are provided in some working places, in which case the time exposed under ambient temperature is the duration from home to working place. For example, the Dutch would spend an average of 50 min on the way to work, considering the variation and the short time duration at home before going to work and when just arrived at the work place, 2 hours is suggested to be another point to distinguish the levels as constructed below.

Level 0: consume immediately, or stored under low environmental temperature e.g. in winter. (no influence on probiotic viability)

When the product are exposed to room temperature:

Level 1: exposure for less than 2 hours (the influence on viability is not obvious at 20°C, but is possibly a problem when temperature is higher).

Level 2: exposure for 2- 6 hours (up to one third or a half of the probiotics die given the temperature at 20°C, equals to 0.2-0.3 log cfu mL^{-1} or g^{-1})

Level 3: exposure for more than 6 hours (more than 0.2-0.3 log cfu mL^{-1} or g^{-1} the probiotics die given the temperature at 20°C).

4.3 Summary of indicator levels and implication for data collection

The levels within each indicator constructed are summarized in table 3. Level 0 favours probiotic survival, and level 1 means the impact on probioitc viability is not obvious, while level 2 indicates space for improvement and level 3 is generally out of tolerance and needs urgent change. The next step is to find out the level of each indicator from supermarket chain to consumer. The indicators in high level represent the problem in real life condition and define the bottleneck.

Table 3: summary of levels of each indicator

Level	Distribution center	Storeroom of supermarket	Supermarket cabinet	On the way home	In the home refrigerator	Out before consumption
0	0 d	≤ 10°C, 0-1 d	≤ 10°C, 0 d	≤ 10°C	1-4 d	≤ 10°C, or consume immediately
1	1-7 d	≤ 10°C, 2-4 d	≤ 10°C, 1-3 d	> 10°C, ≤ 2 h	5-10 d	> 10°C, ≤ 2 h
2	8-14 d	≤ 10°C, ≥ 5 d	≤ 10°C, 4-7 d	> 10°C, 2-6 h	>10 d, < expiration	> 10°C, 2-6 h
3	≥ 15 d	> 10°C	> 10°C or > 7d	> 10°C, > 6 h	> expiration	> 10°C, > 6 h

Chapter 5. Data collection in supermarket chain and consumer chain

The conceptual research model in chapter 4 guided the following investigation from the supermarket chain to the consumer chain. The aim was to find out the level or level distribution of each indicator in real life, and thus narrow down the problem to bottleneck.

5.1 Data collection in supermarket chain

To investigate the scenario in the supermarket chain with emphasis on the temperature-time conditions, an informative base of knowledge of the supermarket chain is necessary. Case study, which provides rich knowledge of a specific context (Yin, 1994), is therefore considered as a suitable method in this project.

5.1.1 Research method

Case selection and information resources A single case study was carried out to gain an in-depth understanding of the case, the representativeness of the case will be discussed in the end. One Dutch supermarket chain "Albert Heijn" was selected as the case, which is by far the oldest existing supermarket chain in the Netherlands with a brand awareness of 99% (Albert Heijn website, http://www.ah.nl/artikel?trg=albertheijn/article.feiten, Access date: 28-10-2010).

The research question was "what are the levels of each indicator in the supermarket chain?". The resources for gathering information were: probiotic yogurt products in the supermarket store, the open supermarket refrigerated display cabinet, the workers and the manager of the dairy section. In order to gather information continuously, the author worked in the supermarket store for three months as a shelf stacker in the dairy section.

Characteristics of the supermarket chain The characteristics of the supermarket chain such as size and performance were understood from the website of it, the operational conditions were mainly investigated during 3 months when the author worked in one of the local supermarket. Especially, products from three brands, Activia and Actimel (Danone, Groupe), and Yakult (Yakult Honsha Co Ltd), were targeted to investigate the levels of each indicator. This is due to their unchallengeable sales from 2004-2008 and 2001-2008, compared products from other brands (Appendix 1).

Time in the distribution center The time of storage in the distribution center was deducted by subtraction methods and gathered through the manager of the supermarket store, because information on the storage duration from the distribution was kept confidential. While working in the supermarket store to fill in the shelf of the dairy section, the batches of probiotic yogurt products from the aforementioned three brands were tracked on different days for a month. The following information was recorded: the date of incoming products, the brand of the products and the expiration date printed on the products. The days the products stored in the distribution center was deducted as "shelf life – remaining shelf life when unloaded – 1 or 2 days". Shelf life for Yakult is 30 days, for Activia and Actimel 32 days. The remaining shelf life of the product when unloaded is calculated by using the expiration date on the package to subtract the unloading date. Moreover, in the deduction, 1 day was subtracted for products of Yakult, and 2 days were subtracted for products of Activia and Actimel. This is basing on the consideration that the products were usually stored in the manufacturing plant overnight and then distributed (Tamime and Robinson, 2007), and Yakult products were produced in the Netherlands, it was assumed that 1 day passed before they reached the distribution center, and for Activia and Actimel products, which were produced in France, 2 days were assumed to be needed for the transportation to the distribution center.

Temperature-time in the storeroom The information on temperature of the storeroom was gained by observation and measurement at 13:30-14:00 on four random days in one week. The temperature displayed on the thermometer of the storeroom was observed; and the temperature of the products in the storeroom was measured by infrared thermometer on three different spots. Measurement was done before the truck with new products arrived, so the products in the storeroom stayed there for at least one day and therefore represented the temperature of the room. The time of storage in the storeroom was not measured systematically, because according to the working experience, the probiotic yogurt products from the target brands stayed less than 1 day in the storeroom.

Temperature-time in the cold supermarket cabinet The information on temperature in the cold supermarket cabinet was gained by observation and measurement at 14:30-15:00 on the same days when checking the storeroom. Observation refers to reading and recording the set temperature of the cold cabinets in which the probiotic yogurt products were stacked; while measurement refers to measuing the temperature of the probiotic yogurt products on the outside of the cabinet by the infrared thermometer. Both observation and measurements are done because it is common that the actual temperature of the cabinet is higher than the set one. What is more, products of Activia occupied a large space of the shelf, so the temperature of 4 products outside was measured randomly; products of Actimel and Yakult was put in the same cabinet in different layers with smaller shelf space comparing to Activia, the temperature of two products of each brand was measured, respectively. Measurement was done before the truck with new products arrived, so the products in the storeroom stayed there for at least one day and therefore represented the temperature of the room.

In order to investigate how long the products stayed on the shelf, seven, three and three products under brands Activia, Actimel and Yakult were selected, respectively. The products within each brand were different in the taste or package size, e.g. 125g × 4 or 125g × 8, etc. The inflow and outflow of these products were checked through the internal FVOS system of the supermarket for 14 days consecutively. Assuming complete obedience to the first-in-first out rule, the days of storage on the shelf of the products was deducted. For example, 6 units of product 1 of Activia was sent to the supermarket on Nov 26th 2010, at the end of that day, 5 old units of the product were before the new 6 units, on the second day, 5 units were sold and 6 more came, from the third day, 1 unit of product 1 arrived on Nov 26th was to be taken by consumers, and on the fourth day, 4 units in that batch was bought, on the fifth day, no unit was taken, on the sixth day, the last unit was bought, so units in batch 1 stayed on the shelf for 2, 3, 3, 3, 3, and 5 days.

5.1.2 Results – supermarket chain

5.1.2.1 Characteristics of the supermarket chain

Albert Heijn is the main supermarket chain in the Netherlands. 78.2% of all Dutch households had been shopping at its supermarkets and the annual turnover reached € 6 billion (2005); it owns 8 distribution centers, and over 700 stores scattered throughout the country (source: http://www.ah.nl/artikel?trg=albertheijn/article.feiten, Access date: 28-10-2010). The warehouse management was supported by the CDC software. The stores were replenished at least twice a day following a fixed delivery schedule, and the time between ordering and delivery was not more than 18 hours. The automated store ordering system was applied for replenishment. The amount of products sent to the supermarkets was predicted automatically basing on the sales in the past week.

In the supermarket store where the author worked, the truck was driven from the distribution center to the local supermarket store three times a day, each time with different categories of products. The truck with dairy products arrived at the backdoor of the supermarket around 2:30pm. The unloading process was done outdoor within half an hour. The temperature inside the truck was controlled and recorded every transportation.

The storeroom of the supermarket was chilled, and there were three small, closed storerooms inside, one of which was for dairy products particularly. The dairy products, including probiotic yogurts, were stored in the small, refrigerated storeroom shortly until 3:30pm when the workers began to fill the products in the cold cabinets by trolley after trolley. The whole process was done within 2 hours. During the process, each time only one or two trolleys with various dairy products were taken out, the next trolley came to the store after finishing the previous one. What is more, the ambient temperature around the supermarket was around 12-14°C. However, the temperature during summer might be higher than measured in winter in this case.

When there was not enough space on shelf for products, they were put back in the small storeroom overnight until next morning; and were filled in by another worker then. However, it seldom happened on the probiotic yogurt products. Compared to the largely demanded products such as milk or conventional yogurt, probiotic yogurts were sent to the supermarket in a small amount and only several varieties within each brand were sent every time.

5.1.2.2 Time in the distribution center

The records of dates of incoming products, brands, and expiration dates are shown in Appendix 2; the deducted time in the distribution center for each brand is shown in table 4, and the box plot of each brand of products are drawn in figure 13.

Table 4: number of days the products stored in the distribution center

	Days stored in the distribution center	N	Range	Mean	Std. Deviation
Activia	14, 11, 2, 11, 9, 8, 16, 14, 9, 12, 15, 11, 14, 16, 9, 13, 15, 17, 5, 4	20	2-17	11.3	4.3
Actimel	10, 8, 9, 10, 12, 13, 13, 12, 8, 10, 11, 9, 5, 13	14	5-13	10.2	2.3
Yakult	6, 5, 5, 5, 6, 4, 8, 6, 5, 5, 6	11	4-8	5.5	1.0

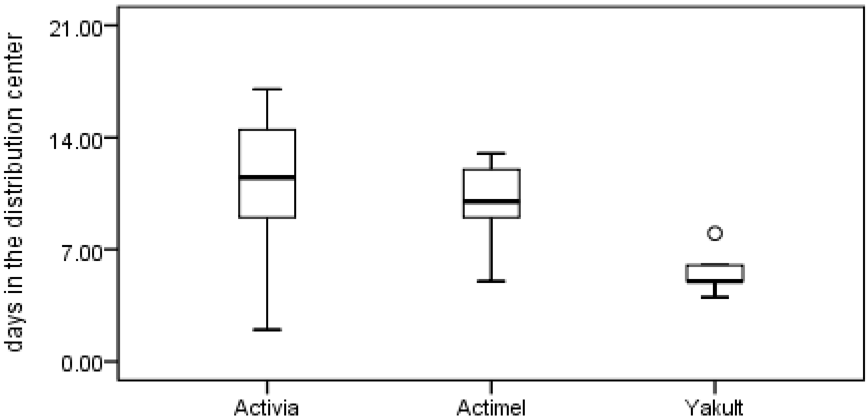


Figure 13: box plot of each brand of products stayed in distribution center

In figure 13, the box represents the interquartile range which contains 50% of the values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median. It is shown that half the products of Activia stayed in the distribution center for 9-14 days. Occasionally, a few products stayed for only 2, or 4 days, while others were stored as long to 15-17 days. Actimel products stayed in the distribution center for 9-12 days. Storage time of Yakult was relatively short and stable, mainly 5-6 days. This is possibly attributed to the low variety of products under this brand, it is easier to predict the demand of the products and control the inflow. Conversely, the reason that Activia had a longer storage might be attributed to the larger varieties of the products, the demand on each product changing and hard to be predicted. From figure 9 it is clear that the boxes of Activia and Actimel lays between 7-14 days, indicating that they are on level 2 according to table 3 in chapter 4. Yakult products are on level 1. The level of storage in the distribution center is brand differing, and there is space for improvement for some brands.

However, according to the manager of the supermarket store, the storage time of products in distribution center does not exceed 7 days and is usually 3-4 days. This is in line with the subtraction method in Yakult product only. Perhaps the products of Activia and Actimel, which are produced in France, spent much more days in the manufacture plant than the estimated one night before they are sent to the distribution center. Since the data is gathered through the manager of the store, rather than the direct interview by the author, the data from deduction is considered with priority. What is more, the two data actually indicate the same problem in another sense: the time before the product reached the supermarket is too long, up to 1-2 log cfu g^{-1} or mL^{-1} of viability is lost in the chain before reaching consumers.

5.1.2.3 Temperature-time in the storeroom

The results of the temperature of the storeroom particularly for dairy products are shown in table 5. Generally, the observed value represented the product temperature in the room as displayed. Considering all the measured values, the temperature condition in the storeroom is controlled well (4.2 ± 0.7°C). According to the operational conditions of the supermarket, the probiotic yogurt products seldom stayed in the storeroom overnight, the storage time was only 1 hour between unloading from the truck and filling in the cabinet. Therefore, the level of the storeroom is considered as 0 and no influence is on probiotic viability.

Table 5: temperature observed and measured in storeroom

	Observed (°C)	Measured (°C)		
Day 1	3.8	4.0	4.2	3.6
Day 2	5.0	5.0	5.4	5.4
Day 3	4.3	4.2	3.8	4.0
Day 4	3.0	3.6	3.8	3.4

5.1.2.4 Temperature-time of supermarket cabinet

The result of temperature conditions of the cabinets for the probiotic yogurt products are shown in table 6. The measured temperature of the products, though generally several degrees higher than the set temperature of the cabinet, was controlled the well (4.8 ± 1.0°C). Under this temperature, the death rate is not accelerated, only time (days) is influential on the probiotic viability.

Table 6: temperature of the cold cabinet and the products

	Activia (°C)				Cabinet (°C)	Actimel (°C)		Yakult (°C)		Cabinet* (°C)
Day 1	3.2	2.2	3.8	2.6	-0.5	3.8	4.4	4.2	4.6	2.0
Day 2	5.0	4.4	6.2	5.6	0.0	4.6	5.4	4.8	4.2	-0.8
Day 3	5.6	5.2	6.2	4.6	0.0	5.8	4.4	3.4	6.6	0.8
Day 4	4.6	4.6	4.2	4.4	0.5	3.6	4.8	3.8	5.8	0.5

* Actimel and Yakult were put closely to each other in the same cabinet, sharing the observed temperature

Based on the daily inflow and outflow of 13 selected products on 14 consecutive days (Appendix 3), the deducted days that the products stayed on the cabinet are shown in table 7. Excluding the data from product 3 of Actimel, the box plot is drawn to describe the feature of the other data (figure 14).

