CHAPTER 3

A STUDY ABOUT INOCULATION LEVELS OF PROBIOTIC BACTERIA IN GOAT AND COW MILK YOGHURT AND THE PRODUCTION AND SHELF LIFE OF THE YOGHURT POWDER

INTRODUCTION

There are some differences in the growth of yoghurt starter culture and probiotic bacteria between addition in yoghurt before and after fermentation. Ishibashi and Shimamura (1993) recommended inoculation of *S. thermophilus* and bifidobacteria simultaneously during fermentation since bifidobacteria are anaerobic and *S. thermophilus* has a high oxygen utilization ability, which results in removal of dissolved oxygen in the product and enhances the viability of bifidobacteria. On the other hand, Modler and Villa-Garcia (1993) suggested that the cells of *Bifidobacterium* spp. should be added into the yoghurt after fermentation.

Several factors have been claimed to affect the viability of probiotic culture in fermented milk products. Acidity and pH have been identified to have an effect during manufacture. Other factors, such as temperature of storage, oxygen content, concentration of lactic acid, etc., have been presumed to affect the viability of probiotic organism in yoghurt (Prajapati and Dave, 1994; Samona and Robinson, 1994; Rybka and Kailasapathy, 1995). Improvement of the shelf life of yoghurt can be obtained by lowering its water content or by draining of whey. Another method is drying, e.g. freeze-, spray or microwave-drying, the primary objective of which is to preserve the product in a shelf-stable powder form of high quality without the need for refrigeration. Dried yoghurt requires less packaging and storage costs because of the reduction of bulk, and no refrigeration is required. Freeze drying generally results in better survival of starter culture when compared with other commonly used methods, e.g. spray drying (Rybka and Kailasapathy, 1995). Spray drying is reported to have marked detrimental effects on the physico-chemical, microbiological characteristics and the flavour of the yoghurt powder (Groux, 1973).

The packaging plays a fundamental role in maintaining the quality and shelf-life of foods. The package is an integrated part of the preservation system and functions as a barrier between the food and the external atmosphere. The package should be designed and developed not only to hold the food product, but also to protect it and add value to it, since its design may directly affect the purchase decision of the consumer (Roberston, 1993). The incorporation and viability of probiotic bacteria in foods throughout storage which result in health benefits for the host is a constant challenge for the food industry and requires the understanding of all intrinsic and extrinsic factors associated with processing, including the selection of type of packaging material. Mattila-Sandholm *et al.* (2002) reported that the packaging materials and the storage conditions are important factors for the quality of products containing probiotic microorganisms. In view of the ecology of the strains normally used in probiotic products – anaerobic and microaerophilic ones – the level of oxygen within the package during storage of the product should be as low as possible in order to avoid toxicity and death of the microorganism and the consequent loss of functionality of the product. Exposure to dissolved oxygen during processing and storage is highly detrimental to *B. bifidum* and *L. acidophilus.* Contrary to aerobic microorganisms, which completely reduce oxygen to water, the absorption system of this substance is minimal or even completely non-existing. The absence of an electron transport chain results in the incomplete reduction of oxygen to hydrogen peroxide. In addition, these probiotic bacteria do not produce catalase, an enzyme essential to the breakdown of hydrogen peroxide, a characteristic that consequently leads to the accumulation of derived toxic metabolites, such as superoxide anion (O_2^-), the hydroxide radical (OH^-), hydrogen peroxide (H_2O_2) in the cell, causing its death. This suggests that probiotic strains may be affected by H_2O_2 produced by other cultures present in the reaction medium. This has motivated several studies aimed at developing alternatives that minimize these negative effects, among which the most promising are those evaluating the addition of antioxidants, such as ascorbic acid and the elimination of peroxide producing strains (Adriano *et al.,* 2007).

In this study section, the viability of yoghurt starter culture and probiotic in goat and cow milk yoghurt was assessed. Differences between addition of microencapsulated probiotic in yoghurt before and after fermentation were

monitoring. The effect of dehydration methods (spray drying and freeze drying) and different packaging materials on the survival of studied microorganisms were also investigated.

3.1 LITERATURE REVIEW

3.1.1 Probiotic Bacteria

Cultured dairy products, along with other lactic acid-fermented foods, have been part of the human diet for thousands of years and are still considered therapeutic food during sickness in many cultures. The Nobel laureate and director of the Pasteur Institute in Paris, Elie Metchnikoff, is generally credited with having formulated a hypothesis explaining the health benefits of yoghurt bacteria in the early part of the 20^{th} century. Studies on yoghurt starter culture and other lactic acid bacteria were conducted throughout the last century. It was only in recent decades, however, that reproducible data began to accumulate which indicated that some microbial preparations could beneficially affect human health. The term "probiotic", defined as live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance, has become widely established. The definition has been broadened since then to include human use for organs and tissues other than the gastrointestinal (GI) tract and to encompass effects not directly mediated by the microflora. In view of evidence that nonviable microbes can also exert health benefits, several definitions no longer require that microbes have to be alive in order to qualify as probiotics (Caglar, 2005).