Table 7: days on the refrigerated display cabinet of each unit in different batches of products

	Days on the shelf in supermarket (d)					
	Batch 1	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 5**	**Batch 6**
Product 1, Activia,	2, 3, 3, 3, 3, 5	2, 3, 3, 3, 3, 3	0, 1, 1, 1, 2, 2	1, 1, 2, 2, 3, 3	-	-
Product 2, Activia	1, 3, 3, 4, 4, 5	4, 4, 4, 5, 5, 5	4, 4, 4, 4, 4, 5	6, 6, 6, 6, 6, 6	4, 5, 5, 5, 5, 5	3, 4, 4, 4, 4, 4
Product 3, Activia	0, 1, 1, 1, 1, 1	0, 0, 1, 2, 2, 3, 3, 3, 3, 3, 4, 4	3, 3, 3, 3, 4, 4,	2, 2, 2, 2, 3, 3	2, 2, 2, 2, 3, 3	
Product 4, Activia	0, 0, 1, 1, 1, 1	0, 1, 1, 1, 1, 3	3, 3, 4, 4, 4, 4	2, 2, 3, 3, 3, 3	3, 3, 3, 3, 3, 6	
Product 5, Activia	5, 6, 6, 6, 6, 6	2, 3, 3, 4, 4, 5	-	-	-	-
Product 6, Activia	5, 5, 6, 6, 6, 6	5, 5, 5, 6, 6, 6	2, 4, 4, 4, 4, 4	2, 3, 3, 3, 4, 4	3, 4, 4, 4, 4, 4	-
Product 7, Activia	0, 0, 0, 2, 3, 4	3, 3, 5, 5, 5, 5	2, 2, 2, 4, 4, 4	-	-	-
Product 1, Actimel	2, 2, 2, 2, 2, 2	1, 1, 2, 2, 2, 3, 4, 5, 6, 7, 7, 7	-	-	-	-
Product 2, Actimel	0, 0, 0, 2	4, 4	4, 4	4, 4	3, 3	-
Product 3, Actimel	1-14 *	-	-	-	-	-
Product 1, Yakult	2, 2, 2, 2, 3, 3, 3, 3, 3, 4	3, 3, 3, 3, 3, 3, 4, 4, 4, 5	3, 3, 3, 4, 4, 4, 4, 5, 5, 5	3, 3, 3, 3, 3, 3, 3, 4, 4, 4	2, 2, 2, 2, 2, 2, 2, 2, 3, 4	3, 3, 3, 3, 3, 4, 4, 4, 5, 5
Product 2, Yakult	1, 1, 1, 1, 1, 1, 2, 2, 2, 2	1, 1, 2, 2, 3, 3, 3, 3, 3, 3	3, 3, 3, 4, 4, 4, 4, 4, 4, 4	3, 3, 4, 4, 4, 4, 4, 4, 4, 4	2, 3, 3, 3, 3, 3, 3, 3, 3, 3	1, 1, 1, 2, 2, 2, 2, 2, 2, 2
Product 3, Yakult	1, 1, 4, 5	6, 6, 6, 6	6, 6, 6, 6	2, 2, 2, 3, 3, 4, 4, 4	-	-

*There were 69 units of product 3 Actimel in the beginning with no incoming in the following days

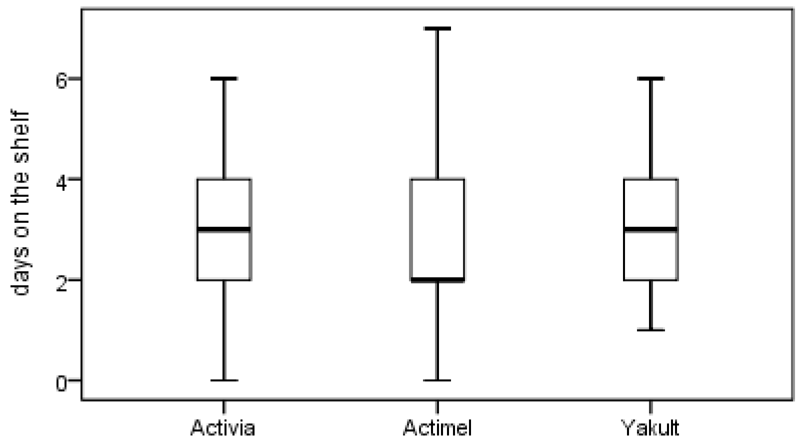


Figure 14: box plot of each brand of products stayed on the cold cabinet of supermarket

The box plot indicates that generally the products (50% of them) were stored on the shelf for 2-4 days, and the range of storage time was 0 to 7. Given the well controlled temperature, the level of this indicator is considered to be 1-2, meaning that the storage time of about half of the products needs improvement, that is, to reduce the time on the cold cabinet. It should be mentioned that the range of products stayed on the cabinet in real life according to the author's working experience in the supermarket. The data in table 6 were deducted assuming the obedience to the first-in-first-out rule, which was actually disrupted often by the workers when filled in the shelves, and it was also observed that some consumers searched inside of the shelves for the freshest products, causing larger variation in storage time. It indicates that in addition to the improvement to shorten the storage time in general, the control is needed to shorten the variation as well.

5.1.3 Impact of supermarket chain on probiotic viability

According to this supermarket chain, since the temperature is controlled well, the probiotics are dying at a nearly constant rate over time. The overall storage time of probiotic yogurt products from distribution center to the supermarket store is 10-20 days in general, a loss in viability of 1-2 log cfu mL^{-1} or log cfu g^{-1} of probiotic viability is estimated according to the death kinetics reviewed in 2.1.3.

5.2 Data collection in consumer chain

5.2.1 Research method

Questionnaire is considered to be efficient to collect the data of the consumer chains because multiple consumers were needed considering the variance in their behaviour etc. The aim was to figure out their handling and consuming patterns, the temperature-time conditions created and correspondingly the level of each indicator; finally the impact of consumer chains was estimated. The relation between some characteristics of the consumers and their behaviour will be explored to give extra information that might lead to further research.

Questionnaire construction A questionnaire (Appendix 4) was formulated by the author, both Dutch and English versions were constructed with the same content. The questionnaire includes the cover (page 1), the questions (page 2), the statements for judging (page 3), the knowledge and tips on probiotic yogurt (page 4) and the announcement of the author (page 5). The purpose of questions and statements on page 2 and 3, and how to translate them into levels of each indicator are written in table 8.

Question 3 asked the respondents about the time duration on the way usually without focusing on the summer situation, because the word ‘summer’ might induce the respondents to bias their answer. It was assumed that no obvious difference exits between the seasons. In addition, questions 2-6 are not only for assessing the levels of each indicator, together they represents the consumer’s “shopping and consumption pattern”, the relationship of which with the age, gender will be explored to elaborate consumer characteristics. Consuming frequency is considered as the most important characteristic of the consumers, because on the one hand, frequent consumers are the target group of the manufacturer and contributed a large portion to the profit, on the other hand, only when consumed frequently (several times per week), the health benefit can be realized (Tamime and Robinson, 2007). Therefore, the relationship between consuming frequency and level distribution in each indicator was investigated by Chi-square test, respectively.

Table 8: purpose of questions and statements constructed, and interpretation of the choices on different answers

	Purpose of the question/statement	Interpretation of choices
Question 1, age, and gender	To describe basic characteristics of the respondents	-
Question 2	To judge the level of the indicator – days in the home fridge	The choices are correspondent to level 0-3.
Question 3	To judge the level of the indicator – on the way until the products are put in the home fridge	The choices are correspondent to levels 1-3 assuming that it is not in winter (in winter the level is considered as 0).

Question 4	To gather extra information for discussing the time on the way home and the consumers' shopping pattern	-
Question 5 & 6	To assess the level of the indicator – out of home refrigerator	The 1st and 3rd choice of question 5 indicates level 0, the choices under question 6 are correspondent to level 1-3.
Statements 1-3	To assess the basic knowledge on probiotic yogurt, especially if respondents know the distinction between normal and probiotic yogurt	Only when questions 1-3 were answered correctly, consumers are supposed to have basic knowledge on probiotic yogurt, and can distinguish normal and probiotic yogurt. The correct answers are T(true), F(false), and T.
Statements 4-6	To examine if the respondents know exactly that the probiotics are dying during the storage and would die easily under room temperature	only when questions 4-6 were all answered correctly, respondents are considered to know exactly the knowledge stated on the left. The correct answers are F, F, and T.
Statement 7	Constructed only to be in accordance with the secrets and tips written on page 4	-

Questionnaire distribution With permission from the supermarket where the author worked in, the questionnaires were distributed to the consumers who took probiotic yogurt from the shelf in order to avoid respondents with no consuming experience of probiotic yogurt. The respondents were asked to finish the questionnaire in front of the author, the cover and page 4 & 5 were given to consumers, which contained the answers to the knowledge assessment part and the purpose of the survey. Additionally, the questionnaires were also distributed to the students and staff in the author's university, who were asked in advance if they had ever consumed probiotic yogurts (provided with several typical examples of the products), while those who had never consumed probiotic yogurt were not given the questionnaire.

5.2.2 Results – consumer chain

In total 141 respondents were gathered, while 87 of them were from the supermarket and the rest 54 were from the university. The results of each respondent on each question are shown in Appendix 5. Distributing questionnaires in the university was to balance the distribution of age and consuming frequency (to facilitate the chi-square test), because according to the primary results from the supermarket, the respondents were mostly older than 50 years, and consume the products 1-4 or 5-7 times per week. In the university, only 2 respondents refused to answer the questionnaire, while consumers in the supermarket were more likely to refuse answering the questionnaire, especially during the peak time in the supermarket, which was around 11:30-13:00 and 16:00-18:00, middle-aged consumers are also less likely to answer the questionnaire, and they tended to appear in the supermarket during peak time, which might be according to their life style.

5.2.2.1 Characteristics of the consumers

Gender, age and consuming frequency Distributions of 141 respondents on their gender, age and consuming frequency are displayed independently in figure 15. Among 141 consumers, 42 were male and 99 were female. 3 were younger than 19 years old (which was not expected when constructing the questionnaire), 55 were 19-34 years, 13 were 35-49 years, 41 were 50-64 years, and 29 were older than 65 years. 57 consumed probiotic yogurt 5-7 times per week, 32 1-4 times per week, 23 1-3 times per month, 29 less than monthly.

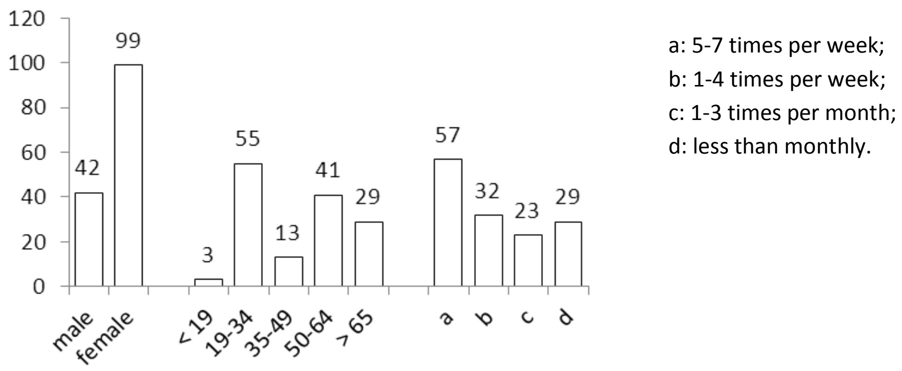


Figure 15: gender, age, and consuming frequency of respondents.

Divided by the consuming frequency, the distribution of age and gender are shown in figure 16. Generally there were more female than male consumers, especially in the groups a & b with higher consuming frequency (a & b). Also, respondents in these two groups tended to be 50-64 years or older than 65. Age was found to be strongly related to consuming frequency ($\gamma = 0.80$), however, in the previous survey of de Jong et al. (2003), people older than 65 years tended to consume probiotic yogurt less, while people in other groups showed no significant difference in consuming frequency. The difference between the findings might be due to the sampling methods used, or the increased familiarity with probiotic yogurts in the recent decade.

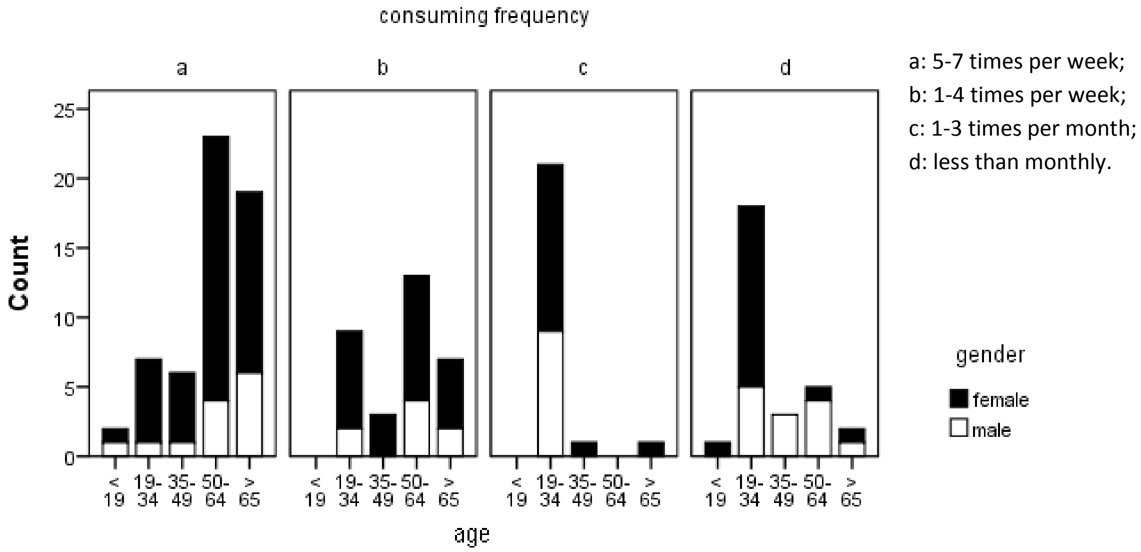


Figure 16: gender and age distribution in groups with different consuming frequency

Age and shopping and consuming pattern Divided according to the age, the results on shopping and consuming pattern are shown in figure 17. The pattern refers to the transportation, the time on the way home, the time stored in home fridge and the time out of fridge before consumption, which are in accordance with question 2-6. In figure 17(a), it is shown that respondents from 19-34 years tended to ride a bike for shopping, because most of them were students from the university in a small town, where bike is the main transporting tool, particularly for students. Respondents older than 65 years tended to walk more, which might be attribute to their physical condition. Figure 17(b) shows that most respondents spent less than two hours on the way home until the products were put in the refrigerator, but the percent of respondents who spent 2-6 hours plus more than 6 hours on the way is larger in the group older than 65 years than other groups. Figure 17(c) indicates that storage time in home refrigerator is not very different among all age groups, but it seems younger respondents tended to spend less time to finish one batch of product. However, age is not the reason of fast consumption. The time might be more related to the amount of products bought by respondents at once. During the questionnaire distribution, the frequent users (often old) used to buy two units which lasts for a consumption of 5-10 days. While the younger respondents, containing those who tried the product once or twice, tended to buy fewer products at one time. After taken out of the refrigerator, as depicted in figure 17(d), although most respondents ate the product immediately, the younger were more possible to keep the products for hours before consumption than the older. This might be explained by that younger people can take the product to school or working place, while the older are retired from their work, the chance to take the product elsewhere is much less. There were also respondents who kept the product outside the fridge before consumption, but they were known to intensely keep in under very cold environment through communication while answering the questionnaire, therefore, the effect on viability is neglected and is the same as consumed immediately.

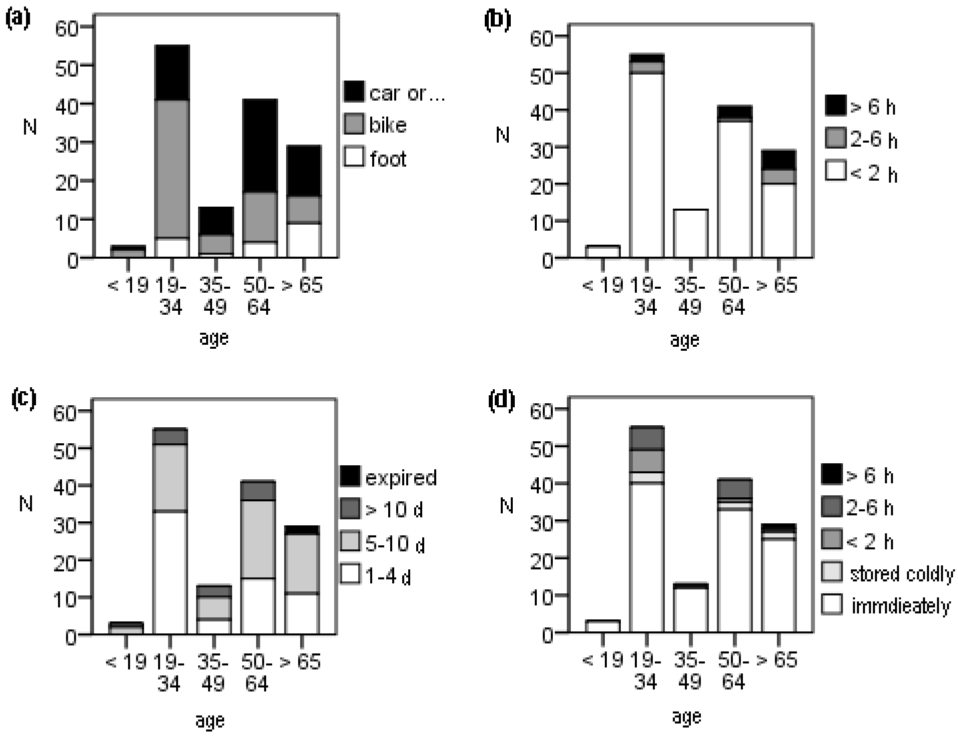


Figure 17: distribution of respondents with respect to their shopping and consumption pattern in different age group.

(a): transportation used; (b): time on the way home, hours; (c): time stored in the home fridge, days; (d): time after taken out before eating, or stored coldly.

Gender and shopping, consuming pattern Similarly, the respondents were divided by gender to analyze its relationship with the shopping and consumption pattern (figure 18). No significant difference is found between male and female. In both groups, about a half respondents used car or public transport; most of the respondents put the products in the home refrigerator within 2 hours, with a storage time of 1-4 days or 5-10 days, and consumed the products immediately after taken out of the refrigerator.