3.1.2 Probiotic Genera and Strains

The two most commonly-used probiotic bacteria are bifidobacteria and lactobacilli, but other lactic acid bacteria and even yeast strains also qualify (Table 17). It has only been after the development of molecular biology methods that many of the individual bacterial strains were identified unambiguously (Klein *et al.,* 1998).

Lactobacilli are Gram positive, non-forming, microaerophilic rods that produce abundant lactate as an end product and can be H_2O_2-producing or not. Several recent studies using molecular biology techniques on fecal samples indicate that the intestinal *Lactobacillus* population of each individual is unique, with

Lactobacillus plantarum, Lactobacillus rhamnosus and *Lactobacillus paracasei* ssp. *paracasei* representing the most frequently-detected bacteria. In some subjects, the *Lactobacillus* population is quite simple with one or two strains predominating over several months. In others, it is markedly more complex, consisting of up to 11 different strains of which none is truly dominant. Considerable fluctuation in the strain composition and size of the *Lactobacillus* population have been observed in some individuals. Interestingly, exogenously-supplied *Lactobacillus* may become transiently established as the predominant *Lactobacillus* species but only in subjects with fluctuations in their endogenous *Lactobacillus* population (Klein *et al*., 1998).

Bifidobacteria are strictly anaerobic Gram positive rods, often shaped in the "Y" or bifid form. They possess a special metabolic pathway that allows them to produce acetic as well as lactic acid. Both classical culture method and recent molecular biology studies have shown that the pattern of bifidobacteria is unique for each human host. Data are somewhat conflicting on the stability of the total numbers and numbers of different strains of bifidobacteria present in individual subjects. Both parameters seem to fluctuate in some humans, but not in others; whereas only one or two species of bifidobacteria are detected in some individuals, others may harbor up to seven different strains (Klein *et al.,* 1998).

Table 16 Microorganism species Used as Probiotics

Genus	Species	Strain
Lactobacillus	*lactis*	
	*plantarum**	
	*rhamnosus**	GG,HN110
	johnsonii	LJ-1 (LA-1), NCFB 1748
	*reuteri**	ATCC 55730
	*casei**	Shirota
	*gasseri**	
Bidifobacterium	*bifidum**	Bb-12
	longum	
	breve	
	infantis	
	*lactis**	HN019
	adolescentis	

Table 16 Microorganism species Used as Probiotics (continue)

Genus	Species	Strain
Streptococcus	*thermophilus*	
Enterococcus	*faecalis*	
	faecium	
Escherichia	*coli*	
Bacillus	*cereus*	
Saccharomyces	*boulardii*	

*With evidence from clinical trials.

Source: Guerra *et al.* (2007)

Probiotic bacteria in fermented milk

Probiotics used in fermented milk production have mostly 3 genus; *Lactobacillus*, *Enterococcus* and *Bifidobacterium*. Probiotics strains in Genus *Lactobacillius* are *L. acidophilus*, *L. crispaths*, *L. amylovorus*, *L. gallinarum*, *L. gasseri*, *L. johnsonii*, *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. reuteri*, and *L. fermentum*. Both *Ec. faecium* and *Ec. faecalis* are probiotic bacteria in Genus *Enterococcus.* In fermented probiotic products it is important that the probiotic culture used contributed to good sensory properties. For milk-based products the probiotic strains are often mixed with *Streptococcus thermophillus* and *Lactobacillus delbrueckii* to achieve the desired flavor and texture. In many cases consumers find products fermented with *L. delbrueckii* are too acidic and too heavy acetaldehyde flavor (yoghurt flavor). Therefore probiotic cultures have been developed to bring out the preferred flavors in the products in which they are used. Examples of such cultures are called ABT cultures (ABT standing for *L. acidophilus*, *Bifidobacterium* and *S. thermophilus*) (Saarela *et al.*, 2000).

The interaction between probiotic and starter bacteria might have an impact on the quality of the product. It has been shown that it is possible to produce fermented dairy products with excellent sensory properties and good survival of the bacteria by using starter and probiotic organisms together. If possible, the probiotic should be able to grow during the fermentation. This will increase the total number of probiotic resulting in a lower process cost and increased adaptation of the probiotics

to the fermented food. As most probiotics grow well at 37oC a thermophillic starter might be preferable to a mesophilic one. The growth rate of the starter should be moderate allowing some growth of the probiotic bacteria. It is also important to add the probiotic before or at the same time as the starter. Addition of the probiotic after fermentation does not allow any growth but instead might result to a reduced viability as shown when *L. acidophilus* was mixed with yoghurt. The starter might improve the growth conditions of probiotic by producing substances favorable to the growth of probiotic or by reducing the oxygen pressure (Saarela *et al.*, 2000).

3.1.3 Spray-dried yoghurt

The positive health attributes associated with the consumption of fermented milk product has stimulated considerable market interest in spray-dried dairy ingredient, including yoghurt powder. Natural flavors produced during fermented milk processing are much valued in their own right and also to complement other added flavor. Traditionally, yoghurt is produced in a hydrated form and, thus, possesses a limited shelf-life even when refrigerated. Consumption within a short time of production is advisable, particularly if advantage is to be taken of putative benefits associated with the ingestion of live yoghurt cultures. The production of an instant yoghurt powder would, thus, provide benefits of shelf-life extension and convenience of preparation and storage (Tamine and Robinson, 1999).

Basically, there are two methods of drying that could be employed commercially for the manufacture of dried yoghurt which are spray drying and freeze drying. Although the latter method of drying would seem to be more attractive because the temperature of drying (20-35^{o}C) is much lower than spray drying (55-60^{o}C), so ultimately causing the least damage to the milk constituents and/or loss of flavor-it is far too expensive to be considered on a commercial scale. At present, powdered yoghurt is produced commercially using spray drying, but some precautionary measures should be considered. First, the concentration of yoghurt, before drying, should be carried out at 50-60^{o}C and second, the drying condition should be moderate to ensure a high viable cell count of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in the dried product (Tamine and Robinson, 1999).

Yoghurt can be dried in a three-stage drying plant, and on average, the yoghurt is concentrated to 35 g/100 g total solid, preheated and atomized into the drying chamber with inlet and outlet temperatures at 160 and 65^{o}C, respectively. The microbial number in commercial dried yoghurt of non-lactic acid bacteria, *S. thermophilus a*nd *L. delbrueckii* ssp. *bulgaricus* were <1x10^{4}, 1x10^{3} and 1x10^{4}, respectively. Pan *et al.* (1995) reported that the count of lactobacilli in dried yoghurt was 7x10^{5}cfu/g. Kim and Bhowmik (1990) reported that the survival rate of the yoghurt organisms was influenced by the product inlet feed temperature to be at 30^{o}C, the air inlet and outlet temperatures to be at 160 and 60^{o}C, respectively, the atomizing air pressure be at 98 kpa and the hot air flow to be 0.23 m/min (Tamine and Robinson, 1999).

Bielecka and Majkowska (2000) reported that synergistic set of the strains *L. delbrueckii* ssp. *bulgaricus* 151 and *S. thermophilus* MK-10 was preserved with a method of spray-drying. Studies were performed on the effect of outlet air temperature in the range of 60-80 ^{o}C on the survival of yoghurt cultures, as well as the moisture content and sensory properties of yoghurt powder. Survival of yoghurt culture was the highest at 60 and 65^{o}C but excessive moisture (10.2%) of yoghurt powder had a negative effect on its texture. The moisture content of the powder was lower at 80^{o}C (4.4%), unfortunately, sensory faults appeared at this temperature and survival of bacteria cultures decreased considerably. Temperatures within the range of 70-75^{o}C ensured satisfactory survival of yoghurt cultures *L. delbrueckii* ssp. *bulgaricus* at 13.7-15.8% and *S. thermophilus* at 51.6-54.7%, maintained the proportion of yoghurt strains *L. bulgaricus* : *S thermophilus* =1:3, a satisfactory moisture content 5.1-6.3% and good sensory properties of yoghurt powder.

Kim *et al.* (1990) reported the survival of *Streptococcus salivarius* ssp. *thermophilus* and *L. delbrueckii* ssp. *bulgaricus* under various processing conditions for spray-drying. Numbers of both microorganisms decreased with increased outlet or inlet air temperature and atomizing air pressure. Outlet air temperature was a major parameter affecting the number of survivors. Suitable conditions were an inlet air at 160^{o}C, an outlet at 60^{o}C, an atomizing at pressure 98 kpa, a hot air flow 0.28 m/min and a feed temperature at 30^{o}C. The spray-dried yoghurt powder showed lower

survival for *S. thermophilus* but a similar survival for *L. delbrueckii* ssp. *bulgaricus* as compared to freeze dried powder.