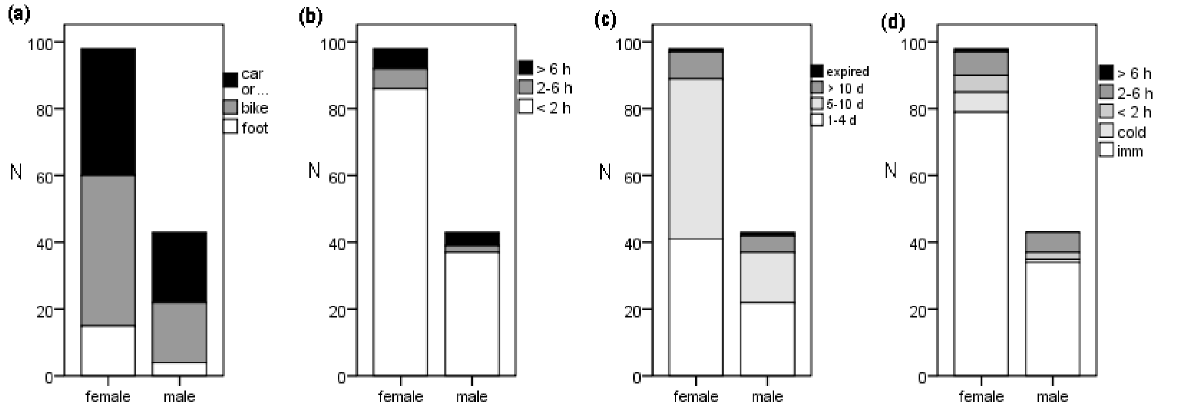


Figure 18: distribution of respondents with respect to their shopping and consumption pattern divided by gender.

(a): transportation used; (b): time on the way home; (c): time stored in the home fridge; (d): time after taken out before eating, or stored coldly.

Knowledge of respondents On page 3 of the questionnaire there were 7 statements for respondents to judge. Their answers (true/false/I don't know) on each statement are shown in Appendix 5. The correct answers for statement 1-7 are T (true), F (false), T, F, F, T, and T, respectively. The number of respondents on each choice of each statement, and the percentage of correct answers are counted; the number of respondents who answered all statements 1-3 or statements 4-6 right are counted as well (table 9).

Table 9: answers on each statement and the percentage of respondents with correct answers

	S1	S2	S3	S1, 2, 3	S4	S5	S6	S4, 5, 6	S7
True	112 √	34	78 √	-	46	83	53 √	-	84 √
False	2	59 √	13	-	25 √	30 √	18	-	15
Don't know	24	45	47	-	67	25	67	-	39
T, F, T	-	-	-	33	-	-	-	-	-
F, F, T	-	-	-	-	-	-	-	0	-
Respondents with correct answers %	81.2%	42.8%	56.5%	23.9%	18.1%	21.7%	38.4%	0%	60.9%

*3 respondent did not finish the questions to access their knowledge level

It is shown that the percentages of correct answers on statement 1, 2 or 3 are generally higher than on statement 4, 5, or 6. This is reasonable because statements 1-3 relate to basic knowledge on the difference between probiotic and normal yogurts, which is often communicated from the producers to the consumers in order to promote sale; statements 4-6 relate to specialized knowledge in this field. However, correct rate on individual statement is not accurate enough to represent respondent knowledge due to the possibility of guessing and deducting the answers from the context, which was observed when distributing them to respondents. This situation was considered while constructing the questionnaire, hence, only when statements 1-3 (or 4-6) are all judged correctly, the respondents are considered to have basic (or advanced) knowledge on probiotic yogurt. The interruption of guessing or deducting answers are excluded, because the chance to guess or deduct all answers right is much less if the consumers do not know exactly the difference between probiotic yogurt, or that the probiotics are dying constantly during storage and easily under room temperature. Statement 7 is not focused due to the reason in table 8.

Table 9 also shows that only one fourth (23.9%) of the respondents judged statements 1-3 all correctly, indicating that they know exactly the basic characteristics of probiotic yogurt and the difference from normal yogurt. Under the Chi-square test, the knowledge level is not related to consuming frequency (P=0.85>0.05), neither gender (P=0.90>0.05). Rather, it seems to be related to age (P<0.05, excluding the data of 2 respondents <19 years). The younger respondents are more likely to know the basic knowledge on probiotic yogurt. However, the significance might be exaggerated due to the sampling, for most respondents 19-34 years were found in the university, some of them were majored in microbiology or food technology etc. with relevant background knowledge. When it comes to statements 4-6, however, none of the respondents judged all three correctly, including several experts related to microbiology field in the university. It indicates none of them knew that probiotics are dying constantly even stored in refrigerator and dying quickly under room temperature. Therefore, the relation between knowledge and handling cannot be linked. However, regarding that none of the respondents judged the statements 4-6 correctly, including even some experts in microbiology or food area (though not specifically in probiotic field), the statements were possibly constructed too tricky for the respondents and induced them to choose the wrong answers.

5.2.2.2 Temperature-time on the way home until the refrigerator

The time on the way home until the products were put in home refrigerator was inevitable. The results of all respondents with respect to their consuming frequency are shown in table 10. Most respondents spent less than 2 hours, but those who consume the yogurt less than monthly tended to spend more time. However, the relation between the consuming frequency and the time duration was not significant under Chi-square test (χ^2=4.63; P=0.59>0.05). Therefore, the distribution of time in each consuming frequency group was comparable.

Table 10: distribution of respondents regarding the time spent on the way home with respect to consuming frequency

Time	On the way home until the products were put in refrigerator (not in winter)							
	5-7 times per week		1-4 times per week		1-3 times per month		Less than monthly	
	N	%	N	%	N	%	N	%
≤ 2 h	50	87.7%	29	90.6%	21	91.3%	23	79.3%
2-6 h	3	5.3%	1	3.1%	2	8.7%	2	6.9%
> 6 h	4	7.0%	2	6.3%	0	0.0%	4	13.8%
total	57	100.0%	32	100.0%	23	100.0%	29	100.0%

The temperature in this indicator depends on the environmental temperature, which is mainly decided by the season. In the Netherlands, the average maximum temperature is below 10°C from November to the next March, and from May to September, the average maximum temperature is around 20°C (resource: http://www.climatetemp.info/netherlands/ Access date: 18-01-2010). As stated before, when the temperature is below 10°C, death rate is not influenced, the time in this step is therefore too short to have an impact on viability and the level is considered as 0. But when it is not in winter, the time duration < 2h, 2-6h, and > 6h corresponds to level 1, 2 and 3 respectively.

5.2.2.3 Time in the home refrigerator

The respondents spent various days to finish all the probiotic yogurts they bought, and the results are shown in table 11 with respect to their consuming frequency. Most respondents finished the products in 1-4 days or 5-10 days. Those who consumed the products often (5-7 or 1-4 times per week) tended to finish the product in 5-10 days rather than 1-4 days, particularly for who consumed 5-7 times per week. Under Chi-square test, the storage time in home refrigerator is related to consuming frequency (χ^2=29.23; P=0.001<0.05). It was observed that respondents with higher consuming frequency usually bought products for consumption in 5-10 days, perhaps in order to avoid frequent visits to the supermarket, while respondents with lower consuming frequency reported that they just bought a small amount to try the product. The respondents who ate the product less than monthly were more possible to finish the product in longer time, which might be related to their lifestyle.

Table 11: distribution of respondents regarding the time they finish the products stored in home refrigerator

Time	Storage in home refrigerator							
	5-7 times per week		1-4 times per week		1-3 times per month		Less than monthly	
	N	%	N	%	N	%	N	%
1-4 d	17	29.8%	13	40.6%	17	73.9%	16	55.2%
5-10 d	35	61.4%	17	53.1%	5	21.7%	6	20.7%
> 10 d	5	8.8%	2	6.3%	1	4.3%	5	17.2%
Expired	0	0.0%	0	0.0%	0	0.0%	2	6.9%
total	57	100.0%	32	100.0%	23	100.0%	29	100.0%

5.2.2.4 Temperature-time out of refrigerator before consumption

The answers to questions 5-6 on the questionnaire of all consumers are shown in table 12 with respect to their consuming frequency. Most respondents ate the products immediately after taken out of home refrigerator; others kept the product under environmental temperature for hours before eating, but some of them intensely kept the product cold, e.g. in winter. Consuming frequency was correlated to the temperature-time behaviour (χ^2=21.8; P=0.04<0.05), however, one assumption of the Chi-square test was interrupted seriously, for 15 (75.0%) cells have the expected count less than 5. Therefore, the relation might not be meaningful. In this indicator, most consumers are on level 0, which had little impact on probiotic viability.

Table 12: distribution and percentage of each level in the indicator “Out of home refrigerator before consumption”

T-t*	Out of refrigerator before consumption							
	5-7 times per week		1-4 times per week		1-3 times per month		Less than monthly	
	N	%	N	%	N	%	N	%
Immediately	53	93.0%	23	71.9%	14	60.9%	23	82.8%
cold	1	1.8%	3	9.4%	2	8.7%	1	3.4%
< 2 h	1	1.8%	1	3.1%	4	17.4%	1	13.8%
2-6 h	1	1.8%	5	15.6%	3	13.0%	4	0.0%
> 6 h	1	1.8%	0	0.0%	0	0.0%	0	0.0%
total	57	100.0%	32	100.0%	23	100.0%	29	100.0%

*T-t: temperature-time. Immediately: time is 0; cold: temperature is not influential.

5.2.2.5 Level distribution in each indicator

The answers of respondents on temperature-time conditions are translated into levels according to table 8, and the higher the level, the more adverse effect on probiotic viability. The level distribution of all 141 respondents is shown below (table 13). In each indicator, about 90% respondents occupied the level 0 and 1.

Table 13: Distribution of levels in each indicator basing on all 141 respondents

Level	On the way home		In the refrigerator		Out of the refrigerator	
	N	%	N	%	N	%
0	0	0.0%	63	45.4%	120	86.5%
1	123	87.2%	63	44.0%	7	4.3%
2	8	7.1%	13	9.2%	13	8.5%
3	10	5.7%	2	1.4%	1	0.7%
Total	141	100%	141	100%	141	100%

Besides, result basing on 57 frequent consumers (5-7 times per week) are split from the whole (table 14) due to both the commercial concern and health benefit concern. On the one hand, those who consume the products often are the target group in marketing so they are put into the primary concern; on the other hand, the health benefit is achieved when probiotics are consumed on a regular level - several times per week (Tamime and Robinson, 2007). Compared with the result of all respondents, the frequent consumers tended to concentrated in level 1 in the indicator "in the refrigerator", and none of them consumed the product after expiration date; they also performed better when the product is out of the refrigerator since 94.7% of them are on level 0; while on the way home, the level distribution of them is comparable with the whole, though there is a slightly higher proportion of respondents on level 3.

Table 14: Distribution of levels in each indicator basing on frequent consumers (5-7 times per week)

Level	On the way home		In the refrigerator		Out of the refrigerator	
	N	%	N	%	N	%
0	0	0.0%	17	29.8%	54	94.7%
1	50	87.7%	35	61.4%	1	1.8%
2	3	5.3%	5	8.8%	1	1.8%
3	4	7.0%	0	0.0%	1	1.8%
Total	57	100%	57	100%	57	100%

5.2.3 Impact of consumer chains on probiotic viability

Estimated basing on the probiotic death kinetics reviewed in chapter 2, the impacts of each level in each indicator are tabled as below (table 15). Taking into account of the level distribution in each indicator (table 13, 15), on the way home until in fridge, about 90% consumer chains did not influence the probiotic viability assuming that the temperature is around 20°C; in the home refrigerator, about 90% consumer chains caused a decrease in viability of 0.1-1 log cfu mL^{-1} or g^{-1}, while the chains of frequent consumers (5-7 times per week) were more likely to cause decrease 0.5-1 log cfu mL^{-1} or g^{-1}; out of the refrigerator, about 90% consumer chains did not impact the probiotic viability, and the percentage of frequent consumers was even high to nighty-seven.

Inspecting each consumer chain (Appendix 5), 99 out of 141 (70.2%) were on level 0 or 1 in all indicators, causing a decrease in viability of 0.1-0.5 or 0.5-1.0 log cfu mL^{-1} or g^{-1} only due to the storage in home refrigerator, which was inevitable regarding the consumption pattern; hence, these chains were good enough to leave space of improvement. 38 chains/respondents (27.0%) caused an extra loss in viability for they were above level 1 in either three indicators. 4 (2.8%) chains/ respondents had levels 2 or 3 in more than one indicator, causing a larger loss. However, the 4 chains with very unfavourable handling are all created by consumers with a frequency less than once a month. Considering 57 chains created by frequent consumers (5-7 times per week), none of them included unfavourable handling (level 2-3) in more than one indicator. 43 (75.2%) caused a viability loss of 0.1-0.5 or 0.5-1.0 log cfu mL^{-1} or g^{-1}, whereas they are more likely to cause a loss of 0.5-1.0 cfu mL^{-1} or g^{-1} since most of the consumers finish the products in 5-10 days; 7 (12.3%) caused extra loss due to the longer time spent on the way home: 5 (8.8%) due to storage in the refrigerator longer than 10d, and 2 (3.5%) due to the exposure to room temperature for hours before consumption. In summary, 70%-75% consumer chains cause an inevitable viability loss of 0.1-1 log cfu mL^{-1} or g^{-1}; 25-30% consumer chains cause extra loss of 0.2-0.3 log cfu mL^{-1} or g^{-1} or more.

Table 15: estimated impact of different levels on probiotic viability

level	**Estimated impact of consumer handling of probiotic viability**		
	On the way home until in fridge	**In the home refrigerator**	**Out of the home refrigerator**
0	< 10°C. Neglected.	1-4 d. A decrease of 0.1-0.4 log cfu mL^{-1} or g^{-1}.	Consumed immediately, or stored coldly (<10°C). Neglected.
1	< 2 h. Not obvious at 20°C.	5-10 d. A decrease of 0.5-1 log cfu mL^{-1} or g^{-1}.	< 2 h. not obvious at 20°C.
2	2-6 h. A loss up to 0.2-0.3 log cfu mL^{-1} or g^{-1} (at 20°C).	More than 10 d. A decrease more than 1 log cfu mL^{-1} or g^{-1}.	2-6 h. a loss up to 0.2-0.3 log cfu mL^{-1} or g^{-1} (at 20°C).
3	> 6 h. A loss more than 0.2-0.3 log cfu mL^{-1} or g^{-1} (at 20°C).	After expiration date, probiotic viability is not guaranteed.	> 6 h. A loss more than 0.2-0.3 log cfu mL^{-1} or g^{-1} (at 20°C).

5.3 Conclusion and discussion

The storage time of the probiotic yogurt products is long in the supermarket chain, which is 5-6 days to 9-14 days in the distribution center and 3-6 days in the supermarket store. When the products are bought by the consumers, the viability has decreased by 1-2 log cfu mL^{-1} or g^{-1} generally.

70-75% consumer chains lead to a loss of 0.1-1.0 log cfu mL^{-1} or g^{-1} in probiotic viability due to the storage in the home refrigerator, which is hardly avoidable. Chains of frequent consumers are more likely to cause 0.5-1.0 log cfu mL^{-1} or g^{-1}. 25-30% consumer chains cause an extra loss of 0.2-0.3 log cfu mL^{-1} or g^{-1} or more in viability either due to exposing the products for hours under room temperature or due to the long storage in home refrigerator. A few consumer chains, which tend to be of the less than monthly consumers, cause a larger loss in viability due to both exposing the products under room temperature for long and storing them in the refrigerators for long.

Counting the loss of viability in the supermarket chain and consumer chain together, in total it can be 1.5-3.0 log cfu mL^{-1} or g^{-1} at the date of consumption, and be more at the date of expiration. If the products are to meet the threshold (6 log cfu mL^{-1} or g^{-1}) for health benefit, the initial probiotic viability should be 7.5-9.0 log cfu mL^{-1} or g^{-1} or even higher. However, not mentioning that the requirement on initial probiotic viability is not established in all countries, even the established requirement in some countries might not be sufficient to ensure the viability of all products on a beneficial level. For example, National Yogurt Association (NYA) of the United States requires 8 log cfu g^{-1} of lactic acid bacteria at the time of manufacture, as a prerequisite to use the NYA 'Live and Active Culture' logo on the containers of products (Kailasapathy & Rybka, 1997), considering that the loss of viability can be 3.0 log cfu mL^{-1} or g^{-1} or more, the probiotic viability is not enough to realize the heath benefit at consumption. This explains the situation that many products in the market did not meet the requirement on viability to reach 6 log cfu mL^{-1} or g^{-1}, and the hypothesis is confirmed.

Improvements on probiotic viability are needed, either in the supermarket chain, in the consumer chain, tracing back to the production or all of them. The improvements in supermarket chains are more important than in the consumer chains, not only because the loss in viability is larger, but the loss is not as necessary as compared with the loss in consumer chain which is due to the consumer's eating pattern. Improvements in the supermarket chain is considered to be more cost-efficient because a better control on logistics to shorten the time duration in the chain benefits all the stakeholders, including the producers, the supermarket chains and the consumers. The improvements in the production phase can also be helpful when the improvements in the chains are not enough to ensure the viability on a beneficial level. The solutions for improvement are discussed in the next chapter.

Chapter 6. Solutions and implications

According to 5.3, three fields can be improved: in the supermarket chain, in the consumer chain, and in the production phase. The solutions are discussed below, with implications for further research.

6.1 Reduce the storage time in supermarket chain

The time of storage in supermarket chain is unnecessarily long; as time passing by, the viability of probiotics is declining. To reduce the time in the supermarket chain means that products appear fresher in the supermarket store, which improves the probiotic viability when the product is bought and consumed by the consumers.

6.1.1 Reduce stock through integration of supplier and customer

Relative large stock and small demand is the direct reason of long storage time of the products. In the supermarket, for example, it was observed that products were sent to the supermarket when there were still enough old ones on the shelf (as shown in table 4 and Appendix 3). High stock on the shelf of the supermarket store is the direct cause of long storage time on the cabinet. However, controlling the inventory of supermarket store pushes the stock back to the distribution center; instead of staying on the shelf, the products are stored in the distribution center, which does not really solve the problem. Therefore, the supermarket chain, and probably the whole logistic from the manufacturer to the consumer, should be considered as a whole when generating the solutions. Exact prediction on demand lowers down inventory both in supermarket stores and distribution center, and the product quality would be further improved if the producers are involved in the chain to share information on inventory control, so the stock is not pushed back from the supermarket chain to the producer. By sending back the information of the demand of consumers to the producer through the supermarket chain, the supermarket chain facilitates the decision-making in the production plant, thus the products sent to the distribution center can be fresher. Actually, it is a lean logistic system that needs to be created through the collaboration of the supplier and the customer. In this project, the three brands from two companies performed distinguishingly on the logistics. Yakult performed better than Activia and Actimel in general considering the shorter storage time in the supermarket chain. The known differences between these brands are the location of the factory plant and product variety; the Yakult products were produced in the Netherlands and had much fewer varieties while the other two were produced in France and had a lot of varieties. As following, a case study on them might give solutions in detail to improve logistics, lowering the storage time in the chain.

6.1.2 Reduce inventory by increasing demand

Increasing demand is a short term solution to lower down inventory and thus to reduce the storage time in the supermarket chain. A simple way to increase demand is to lower down the price. Since the probiotic yogurt is not considered as a necessity for living, the demand elasticity of probiotic yogurts is high, indicating that a small cut on price might increase the demand largely. However, cutting the price is only a supportive solution of inventory control, because it does not reduce the storage time on a continuous basis. What is more, cutting the price harms the profit of the manufacturer and/or the supermarket chain stakeholder, so it is not a cost-efficient method. In order to support the decisions on price, further studies on the relation between price and demand, the impact on profits, and the impact on the quality image etc. are needed.

6.1.3 Control on supermarket shelf

As observed by the author, the first-in-first-out rule, which was assumed to be obeyed when deducing the duration of the products stored on the shelf, was actually often disrupted by the workers, indicating that the time on the shelf had a larger variation than estimated, so part of the products had even lower quality due to the long storage time on shelf. The control on the supermarket shelf reduces the variety of probiotic viability instead of improving the average viability of all the products. According to the author's working experience in the supermarket as shelf filler, three factors hindered the obedience to the first-in-first-out rule: time pressure, top part of the shelf, and a large amount of old products already on the shelf. The workers are usually required to fill the products in the shelf in a certain time, e.g. 2 hours, so when there is a lot of incoming products, under time pressure they are more likely to disrupt the first-in-first-out rule; when the products are placed on the top of the shelf, which is hard to be reached, the rule is possibly not obeyed. When there are a lot of old products already on the shelf, the workers tend to avoid moving them to put the new ones inside. The time pressure can be solved by hiring more workers, the problem from the top shelf can be solved by providing a ladder, or the manufacturer can consider buying the middle shelf for their probiotic yogurts which also benefits the marketing by presenting the products on the height of the eyes. The problem that a lot of old products stood on the shelf is due that the stock is higher than the demand, which actually reflects the problem in inventory control, and the solutions are traced back to 6.1.1 and 6.1.2.

6.2 Improve handling behaviour of consumers

The target group of improvement is 25-30% of consumers who cause an extra loss of in viability either due to exposing the products for hours under room temperature or due to the long storage in home refrigerator, especially those who spent a long time on the way home until the products are put into the home refrigerator. Before any hasty solutions, the reasons why some consumers spent many hours on the way home, or why they consumed all products slowly, or why they exposed the products under room temperature for hours before consumption should be investigated first, e.g. by interviews. The lifestyle, the lack of relevant knowledge, or the unawareness of the favourable handling might be the reasons, thus, educating consumers with good handling pattern and providing relevant knowledge of the products etc. can be possible solutions. However, generally speaking, improvements in this field might not be very cost-effective. On the one hand, a large portion of consumer chains performed well; on the other hand, the handling of consumers is not easy to be changed. Instead of being fully focused, improvements in consumers can be a supplementary solution.

6.3 Improve product properties by the manufacturer

Inspecting the complete probiotic behaviour model (figure 3 in 2.1.3), efforts can be made on reducing probiotic death rate and improving the initial probiotic viability by the manufacturer.

6.3.1 Reduce probiotic death rate

During the storage, the probiotics are dying on a certain rate. Thus, lowering down the death rate improves probiotic viability given the same storage duration. The factors influencing probiotic death rate was described as the pH and the oxidants (H_2O_2, O_2). The pH, though not directly controllable during storage, is indeed controllable by the producers during manufacture. Take into account of the legislation and sensory, pH should be controlled as 4.5 at the end of production and not too much lower, creating an optimal environment at the starting point of storage. Besides, using the H^+-ATPase-defective mutants of lactic acid bacteria for fermentation was suggested to reduce post-acidification of the product and thus favours probiotic viability (Ongol et al., 2007).

Microencapsulation of probiotics is also reported as an effective way to protect the probiotics from post-acidification during storage (Kailasapathy, 2006; Mortazavian et al., 2008). Other reported protectants to be added in product phase were adding whey protein (Antunes et al., 2005), beta-glucan (Rosburg et al., 2010), and insulin (Desai et al., 2004; Capela et al., 2006), etc. However, impact on sensory quality such as texture was also reported with the studies, which should be taken into account when applied to the industry. Package of the product can be improved to prevent the O_2 emission; glass (Dave and Shah, 1997), and other materials such as Nupak™ and Zero$_2$™ (Talwalkar et al., 2004) are reported to better prevent O_2 than the commonly used high-impact polystyrene. However, since each product has specific property and different strains are used, the producers are recommended to fund the study on their own products. It is recommended that sensory properties should be taken into account when adding ingredients to lower the death rate, they should not alter the sensory properties of the yogurt, because consumers are not willing to compromise the taste and overall eating enjoyment for the health benefit of functional foods, irrespective of its type (Krystallis et al., 2008).

6.3.2 Improve initial probiotic viability

Since the probiotics are dying constantly during the storage, high viability of the probiotics at the end of production can compensate for the death and improve the viability in the end as consumed. The fermentation phase decides whether or not the probiotics can multiply to a high amount, so improvements should aim to facilitate probiotic growth during fermentation. A lot of recommendations with promising results are given, including usage of a high level of inoculum at suitable proportion with yogurt bacteria (Anon, 1994), lowering the incubation temperature to 37°C to favour growth of bifidobacteria with increased incubation time (Kneifel et al., 1993), adding supplements to enhance growth ability (Donkor et al., 2007), adapting the heat treatment method to release more free amino acid to initiate growth (Mortazavian et al., 2006), or even excluding *L. delbrueckii* ssp. *bulgaricus* from the fermentation to eliminate antagonistic effects of substances such as hydrogen peroxide (Damin et al., 2008), etc. Worth mentioning, the viability should be high enough to compensate the loss during the storage until consumption. Therefore, it is necessary for the producers to evaluate the loss of viability basing on their own products. Especially, the estimation should be done by imitating the temperature-time conditions of the real life scenario, rather than under the well-controlled lab environment. The methods to enumerate viable cells in the yogurt to test viability are shown in appendix 6.

Chapter 7. Assessment

This project carried out an initial research to describe the scenario from the supermarket chain to the consumer, which was neglected previously. The tests done before were mostly under an ideal temperature condition, which is often interrupted in real life to different extents. In order that the consumers benefit from the product, the possible loss in viability under real life condition should be understood, where the temperature and time are not always favourable for probiotic viability. Though estimated in Chapter 5 on a general level, strictly speaking, the loss of viability is strain specific and product specific. Hence, the manufacturer should assess the probiotic viability of their own products, particularly under the imitated temperature-time conditions of the real life rather than under the standard 4°C. Subsequent solutions can be taken out such as improve logistics, lower down the death rate during logistics, or improve the initial viability at the end of production, etc.

This research was conducted in the Netherlands, wherein the Dutch supermarket chain and the Dutch consumers were chosen, so the scenario in the Netherlands does not necessarily represent the scenario in other countries, where the temperature-time conditions created and the characteristics of both the supermarket chains and the consumers can be very different. For example, the cold chain in the developing countries is still not developed adequately, featured as lacking of adequate cold chain application and infrastructure, and the overall knowledge of proper cold chain practices; erratic (or even lack) of power supply in many regions of the DW, especially in regions with warm climate or during the warm season is a serious problem (Yahia, 2010). Moreover, the area of the country might also influence the logistics since in a country with broad area 2-3 days or more can be spent on the way of transportation. When it comes to the consumer chains, the temperature condition created was actually depending on the weather and the climate. The Netherlands has a mild climate, in summer the maximum average temperature is about 20°C, which can be ten degrees higher in other countries and thus a higher risk for probiotic viability. The time condition created in the consumer chain might be different as well. For example, time spent on the way home can be related to the size of the city and the traffic condition, citizens in large cities with busy traffic might spend more time on the way. Therefore, it is worthwhile to draw the scenario of a different place before assessing the possible effect on probiotic viability, especially the scenario in the countries with undeveloped logistic system, warm climate, but large market potential.

In addition to the implication for the parallel researches to draw the scenarios in different countries or specific areas, other researches on such as the impact of the scenario and the solutions in detail are also needed. For example, in the countries with a warm climate, the impact on probiotic viability can be severe (in summer) by just exposing the product outside for a short time; after generating the general solutions such as creating a lean logistic system or change the consumer behaviour etc., following studies can be on the factors influencing the effectiveness of the logistic system, understanding the relation between the characteristics of certain consumer and their handling behaviour, and reasons of behavioural change, etc.

References

Allgeyer, L.C., Miller, M.J., Lee, S.-Y. 2010. Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics. *Journal of Dairy Science*. 93(10): 4471-4479.

Anon. 1994. Method of analysis for identification of *L. acidophilus* and *Bifidobacteria* (Amedium for differentiating between *L. acidophilus*, *Bifidobacteria* and *S. thermophilus* in ABT products). Chr. Hansen's Laboratorium. Copenhagen, Denmark.

Antunes, A.E.C., Cazetto, T.F., Abolini, H.M. 2005. Viability of probiotic micro-organisms during storage, postacidification and sensory analysis of fat-free yogurts with added whey protein concentrate. *International Journal of Dairy Technology*. 58(3): 169-173.

Arroyo, L., Cotton, L. N., and Martin, J. H. 1994. Evaluation of media for enumeration of *Bifidobacterium adolescentis*, *B. infantis*and *B. longum* from pure culture. *Cultured Dairy Production Journal*. 29:2–24.

Axelsson, L. 1998. Lactic acid bacteria: classification and physiology. In: *Lactic acid bacteria – Microbiology and functional aspects*. Edited: S. Salminen, A. von Wright. New York, Marcel Dekker. 1-72.

Beausoleil, M., Fortier, N., Guénette, S., L'Ecuyer, A., Savoie, M., Franco, M., Lachaîne, J., Weiss, K. 2007. Effect of a fermented milk combining *Lactobacillus acidophilus* CL1285 and *Lactobacillus casei* in the prevention of antibiotic-associated diarrhea: A randomized, double-blind, placebo-controlled trial. *Canadian Journal of Gastroenterology*. 21 (11): 732-736.

Beniwal, R.S., Arena, V.C., Thomas, L., Narla, S., Imperiale, T.F., Chaudhry, R.A., Ahmad, U.A. 2003. A Randomized Trial of Yogurt for Prevention of Antibiotic-Associated Diarrhea. *Digestive Diseases and Sciences*. 48(10): 2077-2082.

Blandon J, Cranfield J, Henson S. 2007. Functional Food and Natural Health Product Issues: The Canadian and World Context. In FERG Working Paper No. 18. Guelph, Ontario, Kanada.

Boutron, M.-C., Faivre, J., Marteau, P., Couillault, C., Senesse, P., Quipourt, V. 1996. Calcium, phosphorus, vitamin D, dairy products and colorectal carcinogenesis: A French case-control study. *British Journal of Cancer*. 74 (1): 145-151.

Capela, P., Hay, T. K. C., & Shah, N. P. 2006. Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yogurt and freezedried yogurt. *Food Research International*. 39: 203–211.

Champagne, C. P., Gardner, N., Brochu, E., & Beaulieu, Y. 1991. The freeze-drying of lactic acid bacteria: A review. *Canadian Institute Food Science and Technology Journal*. 24: 118–128.

Champagne, C. P., Roy, D. and Lafond, A. 1997. Selective enumeration of *Lactobacillus casei* in yogurt-type fermented milks based on a 15°C incubation temperature. Biotechnology Techniques. 11:567–569.

Collins, E. B. 1978. Enumeration of *Lactobacillus acidophilus* with the agar plate count. *Journal of Food Protection*. 41:439–442.

Cogan, T.M., Beresford, T.P., Steele, J., Broadbent, J., Shah, N.P., Ustunol, Z. 2007. Invited review: Advances in starter cultures and cultured foods. *Journal of Dairy Science*. 90(9): 4005-4021.

Condon, S. 1987. Responses of lactic acid bacteria to oxygen. *FEMS Microbiology Reviews*. 46: 269–281.

Damin, M.R., Minowa, E., AlcÂntara, M.R., & Oliveira, M.N. 2008. Effect of cold storage on culture viability and some rheological properties of fermented milk prepared with yogurt and probiotic bacteria. *Journal of Texture Studies*. 39 (1): 40-55.

Dave, R.I., & Shah, N.P. 1996. Evaluation of Media for Selective enumeration of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus*, and Bifidobacteria. *Journal of Dairy Science*. 79: 1524-1536.

Dave, R.I., & Shah, N.P. 1997. Viability of Yogurt and Probiotic Bacteria in yogurt made from commercial cultures. *International Dairy Journal*. 7: 31-41.

de Vries, W., Stouthamer, A.H. 1969. Factors determining the degree of anaerobiosis of Bifidobacterium strains. *Archives of Microbiology*. 65:275–87.

de Jong, N., Ocké, M.C., Branderhorst, H.A.C., Friele, R. 2003. Demographic and lifestyle characteristics of functional food consumers and dietary supplement users. *British Journal of Nutrition*. 89(2): 273-281.

Desai, A. R., Powell, I. B., & Shah, N. P. 2004. Survival and activity of probiotic lactobacilli in skim milk containing prebiotics. *Journal of Food Science*. 69: FM57–FM60.

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 yogurt during cold storage. *International Dairy Journal*. 17(6): 657-665.

del Campo, R., Bravo, D., Cantón, R., Ruiz-Garbajosa, P., García-Albiach, R., Montesi-Libois, A., Yuste, F.-J., Abraira, V., Baquero, F. 2005. Scarce evidence of yogurt lactic acid bacteria in human feces after daily yogurt consumption by healthy volunteers. *Applied and Environmental Microbiology*. 71 (1): 547-549.