Lian *et al.* (2002) reported that microencapsulated cells of *B. longum* B6 and *B. infantis* CCRC14633 were prepared by spray-drying the cell suspension containing the test organism and 10% w/w of the carrier material of either gelatin, soluble starch, skimmed milk or gum arabic. Survival of these microencapsulated and free cells of bifidobacteria in simulated gastric juice at pH 2.0 and 3.0 and bile solution of 0.5% and 2.0% was then examined. *B. infantis* CCRC14633 was more susceptible than *B. longum* B6 to the stimulated gastric environment and bile solution tested. Microencapsulated bifidobacteria exhibited a lower population than free cell when exposed to simulated gastric environment and bile solution. Moreover, it was also observed that the protective effect exerted by encapsulation with spray drying varied with the carriers used and the strains of bifidobacteria.

Peri and Pompi (1976) reported that a spray-dry process for neutralized yoghurt should use a temperature lower than 80 ^{o}C, and a moisture content of the final product was approximately 10%. The shelf life of the product at 5 ^{o}C is more than 8 months.

A sample should be gel or emulsion or slurry that has a total soluble solid between 20 and 50%. This sample is shattered to droplets in the chamber and is passed by hot air. Heat transfer occurs rapidly because of small droplets. This method making a good characteristic of product depends on the shattering of sample and heat transfer rate between the droplets and the hot air. Yoghurt usually has a total soluble solid of 14-16% that is not sufficient for making spray-dry process, so maltodextrin is added to increase the total soluble solid of yoghurt. Maltodextrin is widely used in spray-dry industry because it is more soluble, more dispersive, less absorption of moisture, preserve a product flavor and higher flowability. For example, maltodextrin having dextrose equivalent (DE) 10 is used in cheese powder, fat powder and fruit juice powder (Tamine and Robinson, 1999).

Metwally and Abd-E1-Garad (1989) reported the survival of different microorganisms, using 2 types of atomizer. The research was conducted at an inlet temperature of 190-200^{o}C and an outlet temperature of 80-82^{o}C. When using a centrifugal atomizer, the survival of *Streptococcus lactis* was 0.93%, *S. thermophilus*

7.75% and *L. bulgaricus* 0.92%, whereas using a two-fluid atomizer would give a survival rate for *S. lactis* 2.2%, *S. thermophilus* 11.8% and *L. bulgaricus* 1.14%. The pressure of two-fluid atomizer was 2.5 kg/cm^2 and at this pressure, the microorganism was damaged. The microorganism would be damaged if a pressure higher than 1.6 kg/ cm^2 was used. Using different temperature outlets with the two-fluid atomizer, it was found that at 70-72^{o}C, the survival of *S. lactis* was 22.0%, *S. thermophilus* 46.9% and *L. bulgaricus* 19.5%. At 82-84^{o}C outlet temperature, the survival of *S. lactis* was 1.8%, *S. thermophilus* 12.2% and *L. bulgaricus* 4.8% whereas at 88-90^{o}C outlet temperature, the survival rate of *S. lactis* was 1.57% and *S. thermophilus* was 8.8%.

Cai and Corke (2000) reported that maltodextrin-hydrolyzed starches were effective encapsulating agents when used in the spray-drying of sensitive flavors and carotenoid. Maltodextrins were also a good compromise between cost and effectiveness. The compound was bland in flavor, had low viscosity at high solid ratio and were available in different average molecular weights, DE 4, 10, 15, 20, 25, 30 and 40. A structural analysis revealed that there were many more surface indentations and cracks in wall systems containing lower DE maltodextrins than in those containing higher DE maltodextrins. Most of the spray-dried capsules containing 25 DE maltodextrin looked like smooth sphere with hardly any surface cracks in the wall systems. Moreover, maltodextrin of 10 DE, with the lowest hygroscopicity, could be mixed with 25 DE maltodextrin (25 DE:10 DE = 3:1) in order to improve overall hygroscopicity.