EU. 2006. Regulation (EC) 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. *Official Journal of the European Union*. L404/9, Article 13.

FAO/WHO. 2001. Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Amerian Córdoba Park Hotel, Córdoba, Argentina.

Farber, S., Páez, A., Mercado, R.G., Roorda, M., Morency, C. 2010. A time-use investigation of shopping participation in three Canadian cities: is there evidence of social exclusion? *Transportation*. 1-28.

Foschino, R., Fiori, E., a Galli, A. 1996. Survival and residual activity of *Lactobacillus acidophilus* frozen cultures under different conditions. *Journal of Dairy Research*. 63: 295–303.

Foster, R., and Lunn, J. 2007. 40th Anniversary Briefing Paper: Food availability and our changing diet. British Nutrition Foundation *Nutrition Bulletin*. 32, 187–249.

Greger, J.L. 2001. Dietary supplement use: consumer characteristics and interests. *Journal of Nutrition*. 131: 1339S–1343S.

Gilliland, S. E. 1989. Acidophilus milk products: a review of potential benefits to consumers. *Journal of Dairy Science*. 72: 2483–2494.

Gilliland, S. E., & Lara, R. C. 1988. Influence of storage at freezing and subsequent refrigerator temperature on β-galactosidase activity of *Lactobacillus acidophilus*. *Applied Environmental Microbiology*. 45(4): 898–902.

Gilliland, S.E., & Speck, L.M. 1974. Frozen concentrated cultures of lactic acid bacteria - A review. *Journal of Milk Food Technology*. 37: 107- 111.

Granato, D., Branco, G.F., Cruz, A.G., Faria, J.D.A.F., & Shah, N.P. 2010. Probiotic dairy products as functional foods. *Comprehensive Reviews in Food Science and Food Safety*. 9 (5): 455-470.

Grunert, K.G. 2010. European consumers' acceptance of functional foods. *Annals of the New York Academy of Sciences*. 1190: 166-173.

Guyonnet, D., Chassany, O., Ducrotte, P., Picard, C., Mouret, M., Mercier, C.-H., Matuchansky, C. 2007. Effect of a fermented milk containing Bifidobacterium animalis DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: A multicentre, randomized, double-blind, controlled trial. *Alimentary Pharmacology and Therapeutics*. 26 (3): 475-486.

Heller, K.J. 2001. Probiotic bacteria in fermented foods: Product characteristics and starter organisms. *American Journal of Clinical Nutrition*. 73(2 suppl.): 374S-379S

Hilliam, M. 2000. Functional food—How big is the market? *The World of Food Ingredients*. 12: 50–52.

Ho, S.H., Rosario, L., Rahman, M.M. 2010. Numerical simulation of temperature and velocity in a refrigerated warehouse. *International Journal of Refrigeration*. 33(5): 1015-1025.

Holm, F. 2003. Gut health and diet: the benefits of probiotic and probiotics on human health. *The world of ingredients*. 2: 52-55.

Hull, R. R., and Roberts, A. V. 1984. Differential enumeration of *Lactobacillus acidophilus* in yogurt. *Australian Journal of Dairy Technology*. 39:160–163.

Hunger, W. 1986. Aesculin-cellobiose agar for the isolation and counting of *Lactobacillus acidophilus*. *Milchwissenschaft*. 41:283–285.

Hughes, D.B., Hoover, D.G. 1995. Viability and Enzymatic Activity of Bifidobacteria in Milk. *Journal of Dairy Science*. 78:268-276.

Ishibashi N, Shimamura S. 1993. Bifidobacteria: Research and development in Japan. *Food Technology*. 47(6): 129–34.

Iwana, H., Masuda, H., Fujisawa, H., & Mitsuoka, T. 1993. Isolation AND identification of *Bifidobacterium* spp. in commercial yogurts in Europe. *Bifidobacteria Microflora*. 12: 39-45.

James, C., James, S. 2007. Out of your control - Retailing and domestic handling. *Food Science and Technology*. 21(1): 32-34.

Jayamanne, V.S. & Adams, M.R. 2006. Determination of survival, identity and stress resistance of probiotic bifidobacteria in bio-yogurts. *Letters in Applied Microbiology*. 42(3): 189-194.

Kailasapathy, K. (2006). Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yogurt. *LWT Food Science and Technology*. 39: 1221–1227.

Kailasapathy, K., a Rybka, S. 1997. *L. acidophilus* and *Bifidobacterium* spp. - their therapeutic potential and survival in yogurt. *The Australian Journal of Dairy Technology*. 52: 28–35.

Kneifel, W., and Pacher, B. 1993. An X-Glu based agar medium for the selective enumeration of *L. acidophilus* in yogurt related milk products. *International Dairy Journal*. 3:277–291.

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

Korbekandi, H., Jahadi, M., Maracy, M., Abedi, D., Jalali, M. 2009. Production and evaluation of a probiotic yogurt using *Lactobacillus casei* ssp. *Casei*. *International Journal of Dairy Technology*. 62(1): 75-79.

Krishnakumar, V. & Gordon, IR. 2001. Probiotics: challenges and opportunities. Dairy industries international. 66(2): 38-40.

Krystallis, A., Maglaras, G., and Mamalis, S. 2008. Motivations and cognitive structures of consumers in their purchasing of functional foods. *Food Quality and Preference*. 19, 525–538.

Kociubinski, G. and Salminen, S. 2006. Probiotics: Basis, state of the art and future perspectives. *Functional food network general meeting* Turku, Finland, March 8–10, 2006.

Landström, E., Koivisto Hursti, U.-K., Becker, W., Magnusson, M. 2007. Use of functional foods among Swedish consumers is related to health-consciousness and perceived effect. *British Journal of Nutrition*. 98(5): 1058-1069.

Lankaputhra, W.E.V., Shah, N. P. and Britz, M.L. 1996a. Evaluation of media for selective enumeration of *Lactobacillus acidophilus* and *Bifidobacterium* spp. *Food Australia*. 48:113–118.

Lankaputhra, W.E.V., Shah, N.P., Britz, M.L. 1996b. Survival of bifidobacteria during refrigerated storage in the presence of acid and hydrogen peroxide. *Milchwissenschaft*. 51: 65-70.

Laroia, S., and Martin, J. H. 1991. Methods for enumerating and propagating bifidobacteria. *Cultured Dairy Production Journal*. 26:32–33.

Lin, W.-H., Hwang, C.-F., Chen, L.-W., Tsen, H.-Y. 2006. Viable counts, characteristic evaluation for commercial lactic acid bacteria products . *Food Microbiology*. 23 (1): 74-81.

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

Lourens-Hattingh, A., Viljoen, B.C. 2002. Survival of probiotic bacteria in South African commercial bio-yogurt. *South African Journal of Science*. 98 (5-6): 298-300.

Luning, P. A., & Marcelis, W. J. 2006. A techno-managerial approach in food quality management research. *Trends in Food Science & Technology*. 17: 378-385.

Luning, P.A. & Marcelis, W.J. 2007. A conceptual model of food quality management functions based on a techno-managerial approach. *Trends in Food Science & Technology*. 18: 159-166

Luning, P.A. & Marcelis, W.J. 2009, *Food Quality Management, technological and managerial principles and practices*. Wageningen: Wageningen Academic Publishers.

Luning, P.A., Marcelis, W.J., & Jongen, W.M.F. 2002. Food quality management: a techno-managerial approach. Wageningen: Wageningen Academic Publishers.

Marklinder, I.M., Lindblad, M., Eriksson, L.M., Finnson, A.M., Lindqvist, R. 2004. Home storage temperatures and consumer handling of refrigerated foods in Sweden. *Journal of Food Protection*. 67(11): 2570-2577.

Masco, L. ; Vanhoutte, T. ; Temmerman, R. ; Swings, J. ; Huys, G. 2007. Evaluation of real-time PCR targeting the 16S rRNA and recA genes for the enumeration of bifidobacteria in probiotic products. *International Journal of Food Microbiology*. 113(3): 351-357.

Mattila-Sandholm, T., Myllärinen, P., Crittenden, R., Mogensen, G., Fondén, R., Saarela, M. 2002. Technological challenges for future Probiotic foods. *International Dairy Journal*. 12 (2-3): 173-182.

Medina, L.M., Jordano, R., 1995. Population dynamics of constitutive microbiota in BAT type fermented milk products. *Journal of Food Protection*. 58: 70–76.

Micanel, N., Haynes, I.N., & Playne, M.J. 1997. Viability of probiotic cultures in commercial Australian yogurts. *International Dairy Journal*. 7 (1): 31-41.

Miller, C.W., Nguyen, M.H., Rooney ,M. 2002. The influence of packaging materials on the dissolved oxygen content of probiotic yogurt. *Packaging Technology and Science*. 15(3): 133-138.

Monnet, C., Béal, C., & Corrieu, G. 2003. Improvement of the resistance of *Lactobacillus delbrueckii* ssp. *bulgaricus* to freezing by natural selection. *Journal of Dairy Science*. 86: 3048-3053.

Mortazavian, A.M., Ehsani, M.R., Azizi, A., Razavi, S.H., Mousavi, S.M., Sohrabvandi, S., Reinheimer, J.A. 2008. Viability of calcium-alginate microencapsulated probiotic bacteria in Iranian yogurt drink (Doogh) during refrigerated storage and under simulated gastrointestinal conditions. *Australian Journal of Dairy Technology*. 63 (1): 25-30.

Mortazavian, A.M., Ehsani, M.R., Mousavi, S.M., Sohrabvandi, S., Reinheimer, J.V. 2006. Combined effects of temperature-related variables on the viability of probiotic micro-organisms in yogurt. *Australian Journal of Dairy Technology*. 61(3): 248-252.

Mortazavian, A.M., Ehsani, M.R., Sohrabvandi, S., Reinheimer, J.A. 2007. MRS-bile agar: Its suitability for the enumeration of mixed probiotic cultures in cultured dairy products. *Milchwissenschaft*. 62(3): 270-272.

Munoa, F. J., and Pares, R. 1988. Selective medium for isolation and enumeration of *Bifidobacterium* spp. *Applied Environmental Microbiology*. 54:1715–1718.

Nissen, H. and Dainty, R. 1995. Comparison of the use of rRNA probes and conventional methods in identifying strains of *Lactobacillus sake* and *Lactobacillus curratus* isolated from meat. *International Journal of Food Microbiology*. 25. 311-315.

Olsen, J.E., Aabo, S., Hill, W., Notcrmans, S., Wcrnars, K., Granum, P.E., Popobic, T., Rasmussen, H.N. and Olsvik, ϕ. 1995. Probes and polymerasc chain reaction for the detcctlon of food-borne bacterial pathogcnh. . *International Journal of Food Microbiology*. 22. I-78.

Ongol, M.P., Sawatari, Y., Ebina, Y., Sone, T., Tanaka, M., Tomita, F., Yokota, A., Asano, K. 2007. Yogurt fermented by *Lactobacillus delbrueckii* subsp. *bulgaricus* H+-ATPase-defective mutants exhibits enhanced viability of *Bifidobacterium breve* during storage. *International Journal of Food Microbiology*. 116(3): 358-366.

Ouwehand, A. 2007. Success in applying pro- and prebiotics in dairy products. *Proceedings of the fourth international FFNet meeting on functional foods.* Budapest, Hungary, March 26–27.

Playne, M.J., Bennett, L.E., Smithers, G.W. 2003. Functional dairy foods and ingredients. Australian Journal of Dairy Technology. 60 (2 SPEC. ISS.): 154-156.

Radimer, K.L., Subar, A.F. & Thompson, F.E. 2000. Non-vitamin, non-mineral dietary supplements: issues and findings from NHANES III. *Journal of the American Dietetic Association.* 100: 447–454.

Ramchandran, L. & Shah, N.P. 2010. Characterization of functional, biochemical and textural properties of symbiotic low-fat yogurts during refrigerated storage. *Food Science and Technology*. 43: 819–827

Rasic, J. L. 1983. The role of dairy foods containing bifido and acidophilus bacteria in nutrition and health? *North European Dairy Journal*. 4: 1–5.

Ravula, R.R., Shah, N.P. 1998. Selective enumeration of *Lactobacillus casei* from yogurts and fermented milk drinks. *Biotechnology Techniques*. 12(11): 819–822.

Rosburg, V., Boylston, T., White, P. 2010. Viability of bifidobacteria strains in yogurt with added oat beta-glucan and corn starch during cold storage. *Journal of Food Science*. 75(5): C439-C444.

Ruiz-Garcia, L., Barreiro, P., Robla, J.I., Lunadei, L. 2010. Testing zigBee motes for monitoring refrigerated vegetable transportation under real conditions. *Sensors*. 10(5): 4968-4982.

Rybka, S.,& Kailasapathy, K. 1995. The survival of culture bacteria in fresh and freeze-dried AB yogurts. The Australian Journal of Dairy Technology, 50(2): 51–57.

Salminen, S., Bouley, M.C., Boutron-Rualt, M.C., et al. 1998. Functional food science and gastro-intestinal physiology and function. *Britain Journal of Nutrition*. 80: 147–71.

Sanders, M.E. 1999. Probiotics. *Food technology*. 53: 67-77.

Scharl, M., Geisel, S., Vavricka, S.R., Rogler, G. 2010. Dying in Yogurt: The Number of Living Bacteria in Probiotic Yogurt Decreases under Exposure to Room Temperature. *Digestion*. 83(1-2): 13-17.

Shimamura, S., Abe, F., Ishibashi, N., Miyakawa, H., Yaeshima, T,A,, Tomita, M. 1992. Relationship between oxygen sensitivity and oxygen metabolism of *Bifidobacterium* species. *Journal of Dairy Science*. 75(12): 296–306.

Shah, N.P. 2000. Probiotic bacteria: Selective enumeration and survival in dairy foods. *Journal of Dairy Science*. 83(4): 894-907.

Shah, N.P. 2006. Probiotics and fermented milks. In: *Manufacturing Yogurt and Fermented Milks*. Edited by: Chandan, R.C. Blackwell Publishing Professional: USA.

Shah, N., Jellen, P., 1990. Survival of lactic acid bacteria and their lactases under acidic conditions. *Journal of Food Science*. 55 (2), 506–509.

Shah, N.P., Lankaputhra, W.E.V., Britz, M.L., & Kyle, W.S.A. 1995. Survival of Lactobacillus acidophilus and Bifidobacterium bifidum in commercial yogurt during refrigerated storage. *International Dairy Journal*. 5 (5): 515-521.

Shah, N.P., Ravula, R.R. 2000. Influence of water activity on fermentation, organic acids production and viability of yogurt and probiotic bacteria. *Australian Journal of Dairy Technology*. 55 (3): 127-131.

Shin, S.Y., Park, J.H. 1997. Activities of oxidative enzymes related with oxygen tolerance in *Bifidobacterium* spp. *Journal of Microbiology and Biotechnology*. 7(5):356-359 .

Siuta-Cruce, P., Goulet, J. 2001. Improving probiotic survival rates. *Food Technology*. 55(10): 36.

Succi, M., Tremonte, P., Reale, A., Sorrentino, E., Coppola, R. 2007. Preservation by freezing of potentially probiotic strains of *Lactobacillus rhamnosus*. *Annals of Microbiology*. 57 (4): 537-544.

Tahir, A., Bansal, P.K. 2005. MEPR versus EEPR valves in open supermarket refrigerated display cabinets. *Applied Thermal Engineering*. 25: 191-203.

Talwalkar, A., Kailasapathy, K. 2004. Comparison of selective and differential media for the accurate enumeration of strains of Lactobacillus acidophilus, Bifidobacterium spp. and Lactobacillus casei complex from commercial yogurts. *International Dairy Journal*. 14(2): 143-149.

Talwalkar, A., Miller, C.W., Kailasapathy, K., Nguyen, M.H.2004. Effect of packaging materials and dissolved oxygen on the survival of probiotic bacteria in yogurt. *International Journal of Food Science and Technology*. 39(6): 605-611.

Tamime, A.Y. and Robinson, R.K. 2007. Tamime and Robinson's Yogurt: Science and technology (Third edition). England: Woodhead Publishing.

Tharmaraj, N., Shah, N.P. 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): 2288-2296.

Terpstra, M.J., Steenbekkers, L.P.A., De Maertelaere, N.C.M., Nijhuis, S. 2005. Food storage and disposal: Consumer practices and knowledge. *British Food Journal*. 107(7): 526-533.

Uesugi I, Yajima M. 1978. Oxygen and “strictly anaerobic” intestinal bacteria. 2. Oxygen metabolism in strictly anaerobic bacteria. *Zeit Allg Mikrobiol*. 18(8): 593-601.

van De Water, J., Keen, C.L., Gershwin, M.E. 1999. The influence of chronic yogurt consumption on immunity. *Journal of Nutrition.* 129 (7): 1492S-1495S.

van Donselaar, K.H., Gaur, V., van Woensel, T., Broekmeulen, R.A.C.M., Fransoo, J.C. 2010. Ordering Behaviour in Retail Stores and Implications for Automated Replenishment. *Management Science*. 56 (5): 766-784.

van Donselaar, K., van Woensel, T., Broekmeulen, R., Fransoo, J. 2006. Inventory control of perishables in supermarkets. *International Journal of Production Economics*. 104(2): 462-472.

van Kleef, E., van Trijp, H.C.M., Luning, P. 2005. Consumer research in the early stages of new product development: A critical review of methods and techniques. *Food Quality and Preference*. 16 (3): 181-201.

Varga, L. 2006. Effect of acacia (*Robinia pseudo-acacia* L.) honey on the characteristic microflora of yogurt during refrigerated storage. International Journal of Food Microbiology. 108(2): 272-275.

Varga, L., Szigeti, J., Kovács, R., Földes, T., Buti, S. 2002. Influence of a Spirulina platensis biomass on the microflora of fermented ABT milks during storage (R1). *Journal of Dairy Science*. 85(5): 1031-1038.

Vinderola, C.G., Bailo, N., Reinheimer, J.A. 2000. Survival of probiotic microflora in Argentinian yogurts during refrigerated storage. *Food Research International*. 33 (2): 97-102.

Vinderola, G., Prosello, W., Molinari, F., Ghiberto, D., Reinheimer, J. 2009. Growth of Lactobacillus paracasei A13 in Argentinian probiotic cheese and its impact on the characteristics of the product. *International Journal of Food Microbiology*. 135(2): 171-174.

Warton, M.A., Wills, R.B.H. 2002. Survey of storage conditions and quality of minimally processed packaged lettuce in supermarkets. *Food Australia*. 54(5): 191-192.

Weinbreck, F., Bodnár, I., Marco, M.L. 2010. Can encapsulation lengthen the shelf-life of probiotic bacteria in dry products? *International Journal of Food Microbiology*. 136(3): 364-367.

Wolters, G.W., Steenbekkers, L.P.A. 2006. The scenario method to gain insight into user actions. In: *User Behavior and Technology Development: Shaping Sustainable Relations Between Consumers and Technologies*. Editors: Peter-Paul Verbeek, Adriaan Slob. Dordrecht : Springer.

Yahia, E.M. 2010. Cold chain development and challenges in the developing world. *Acta Horticulturae*. 877: 127-132.

Yin, R. 1994. *Case Study Research*. Sage Publications. Beverly Hills, CA.

Ying, D.Y., Phoon, M.C., Sanguansri, L., Weerakkody, R., Burgar, I., Augustin, M.A. 2010. Microencapsulated *Lactobacillus rhamnosus* GG Powders: Relationship of Powder Physical Properties to Probiotic Survival during Storage. *Journal of Food Science*. 75 (9): E588-E595.

Zubillaga, M., Weill, R., Postaire, E., Goldman, C., Caro, R., Boccio, J. 2001. Effect of probiotics and functional foods and their use in different diseases. *Nutrition Research*. 21 (3): 569-579.

Appendix 1 Functional yogurt manufacture's performance

(source: Euromonitor International, http://www.portal.euromonitor.com/Portal/ResultsList.aspx, access date: 01-12-2010)

The two figures shows Danone's leading position in functional spoonable yogurt and Yakult and Danone's leading position in functional drinking yogurt, respectively. Functional spoonable yogurt products of Danone are named under the brand Activia, which often occupies a place on shelf in supermarkets, especially in western Europe. Functional drinking yogurt products are generally Actimel from Danone and Yakult from Yakult Honsha, which is also common in the market.

Appendix 2 Inflow of probiotic yogurt products on different days

Date	Brand	Shelf life	Expiration date	Days in distribution center
Dec. 9. 2010	Yakult	30 d	Jan. 1. 2011	6 d
	Activia	32 d	Dec. 25. 2010	14 d
			Dec. 28. 2010	11 d
			Jan. 6. 2011	2 d
Dec. 11. 2010	Activia	32 d	Dec. 30. 2010	11 d
			Jan. 1. 2011	9 d
			Jan. 2. 2011	8 d
Dec. 13. 2010	Yakult	30 d	Jan. 6. 2011	5 d
Dec. 14. 2010	Yakult	30 d	Jan. 7. 2011	5 d
Dec. 16. 2010	Activia	32 d	Dec. 30. 2010	16 d
			Jan. 1. 2011	14 d
			Jan. 6. 2011	9 d
	Yakult	30 d	Jan. 9. 2011	5 d
			Jan. 8. 2011	6 d
	Actimel	32 d	Jan. 5. 2011	10 d
			Jan. 7. 2011	8 d
Dec. 21. 2010	Activia	32 d	Jan. 8. 2011	12 d
			Jan. 5. 2011	15 d
	Yakult	30 d	Jan. 15. 2011	4 d
			Jan. 11. 2011	8 d
Dec. 22. 2010	Actimel	32 d	Jan. 12. 2011	9 d
			Jan. 11. 2011	10 d
			Jan. 9. 2011	12 d
			Jan. 8. 2011	13 d
Dec. 23. 2010	Yakult	30 d	Jan. 15. 2011	6 d
Dec. 30. 2010	Activia	32 d	Jan. 18. 2011	11 d
	Yakult	30 d	Jan. 23. 2011	5 d
Jan. 4. 2011	Yakult	30 d	Jan. 28. 2011	5 d
	Actimel	32 d	Jan. 21. 2011	13 d
			Jan. 20. 2011	12 d
			Jan. 26. 2011	8 d
			Jan. 24. 2011	10 d
			Jan. 23. 2011	11 d
	Activia	32 d	Jan. 20. 2011	14 d
			Jan. 18. 2011	16 d
			Jan. 25. 2011	9 d
Jan. 6. 2011	Actimel	32 d	Jan. 27. 2011	9 d
			Jan. 31. 2011	5 d
			Jan. 23. 2011	13 d
	Activia	32 d	Jan. 23. 2011	13 d
			Jan. 21. 2011	15 d
			Jan. 18. 2011	17 d
			Jan. 31. 2011	5 d
			Feb. 1. 2011	4 d
	Yakult	30 d	Jan. 29. 2011	6 d

Appendix 3 Inflow and outflow of probiotic yogurt products of 14 days

Product 1, Activia	At the begin of day	At the end of day	Sale	incoming
25.11.2010	11	8	3	0
26.11.2010	8	11	3	6
27.11.2010	11	6	5	0
28.11.2010	6	5	1	0
29.11.2010	5	7	4	6
30.11.2010	7	7	0	0
01.12.2010	7	5	2	0
02.12.2010	5	5	6	6
03.12.2010	5	8	3	6
04.12.2010	8	11	3	6
05.12.2010	11	16	7	12
06.12.2010	16	14	2	0
07.12.2010	14	5	9	0
08.12.2010	5	14	3	12

Product 2, Activia	At the begin of day	At the end of day	Sale	incoming
25.11.2010	20	16	4	0
26.11.2010	16	12	10	6
27.11.2010	12	5	7	0
28.11.2010	5	11	0	6
29.11.2010	11	15	2	6
30.11.2010	15	19	2	6
01.12.2010	19	18	1	0
02.12.2010	18	15	3	0
03.12.2010	15	13	2	0
04.12.2010	13	18	1	6
05.12.2010	18	24	0	0
06.12.2010	24	17	7	0
07.12.2010	17	23	6	12
08.12.2010	23	19	16	12

Product 3, Activia	At the begin of day	At the end of day	Sale	incoming
25.11.2010	11	10	1	0
26.11.2010	10	5	11	6
27.11.2010	5	10	7	12
28.11.2010	10	15	1	6
29.11.2010	15	13	2	0
30.11.2010	13	8	5	0
01.12.2010	8	8	6	6
02.12.2010	8	13	1	6
03.12.2010	13	8	5	0
04.12.2010	8	14	6	12
05.12.2010	14	48	2	36
06.12.2010	48	47	13	12
07.12.2010	47	48	11	12
08.12.2010	48	47	7	0

Product 4, Activia	At the begin of day	At the end of day	Sale	incoming
25.11.2010	10	5	5	0
26.11.2010	5	4	7	6
27.11.2010	4	5	5	6
28.11.2010	5	7	4	6
29.11.2010	7	7	0	0
30.11.2010	7	6	1	0
01.12.2010	6	10	2	6
02.12.2010	10	6	4	0
03.12.2010	6	10	2	6
04.12.2010	10	6	4	0
05.12.2010	6	10	2	6
06.12.2010	10	6	4	0
07.12.2010	6	12	0	6
08.12.2010	12	7	5	0

Product 5, Activia	At the begin of day	At the end of day	Sale	incoming
25.11.2010	9	7	2	0
26.11.2010	7	13	0	6
27.11.2010	13	9	4	0
28.11.2010	9	8	1	0
29.11.2010	8	7	1	0
30.11.2010	7	6	1	0
01.12.2010	6	11	1	6
02.12.2010	11	6	5	0
03.12.2010	6	5	1	0
04.12.2010	5	3	2	0
05.12.2010	3	1	2	0
06.12.2010	1	6	1	6
07.12.2010	6	9	3	6
08.12.2010	9	5	4	0

Product 6, Activia	At the begin of day	At the end of day	Sale	incoming
25.11.2010	6	9	3	6
26.11.2010	9	9	0	0
27.11.2010	9	14	1	6
28.11.2010	14	12	2	0
29.11.2010	12	12	0	0
30.11.2010	12	10	2	0
01.12.2010	10	6	4	0
02.12.2010	6	9	3	6
03.12.2010	9	6	3	0
04.12.2010	6	11	1	6
05.12.2010	11	17	0	6
06.12.2010	17	17	6	6
07.12.2010	17	20	3	6
08.12.2010	20	17	3	0

Product 7, Activia	At the begin of day	At the end of day	Sale	incoming
25.11.2010	9	7	2	0
26.11.2010	7	1	6	0
27.11.2010	1	3	4	6

28.11.2010	3	9	0	6
29.11.2010	9	8	1	0
30.11.2010	8	7	1	0
01.12.2010	7	4	3	0
02.12.2010	4	10	6	0
03.12.2010	10	6	4	0
04.12.2010	6	9	3	6
05.12.2010	9	57	0	48
06.12.2010	57	57	12	12
07.12.2010	57	54	3	0
08.12.2010	54	47	7	0

Product 1, Actimel	**At the begin of day**	**At the end of day**	**Sale**	**incoming**
25.11.2010	15	13	8	6
26.11.2010	12	22	3	12
27.11.2010	22	10	12	0
28.11.2010	10	7	3	0
29.11.2010	7	6	1	0
30.11.2010	6	5	1	0
01.12.2010	5	4	1	0
02.12.2010	4	3	1	0
03.12.2010	3	5	4	6
04.12.2010	5	3	2	0
05.12.2010	3	3	0	0
06.12.2010	3	3	0	0
07.12.2010	3	3	0	0
08.12.2010	3	3	0	0

Product 2, Actimel	**At the begin of day**	**At the end of day**	**Sale**	**incoming**
25.11.2010	6	3	3	0
26.11.2010	3	1	5	6
27.11.2010	1	3	0	2
28.11.2010	3	4	1	2
29.11.2010	4	6	0	2
30.11.2010	6	8	0	2
01.12.2010	8	6	2	0
02.12.2010	6	8	2	4
03.12.2010	8	4	6	2
04.12.2010	4	4	0	0
05.12.2010	4	4	0	0
06.12.2010	4	4	0	0
07.12.2010	4	4	0	0
08.12.2010	-	-	-	-

Product 3, Actimel	**At the begin of day**	**At the end of day**	**Sale**	**incoming**
25.11.2010	69	67	2	0
26.11.2010	67	64	3	0
27.11.2010	64	57	7	0
28.11.2010	57	50	7	0
29.11.2010	50	47	3	0
30.11.2010	47	46	1	0
01.12.2010	46	44	2	0
02.12.2010	44	39	5	0
03.12.2010	39	36	3	0
04.12.2010	36	30	3	0

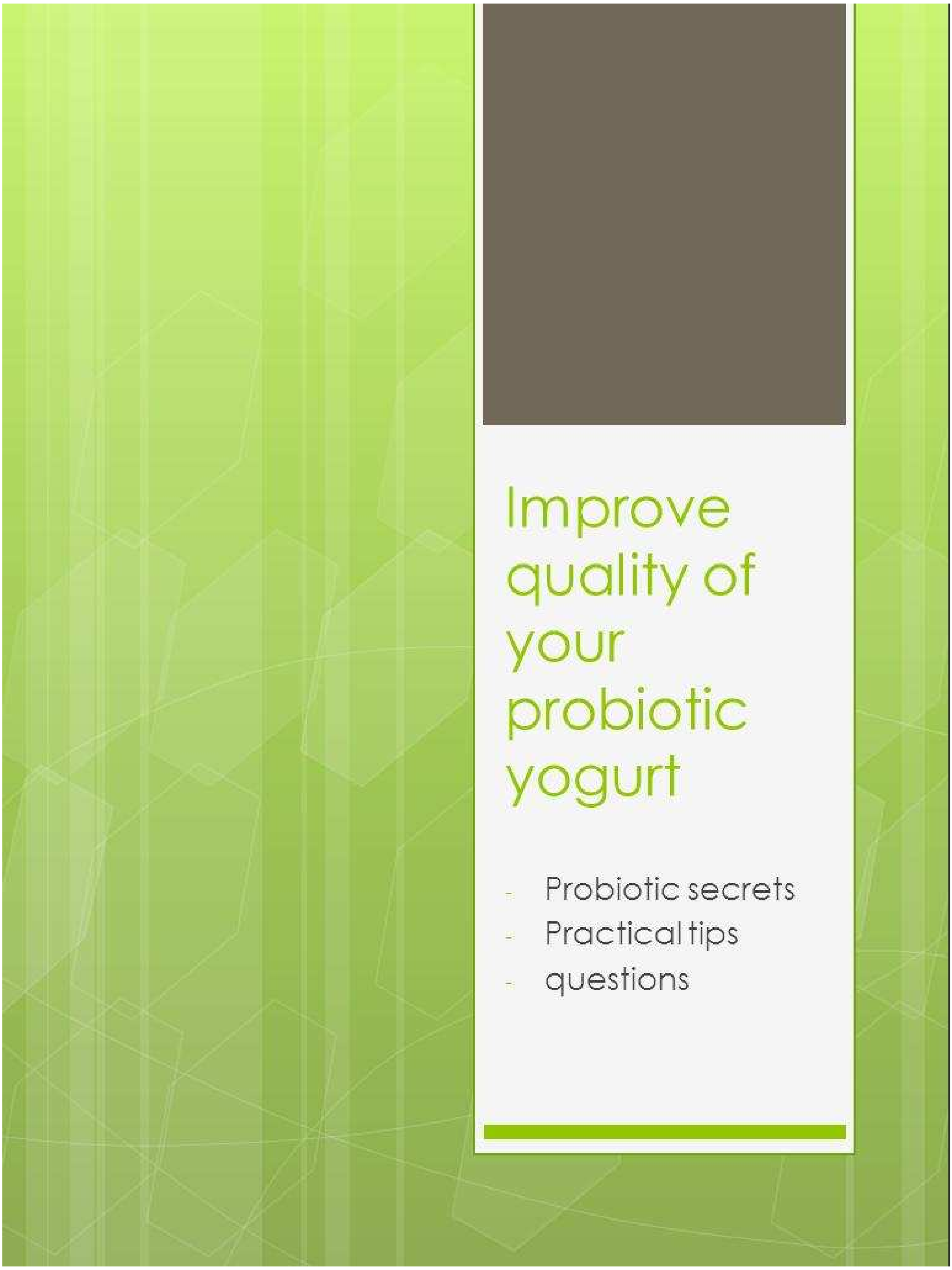
05.12.2010	30	28	6	0
06.12.2010	28	23	2	0
07.12.2010	23	19	5	0
08.12.2010	19	15	4	0

Product 1, Yakult	**At the begin of day**	**At the end of day**	**Sale**	**incoming**
25.11.2010	20	24	6	10
26.11.2010	24	24	10	10
27.11.2010	24	16	8	0
28.11.2010	16	21	5	10
29.11.2010	24	14	7	0
30.11.2010	14	21	3	10
01.12.2010	21	17	4	0
02.12.2010	17	23	4	10
03.12.2010	23	23	10	10
04.12.2010	23	12	11	0
05.12.2010	12	21	1	10
06.12.2010	21	15	6	0
07.12.2010	15	22	3	10
08.12.2010	22	13	9	0

Product 2, Yakult	**At the begin of day**	**At the end of day**	**Sale**	**incoming**
25.11.2010	15	16	9	10
26.11.2010	16	14	12	10
27.11.2010	14	18	6	10
28.11.2010	18	26	2	10
29.11.2010	26	20	6	0
30.11.2010	20	27	3	10
01.12.2010	27	18	9	10
02.12.2010	18	19	9	10
03.12.2010	19	17	12	10
04.12.2010	17	19	8	10
05.12.2010	19	27	2	10
06.12.2010	27	20	7	0
07.12.2010	20	23	7	10
08.12.2010	23	20	3	0

Product 3, Yakult	**At the begin of day**	**At the end of day**	**Sale**	**incoming**
25.11.2010	6	4	2	0
26.11.2010	4	7	1	4
27.11.2010	7	6	5	4
28.11.2010	6	10	0	4
29.11.2010	10	10	0	0
30.11.2010	10	9	1	0
01.12.2010	9	8	1	0
02.12.2010	8	8	0	0
03.12.2010	8	4	4	0
04.12.2010	4	8	4	8
05.12.2010	8	12	0	4
06.12.2010	12	9	3	0
07.12.2010	9	7	2	0
08.12.2010	7	4	3	0

Appendix 4 A sample questionnaire distributed to consumers in supermarkets



Questions

1. How often do you consume probiotic yogurt?
   - 5-7 times per week
   - 1-4 times per week
   - 1-3 times per month
   - less once a month
2. Within how many days do you finish all the probiotic yogurts bought once?
   - 1-4 days
   - 5-10 days
   - More than 10 days, but finished before expiration date
   - Consume after expiration date
3. How long does it take from shopping to finally the product are put in home refrigerator?
   - Less than 2 hours
   - 2-6 hours
   - More than 6 hours
4. Which transportation do you usually use for shopping?
   - Car or public transportation
   - Bike
   - On foot
5. Do you eat or drink the product immediately after taken out of home refrigerator?
   - Yes – no need to answer question 6
   - No, I would take it to work place or school, etc. – go to question 6
   - No, I would take it to work place or school, but I intensely keep it under a low temperature comparable to the refrigerator – go to question 6
6. If not consumed immediately, how long does it take before consumption?
   - Less than 2 hours
   - 2-6 hours
   - Longer than 6 hours
7. Age: ☐ 19-34; ☐ 35-49; ☐ 50-64; ☐ 65 or over
8. Gender: ☐ male; ☐ female

Do you know?
True or false?

		I don't know	True	False
1.	Probiotics are microorganisms that benefit your health.	❑	❑	❑
2.	All kinds of yogurt have probiotics inside.	❑	❑	❑
3.	Probiotics should be alive to benefit your health.	❑	❑	❑
4.	Probiotic amount is constant in yogurt if you keep it in refrigerator.	❑	❑	❑
5.	Keeping yogurt out of refrigerator enhances probiotic growth.	❑	❑	❑
6.	The fresher the yogurt, the more live probiotics are inside.	❑	❑	❑
7.	Generally, probiotic yogurt should be consumed often to achieve health benefit.	❑	❑	❑

Secrets of probiotics

- Probiotics are living microorganisms that have various health benefits.
- They are often added to yogurt for additional health effect, so normal yogurt do not includes probiotics inside.
- Only when live cells of probiotics reach a certain level (≥ 1 million per gram or milliliter) in yogurt are they able benefit your health.
- They need to be consumed regularly in order to achieve health effect, preferably more than 3-4 times per week.
- They die gradually in yogurt over time even if you store it in your refrigerator (so the fresher the better).
- They die quickly if your yogurt is not stored under low temperature.

Tips to improve your probiotic yogurt quality

- ✓ Consume up earlier before expiration date, the fresher the more probiotics are still alive, so the quality is better.
- ✓ Put your probiotic yogurt in refrigerator immediately after you arrive at home.
- ✓ Avoid leaving your probiotic yogurt under room temperature or even higher temperature, particularly for a long time. They die quickly.
- ✓ Eat or drink it immediately after taken out of the refrigerator.
- ✓ Consume preferably daily or 4-5 times per week to achieve health effect in your body.

Questions for me?

- Thank you for answering the questionnaire.
- This survey is part of a MSc thesis project from Wageningen University.
- The "secrets" part is based on knowledge from scientific literature, and the practical tips are generated accordingly by the author of this survey.
- If you are interested in this topic and have more questions, feel free to contact me: jingyu.peng@wur.nl
- If you are interested in the result of the survey, feel free to send a requisition to the same email. The result will be available in Mar. 2011.

Appendix 5 Answers of 141 respondents on the questionnaire

Translation of some words in the table

Frequency: consuming frequency; **5-7**: 5-7 times per week; **1-4**: 1-4 times per week; **1-3**: 1-3 times per month; **< 1**: less than monthly;

Days: within how many days the respondent finished one batch of product

Home: time spend from shopping until the products were put in home refrigerator

Transport: which transportation the respondent usually use for shopping

Out fridge: within how long the product are consume when took out of the home refrigerator;

S1, 2, ... 7: statement 1, 2, ... 7; **t**: true; **f**: false; **d**: don't know

No.	frequency	days	home	transport	Out fridge	age	gender	s1	s2	s3	s4	s5	s6	s7
1	5-7	5-10	< 2	car or...	> 6	> 65	female	t	t	t	t	d	t	d
2	5-7	1-4	< 2	bike	cold	50-64	female	d	d	t	d	d	d	d
3	5-7	1-4	< 2	car or...	immediately	35-49	female	t	d	t	t	d	t	t
4	1-3	> 10	< 2	bike	< 2	19-34	female	t	t	t	f	f	d	t
5	1-3	1-4	< 2	bike	< 2	19-34	female	t	t	t	d	d	d	t
6	1-4	1-4	< 2	car or...	2-6	50-64	male	t	f	t	t	t	t	t
7	5-7	1-4	2-6	car or...	immediately	50-64	female	t	t	t	t	t	t	d
8	1-4	5-10	< 2	foot	immediately	50-64	female	t	f	d	t	d	d	t
9	5-7	5-10	< 2	bike	immediately	35-49	female	t	d	t	d	d	d	t
10	5-7	5-10	< 2	foot	immediately	> 65	female	t	f	t	d	d	t	t
11	5-7	5-10	< 2	bike	immediately	> 65	female	t	f	t	f	t	t	t
12	5-7	1-4	< 2	bike	immediately	50-64	female	t	d	d	d	d	d	d
13	5-7	1-4	2-6	foot	immediately	> 65	female	t	d	t	d	d	t	t
14	5-7	1-4	2-6	foot	immediately	> 65	male	t	d	t	t	d	t	t
15	5-7	5-10	< 2	foot	immediately	50-64	male	t	t	t	t	f	t	t
16	< 1	5-10	< 2	car or...	immediately	50-64	male	t	f	f	d	d	d	f
17	< 1	> 10	> 6	bike	immediately	50-64	male	d	d	d	d	d	d	d
18	< 1	expired	> 6	car or...	2-6	> 65	male	d	d	t	t	t	d	t
19	< 1	5-10	< 2	car or...	immediately	19-34	male	t	d	t	f	d	t	f
20	< 1	5-10	< 2	bike	immediately	19-34	female	t	f	t	t	f	d	d
21	< 1	expired	< 2	car or...	cold	> 65	female	d	d	d	d	d	d	d
22	1-3	5-10	2-6	car or...	immediately	> 65	female	t	d	d	d	d	d	t
23	1-3	1-4	< 2	bike	immediately	35-49	female	t	f	t	f	t	t	t
24	1-4	5-10	< 2	bike	immediately	19-34	male	t	f	t	f	f	d	t
25	1-3	1-4	2-6	bike	immediately	19-34	male	t	t	t	t	f	t	t
26	1-4	5-10	< 2	car or...	immediately	50-64	male	t	d	d	d	d	d	t
27	1-4	1-4	< 2	car or...	immediately	50-64	male	t	f	t	t	t	t	d
28	1-4	> 10	< 2	car or...	immediately	35-49	female	t	t	d	d	d	t	t
29	1-4	5-10	< 2	bike	immediately	> 65	female	d	t	t	f	f	f	d
30	1-4	1-4	< 2	car or...	immediately	> 65	male	t	d	t	t	d	d	d
31	1-4	1-4	< 2	bike	immediately	> 65	female	t	f	d	d	f	d	t
32	1-4	> 10	< 2	car or...	2-6	50-64	female	d	f	d	d	d	d	d
33	1-4	5-10	< 2	car or...	immediately	35-49	female	t	f	t	d	d	d	t

34	1-4	1-4	2-6	car or...	immediately	> 65	female							
35	1-4	5-10	< 2	car or...	immediately	50-64	female	t	f	t	d	t	t	t
36	1-4	1-4	< 2	foot	2-6	50-64	female	d	t	d	t	f	d	d
37	1-4	5-10	< 2	bike	cold	50-64	female	t	f	f	t	f	f	t
38	1-4	5-10	< 2	car or...	immediately	50-64	male	t	f	d	d	d	d	t
39	1-4	5-10	< 2	foot	immediately	50-64	female	t	f	d	d	d	t	t
40	1-4	5-10	< 2	car or...	immediately	50-64	female	t	d	d	d	d	d	d
41	1-4	5-10	< 2	bike	immediately	35-49	female	t	f	t	d	d	d	t
42	1-4	5-10	> 6	foot	cold	> 65	female	t	t	d	d	d	d	d
43	1-4	1-4	< 2	car or...	< 2	19-34	male	t	f	t	t	d	t	t
44	1-4	1-4	< 2	car or...	immediately	> 65	male	t	d	d	d	d	d	d
45	1-4	5-10	< 2	bike	immediately	> 65	female	t	d	d	t	f	d	t
46	1-4	1-4	< 2	car or...	immediately	50-64	female	t	d	d	d	d	d	d
47	1-4	5-10	< 2	car or...	cold	19-34	female	t	t	t	t	f	t	t
48	1-4	1-4	< 2	bike	2-6	50-64	female	t	f	f	t	d	t	t
49	5-7	1-4	< 2	foot	immediately	> 65	female	d	t	t	t	t	f	t
50	5-7	5-10	< 2	bike	immediately	50-64	female	t	d	d	t	f	t	t
51	5-7	5-10	< 2	bike	immediately	19-34	female	t	f	d	d	d	d	t
52	5-7	5-10	< 2	car or...	immediately	19-34	female	t	t	d	f	f	f	t
53	5-7	> 10	< 2	bike	immediately	50-64	female	t	d	t	d	d	d	t
54	5-7	5-10	< 2	bike	immediately	19-34	female	d	f	t	f	d	d	d
55	5-7	5-10	< 2	bike	immediately	< 19	female							
56	5-7	5-10	< 2	car or...	immediately	50-64	female							
57	5-7	5-10	< 2	car or...	immediately	50-64	female	t	f	f	f	t	f	f
58	5-7	1-4	< 2	car or...	immediately	19-34	female	t	f	d	f	d	t	t
59	5-7	5-10	< 2	bike	immediately	< 19	male	t	f	t	t	t	t	t
60	5-7	5-10	< 2	foot	immediately	35-49	female	t	f	t	t	d	t	t
61	5-7	5-10	< 2	bike	immediately	50-64	female	t	d	t	t	d	t	t
62	5-7	5-10	< 2	car or...	immediately	> 65	female	t	f	d	f	d	d	t
63	5-7	5-10	< 2	car or...	immediately	> 65	female	t	d	t	d	f	d	t
64	5-7	5-10	< 2	car or...	immediately	19-34	male	t	t	t	d	t	d	t
65	5-7	1-4	< 2	car or...	immediately	50-64	female	t	t	t	d	d	t	t
66	5-7	5-10	< 2	bike	immediately	50-64	female	t	f	t	t	d	d	t
67	5-7	5-10	< 2	car or...	immediately	50-64	female	t	f	d	d	d	d	t
68	5-7	5-10	< 2	car or...	immediately	50-64	male	t	f	t	f	d	t	t
69	5-7	5-10	< 2	foot	immediately	> 65	female	t	f	t	d	f	d	t
70	5-7	5-10	< 2	car or...	immediately	> 65	female	t	t	d	d	t	f	t
71	5-7	5-10	< 2	bike	immediately	19-34	female	d	d	f	f	d	t	t
72	5-7	5-10	< 2	car or...	immediately	50-64	female	t	f	t	t	d	t	t
73	5-7	5-10	> 6	bike	immediately	50-64	female	d	d	d	d	d	d	d
74	5-7	> 10	< 2	car or...	immediately	35-49	male	t	f	t	f	d	t	t
75	5-7	1-4	< 2	foot	immediately	> 65	female	d	d	d	d	d	d	d
76	5-7	1-4	> 6	bike	immediately	> 65	female	d	d	d	d	d	d	d
77	5-7	5-10	< 2	bike	immediately	> 65	female	t	t	d	d	d	d	d
78	5-7	5-10	< 2	car or...	< 2	50-64	female	t	d	d	d	d	d	d
79	5-7	5-10	< 2	car or...	immediately	50-64	female	t	d	t	t	f	t	t

80	5-7	1-4	< 2	car or...	2-6	50-64	male	t	f	d	t	t	f	t
81	5-7	5-10	< 2	bike	immediately	50-64	female	t	f	t	t	f	f	f
82	5-7	1-4	< 2	car or...	immediately	50-64	female	t	f	d	f	d	t	t
83	5-7	5-10	< 2	car or...	immediately	> 65	male	t	f	t	d	d	t	t
84	5-7	1-4	< 2	car or...	immediately	50-64	female	d	t	d	d	d	d	t
85	5-7	> 10	< 2	car or...	immediately	35-49	female	d	f	d	d	d	d	f
86	5-7	5-10	< 2	car or...	immediately	> 65	male	t	t	t	t	d	t	d
87	5-7	5-10	< 2	bike	immediately	> 65	male	t	d	d	t	d	d	t
88	5-7	5-10	> 6	foot	immediately	> 65	male	d	f	f	d	d	d	d
89	1-3	5-10	< 2	bike	immediately	19-34	female	t	t	t	d	d	f	t
90	1-3	1-4	< 2	bike	immediately	19-34	female	t	t	f	f	t	f	f
91	5-7	1-4	> 6	foot	immediately	> 65	female	d	d	d	d	d	d	d
92	1-3	5-10	< 2	bike	immediately	19-34	male	t	d	t	d	f	d	t
93	< 1	1-4	< 2	bike	immediately	19-34	male	t	f	t	t	d	t	f
94	1-3	5-10	< 2	bike	immediately	19-34	female	d	t	t	f	d	f	d
95	< 1	1-4	< 2	bike	immediately	50-64	male	d	f	f	d	d	f	f
96	1-3	1-4	< 2	bike	immediately	19-34	female	t	f	t	f	d	d	t
97	< 1	1-4	< 2	bike	2-6	19-34	female	t	d	d	d	d	d	d
98	< 1	> 10	< 2	bike	2-6	19-34	female	t	f	t	d	d	d	d
99	1-3	1-4	< 2	bike	< 2	19-34	male	t	d	t	t	f	t	f
100	1-3	1-4	< 2	bike	immediately	19-34	male	t	f	t	t	f	t	t
101	1-3	1-4	< 2	foot	immediately	19-34	female	t	f	t	t	f	t	t
102	1-3	1-4	< 2	bike	< 2	19-34	female	t	f	t	d	t	f	t
103	5-7	1-4	< 2	car or...	immediately	19-34	female	t	f	t	d	d	d	t
104	1-3	1-4	< 2	bike	cold	19-34	male	t	t	d	t	f	t	d
105	1-3	1-4	< 2	bike	2-6	19-34	male	t	t	t	d	d	d	d
106	1-4	5-10	< 2	car or...	immediately	19-34	female	d	d	d	d	d	d	d
107	1-4	1-4	< 2	bike	2-6	19-34	female	t	d	f	t	f	t	t
108	1-4	5-10	> 6	bike	immediately	19-34	female	t	t	t	d	f	t	t
109	1-4	1-4	< 2	car or...	immediately	19-34	female	t	f	d	d	t	d	t
110	< 1	1-4	< 2	bike	immediately	19-34	female	t	d	t	d	d	d	d
111	< 1	1-4	< 2	foot	immediately	19-34	female	t	t	d	f	t	d	t
112	< 1	1-4	< 2	bike	immediately	19-34	female	t	t	t	d	d	t	t
113	< 1	1-4	< 2	bike	immediately	19-34	female	t	f	t	f	t	d	d
114	5-7	> 10	< 2	car or...	immediately	50-64	female	d	d	t	d	d	d	t
115	1-3	1-4	< 2	bike	immediately	19-34	male	t	t	d	t	d	t	t
116	< 1	1-4	2-6	car or...	immediately	19-34	female	t	t	d	d	d	t	t
117	< 1	> 10	< 2	foot	immediately	19-34	male	t	f	t	d	d	d	f
118	1-4	5-10	< 2	bike	immediately	19-34	female	t	f	t	t	f	t	t
119	< 1	> 10	< 2	car or...	immediately	19-34	male	t	f	f	t	f	f	t
120	5-7	5-10	< 2	car or...	immediately	35-49	female	t	f	t	d	d	t	d
121	< 1	> 10	< 2	car or...	immediately	< 19	female	t	t	d	t	f	f	f
122	1-4	1-4	< 2	bike	immediately	19-34	female	t	f	t	f	t	t	f
123	1-3	1-4	< 2	bike	2-6	19-34	male	t	d	t	t	t	d	t
124	1-3	1-4	< 2	foot	immediately	19-34	female	d	d	d	d	d	d	d
125	< 1	5-10	< 2	bike	immediately	19-34	female	t	f	t	f	t	f	t

126	< 1	1-4	< 2	bike	immediately	19-34	female	f	t	t	f	f	d	f
127	< 1	1-4	< 2	car or...	immediately	19-34	female	t	t	t	d	d	d	t
128	1-3	1-4	< 2	bike	immediately	19-34	male	t	d	t	d	t	t	t
129	< 1	1-4	< 2	bike	2-6	35-49	male	t	d	t	d	d	t	t
130	< 1	5-10	< 2	car or...	< 2	19-34	female	t	f	t	t	t	f	d
131	< 1	1-4	< 2	car or...	immediately	50-64	male	t	t	t	t	d	t	t
132	< 1	1-4	< 2	car or...	immediately	35-49	male	t	f	f	d	t	t	t
133	< 1	5-10	< 2	bike	immediately	35-49	male	f	f	f	f	t	f	f
134	1-3	1-4	< 2	bike	cold	19-34	female	t	f	t	f	d	d	t
135	< 1	1-4	> 6	bike	immediately	19-34	male	d	d	d	d	d	d	d
136	< 1	1-4	2-6	car or...	immediately	19-34	female							
137	1-3	1-4	< 2	bike	2-6	19-34	female	t	d	f	t	f	t	d
138	< 1	1-4	> 6	bike	immediately	50-64	female	t	d	d	t	d	d	t
139	5-7	> 10	< 2	car or...	immediately	50-64	male	t	f	t	t	d	t	t
140	1-3	5-10	< 2	foot	immediately	19-34	female	d	t	d	d	d	d	d
141	5-7	1-4	< 2	car or...	immediately	> 65	male	t	d	t	d	f	d	t

Appendix 6 Enumeration Methods of Probiotics

Enumeration of probiotics means counting the viable cells of selected probiotic bacterium in order to determine probiotic viability, which is usually presented as cfu·mL^{-1} or cfu·g^{-1}. 'cfu' is the abbreviation of 'colony forming unit' and only the viable cell is able to form a colony; 'mL' is used when the yogurt is more liquid, and 'g' is used when the yogurt is more solid. Finding a suitable way to enumerate the target probiotic from a mixture of probiotics and yogurt bacteria is the prerequisite to judge the quality of probiotic yogurt. The traditional and the newly developed methods are reviewed and compared below. Advices are given to choose the suitable enumeration methods.

Traditional Methods

The traditional method for selective enumeration is to culture the organism on selective medium under certain environment. The composition of the medium and the environmental conditions are made favourable to support growth of the target organism and inhibit growth of the unwanted organisms. Because probiotics are added as supplements to the yogurt bacteria *L. delbrueckii* ssp. *Bulgaricus* and *Streptococcus thermophilus*, usually there is a mix of cultures in the commercial products, i.e. AY (*L. acidophilus* and yogurt bacteria), CY (*L. casei* and yogurt bacteria), BY (bifidobacteria and yogurt bacteria), ABY (*L. acidophilus*, bifidobacteria and yogurt bacteria), ACY (*L. acidophilus*, *L. casei* and yogurt bacteria) and BCY (bifidobacteria, *L. casei* and yogurt bacteria).

NNLP (nalidixic acid, neomycin sulfate, lithium chloride, paromomycin sulfate agar) is usually used as a medium for the enumeration of pure cultures of *Bifidobacterium* spp. (Munoa and Pares, 1988; Laroia and Martin, 1991; Arroyo et al., 1994; Shah, 2000), but with the presence of AY, they are not selective enough. Several media have been suggested to enumerate *L. acidophilus* since long ago, Such as bile medium (Collins, 1978), Rogosa agar, deMan Rogosa Sharpe (MRS) medium containing maltose, raffinose or melibiose in place of dextrose (Hull and Roberts, 1984), cellobiose-esculin agar (Hunger, 1986), agar medium based on X-Glu (Kneifel and Pacher, 1993) and MRS-maltose agar (Lankaputhra et al., 1996a). However, some are not able to enumerate *L. acidophilus* with the presence of Bifidobacteria. Concerning the different sugar utilization characteristics among probiotics and yogurt bacteria, a certain probiotic can be selectively cultured by using specific sugar in the medium that is only capable to be used by this probiotic. Besides the strategy of sugar utilization, antibiotics can also be used to inhibit the growth of unwanted species. For example, vancomycine is found to be suitable for enumeration of *L. rhamnosus*, and also *L. casei* if the former organism is not present. Though Champagne et al. (1997) suggested that incubation at 15°C for 14 days can enumerate *L. casei*, it is not practical considering the long incubation time.

When it is difficult to enumerate a single species at one time, the subtraction method can be applied as the redemption. The principle is to use one culture medium to enumerate two cultures, using another medium to enumerate one of the two cultures, so the number of another culture is obtained when using the former to subtract the latter. Take the mixture of *L. rhamnosus*, and *L. casei* as an example, the counts of *L. rhamnosus* on MRS-vancomycine agar under anaerobic incubation at 43°C for 72 h could be gained, revising the temperature to 37°C, the total counts of *L. casei* and *L. rhamnosus* on MRS-vancomycine agar at 37°C for 72 h under anaerobic incubation is gained, therefore subtraction of the *L. rhamnosus* count from the total count is the *L. casei* count (Tharmaraj and Shah, 2003). Mortazavian et al. (2007) also reported that the number of bifidobacteria in ABY culture composition was well determined by the subtraction method.

With the development of the traditional method, the frequently used culture media is shown in table 3, and the composition of the media is shown in the annex in the end. It is shown that generally 72h is needed for the incubation, which is a disadvantage especially when it is to be applied to the commercial industry. Besides, the accuracy and reliability of traditional method is suspected by some researchers, because it is based on the characteristics of microbial morphology, physiology and biochemistry, which only reflect that portion of the genome expressed under a particular set of cultivation conditions. Talwalkar and Kailasapathy (2004) evaluated various media for their suitability to provide reliable counts of Lactobacillus acidophilus, Bifidobacterium spp. and L. casei from nine different commercial probiotic yogurts. Selective media reported to develop only single type of colonies, demonstrated two types of colonies with some yogurts, making it difficult to conclusively enumerate the probiotic colonies. Counts of each probiotic strain from the same yogurt sample also varied on the different media. Except for LC agar, no selective or differential medium provided reliable counts of probiotic bacteria in all yogurts. This study demonstrates the urgent need to develop reliable techniques to accurately enumerate probiotic bacteria in commercial yogurts.

Selective media and incubation conditions for enumeration of probiotics and yogurt bacteria from a mixture of them (Tharmaraj & Shah, 2003; Dave & Shah, 1996).

Bacterial group	Agar medium	Incubation conditions
L. acidophilus	MRS-agar	anaerobic, 43°C, 72 h
	BA-maltose agar	anaerobic, 43°C, 72 h
	BA-sorbitol agar	anaerobic, 37°C, 72 h
L. rhamnosus	MRS-vancomycine agar	anaerobic, 43°C, 72 h
L. casei	MRS-vancomycine agar	anaerobic, 37°C, 72 h, when *L.. rhamnosus* was not present; otherwise a subtraction method can be used
	LC agar	anaerobic, 27°C, 72 h, when *L.. rhamnosus* was not present.
	MRS-NaCl (4%)	anaerobic, 37°C, 72 h
	MRS-LiCl (0.5%)	anaerobic incubation at 37 degrees C for 72 h
Bifidobacteria	MRS-NNLP agar	anaerobic incubation at 37 degrees C for 72 h
S. thermophilus	S. thermophilus agar	aerobic, 37°C, 24 h
L. delbrueckii ssp. bulgaricus	MRS agar (pH 4.58)	anaerobic, 45°C, 72 h
		if L.. rhamnosus was present in a product., it is easily distinguished by the morphology
	MRS agar (pH 5.20)	anaerobic, 45°C, 72 h
		if L.. rhamnosus and L.. acidophilus were not present in a product.
	reinforced clostridial agar at pH 5.3	45°C for 72 h

Molecular identification

The development of molecular method in recently decades demonstrate a possible innovation in enumeration of probiotics. Unlike the conventional method, molecular biological identification is based on the constitutive composition of nucleic acids rather than on the products of their expression and is therefore considered more reliable for identification purposes (Nissen and Dainty, 1995). As early to more than a decade ago, 16s and 23s rRNA probe hybridisation had become widely adopted for detection of specific bacterial groups in mixed populations, especially foodborne bacterial pathogens (Olsen et al., 1995). Recently the application of molecular methods in enumeration of probiotic cells in probiotic yogurt were studied in several articles. Masco et al. (2007) evaluated the usage of real-time PCR to target the multicopy 16S rRNA gene and the single copy recA gene for the enumeration of bifidobacteria in 29 probiotic products claimed to contain these organisms. Both assays relied on the use of genus-specific primers and the nonspecific SYBR Green I chemistry. For both applications, the calibration curve was constructed using the type strain of

Bifidobacterium animalis subsp*. lactis*. Both assays generally produced comparable enumeration results, they are proved to be rapid and reproducible alternatives for culture-based detection and quantification of bifidobacteria in probiotic products. Meng et al. (2010) evaluated the usage of direct quantitative EMA real-time PCR assay based on molecular beacon to detect viable bifidobacteria in probiotic yogurt, and concluded that it is a rapid and quantitative method. Lahtinen et al. (2005) compared four methods to enumerate probiotic bifidobacteria in a fermented food product, Strains of *Bifidobacterium longum* and *B. lactis* were quantified by plate counts, fluorescent in situ hybridization (FISH), quantitative real-time PCR and commercial LIVE/DEAD® *Bac*Light™ bacterial viability kit, and the methods were further developed to suit the enumeration of bifidobacteria in fermented foods. The choice of enumeration method for probiotic bacteria may have significant effect on the results of the analysis. The strain-specific properties and the objects of the analysis should be taken into account when enumeration methods for different probiotic strains are chosen.

Conclusion

Molecular identification is much faster than the traditional one, which is a valued property in the commercial field. From the logistics view, it is inefficient and uneconomic to keep the products in the factory for three days or more; meanwhile, the probiotic viability kept on losing during the days of test. Besides saving the time, the molecular method is more sensitive than the traditional one when the viable cells is in low content. However, this might cause inconsistency when comparing the results generated from both methods, so if one wants to compare or verify viability of the a product that tested before, a change in enumeration method might be confusing. In conclusion, the selection on enumeration method should be based on the aim, time, and cost-efficiency.

Annex

Agar compound Method (Sources: Tharmaraj & Shah, 2003; Dave & Shah, 1996; Ravula & Shah, 1998).

Bacteriological peptone andwater diluent Bacteriological peptone and water diluent (0.15%) were prepared by dissolving 1.5 g of bacteriological peptone (Oxoid (Australia) Pty. Ltd., West Heidleberg, Australia) in 1 L of distilled water. The pH was adjusted to 7.0 ± 0.2, followed by autoclaving 9ml aliquots at 121°C for 15 min.

***Streptococcus thermophilus* (ST) agar** The ingredients of S. thermophilus agar (10.0 g of tryptone, 1.0.0 g of sucrose, 5.0 g of yeast extract, and 2.0 g KzHP04) were dissolved in 1 L of distilled water. The pH was adjusted to 6.8 ± 0.1, and 6 ml of 0.5% bromocresol purple and 12 g of agar were added to the medium, The medium was sterilized at 121°C for 15 min.

MRS agar (deMan Rogosa Sharpe agar) Rehydrated MRS broth (Oxoid) was prepared according to the manufacturer instructions. The pH of the broth was adjusted to 5.20 and 4.58 using 1.0 M HCl to obtain the pH-modified agar. Two and five grams of pure bile salts (Amyl Media, Dandenong Australia)/L were added to obtain 0.2% and 0.5% MRS-bile agar.

pH-modified (pH 5.20, 4.58) MRS agar Forty grams of NaCl/L was added for MRS-NaCl agar (4% final concentration) and 5 g/L lithium chloride (LiCl) was added for MRS-LiCl agar (0.5% final concentration).

MRS-vancomycine agar To prepare MRS-vancomycine (MRS-V) agar, 2 ml of 0.05 g vancomycine (Sigma Chemical Co., Castle Hill, Australia)/ 100 ml solution was added to 1 L of MRS broth to obtain 1 mg/L final concentration. Agar powder was added to each broth at the rate of 1.2% and the media were autoclaved at 121°C for 15 min. Inoculated plates in duplicates were incubated anaerobically at 37°C and 43°C for 72 h.

MRS-bile (0.2% and 0.5%) agar To prepare MRS-bile agar, 2.0 g of pure bile salts (Amyl Media, Dandenong, Australia)/L of MRS broth were added; for MRS-oxgall agar, 10.0 g of oxgall powder

(Oxoid)/L of MRS broth were added. Twenty grams of NaCl/L (2% final concentration) of MRS broth were dissolved to obtain MRS-NaC1 agar. After the broth was prepared, agar powder was added at the rate of 1.2%, and the media were autoclaved at 121°C for 15 min.

MRS-NaCl agar, Twenty grams of NaCl/L (2% final concentration) of MRS broth were dissolved to obtain MRS-NaC1 agar. After the broth was prepared, agar powder was added at the rate of 1.2%, and the media were autoclaved at 121°C for 15 min.

MRS-NNLP agar The MRS-NNLP (nalidixic acid, neomycin sulfate, lithium chloride, and paromomycin sulfate (all from Sigma Chemical Co., Castle Hill, Australia) agar was prepared according to the method described by Laroia and Martin (1991).The basal medium was MRS agar. Filter-sterilised NNLP was added to the autoclaved MRS basal medium just before pouring. Filter-sterilised L-cysteine(HCl (0.05% final concentration) was also added. Plates in duplicate were incubated anaerobically at 37°C for 72 h.

LC agar The ingredients of LC agar (10.0 g bacteriological peptone; 1.0 g yeast extract; 4.0 g Lab Lemco (Oxoid); 2.0 g KH_2PO_4; 3.0 g sodium acetate; 1.0 g tri-ammonium citrate; 0.2 g $MgSO_4$; 0.05 g $MnSO_4$ (BDH Chemicals Pty. Ltd., Kilsyth, Australia); 1.0 g acid casein hydrolysate; 1.0 g tween 80 (Sigma Chemicals Co. St. Louis, USA)) were dissolved in 1 L of distilled water. The pH was adjusted to 5.1±0.1, and 6 ml of bromocresol green and 12 g of bacteriological agar (Amyl Media, Dandenong, Australia) were added to the medium. The medium was sterilised at 121°C for 15 min. Ten millilitre of membrane-filtered sterile solution of 10% D (-) ribose (Sigma) was added per 90 ml of the medium (1% final concentration) just before pouring the agar medium. Plates in duplicate were incubated anaerobically at 27°C for 72 to 96.