3.1.4 Freeze-drying

Freeze-drying is an important operation for drying expensive foods, which have delicate aromas or textures, for example, coffee, mushrooms, herbs and spices, fruit juices, meat, seafood, vegetables and complete meals for military rations. Also for the expeditions of which consumers are willing to pay higher prices for superior quality. The first stage of freeze-drying is to freeze the food in conventional freezing equipment. Small pieces of food are frozen rapidly to produce small ice crystals and to reduce damage to the cell structure of the food. In liquid foods, slow freezing is used to form an ice crystal lattice which provides channels for the movement of water vapor. The next stage is to remove water during subsequent

drying and hence, dry the food. If the water vapor pressure of a food is held below 4.58 Torr (610.5 Pa) and the water is frozen, the solid ice in heated food sublimes directly to vapor without melting. The water vapor is continuously removed from the food by keeping the pressure in the freeze drier cabinet below the vapor pressure at the surface of the ice. Vapor is removed with a vacuum pump and condensed on refrigeration coils. As drying proceeds, a sublimation front moves into the frozen food, leaving partly-dried porous food behind it. Foods are dried in two stages. First by sublimation to approximately 15% moisture content. Second, by evaporative drying or desorption of unfrozen water to 2% moisture content (Fellow, 2000). Freeze-drying yields a high-quality, lightweight and easily-rehydrated product that retains the original shape of the starting material, unlike conventional drying, in which shrinking and surface hardening can occur (Clark, 2003).

Freeze-drying has been used for microbial cultures in food processing to prolong shelf life prior to inoculum generation (Fellow, 2000). Saxelin *et al*. (1999) reported that *B. lactis* BB12 could survive very well during the freezing in liquid nitrogen (-176 ^{o}C) or in a freezer (-80 ^{o}C) for the concentrated cultures in liquid nitrogen and during freeze drying with the cell counts higher than 10^{11} CFU/g. *B. lactis* BB12 tolerated well the two-week period at 25 ^{o}C that might possibly be needed for transportation. Freeze-dried *B. lactis* BB12 could be stored for one year at -18 ^{o}C without any significant loss in viability. The results demonstrated that freeze-drying is a very effective way of storing probiotic cultures.

Maitrot *et al.* (1997) studied the immobilization of *B. longum* in κ-carrageenan and locust bean gum gel beads, and cultured in a medium containing MRS broth and whey-permeate. The same beads were incubated for 5 successive batch fermentations and freeze-dried following mixing with a protective solution. Viable population in the beads increased from 8 x 10^{7} to 4.7 x 10^{10} CFU/g or 10.67 log CFU/g after three batch fermentations, but no further increase in viable cell population could be achieved in the last two fermentations. However, increasing bifidobacterial cells inside the beads by using 3-5 successive batch fermentation consumed a lot of time.

Freeze-drying has been used to modify the structure of the hydrocolloid beads for using in many biotechnological purposes, such as water

denitrification, matrices for the immobilization of denitrifying isolates, carriers of bacteria or spores for biological control of soil-borne root diseases, and carriers of Gram positive lactic acid bacteria starter cultures involving in dairy and food fermentation. The dried hydrocolloid beads are also used as a vehicle to deliver drug into the human gastrointestinal tract and control-release drug in the target organ (Zohar pereza *et al*., 2004).

Shan-Yang *et al*. (1999) studied the effect of quick and slow freezing on the porosity of freeze-dried poly (*N*-isopropylacrylamide) beads and reported that QF-beads had more porosity than SF-beads after drying. However, poly (*N*-isopropylacrylamide) microgel beads had a diameter ca 50 μm, then the large ice crystal of slow freezing caused the structure of dried beads to collapse.

Fwu-Long *et al*. (2002) produced the freeze-dried chitosan beads for immobilizing an anti-inflammatory drug. The morphology of the freeze-dried chitosan bead had an interconnected porous structure comprising particulates around the pores.

Tal *et al*. (1997) modified the structure of alginate beads by freezing the gel beads at -80^{o}C for 24 h before freeze drying and studied the effect of potato starch as a filler and carbon source at concentrations of 10, 20, 30 and 40% (w/w) to improve the mechanical and biological properties of freeze dried denitrifying alginate beads. The denitrifying bacterium was *Pseudomonas* spp. Freeze-dried beads containing high concentrations of starch were found to have higher ($p<0.05$) mechanical and denitrifying properties than beads containing low concentrations of the starch filler.

3.2 EXPERIMENTAL

3.2.1 Effects of initial concentrations and addition steps of probiotic microorganisms on the production of goat and cow milk yoghurt

3.2.1.1 To find the appropriate amount and addition steps of microencapsulated probiotic microorganism in the production of goat and cow milk yoghurt

Prepare the optimum ingredients for the production of goat and cow milk yoghurt from prototype formula and divide the mixture ingredients into 2 groups :

- Group 1, add *B. bifidum* and *L. acidophilus* which were microencapsulated at the concentration levels of 0, 2, 4 and 8% (w/v) at the same time with yoghurt starter culture.

- Group 2, add *B. bifidum* and *L. acidophilus* which were microencapsulated as the same concentration as the first group but after the fermentation process.

- Keep the yoghurt at the temperature of 4^{o}C for 30 days.

3.2.1.2 Physical analysis

The physical analysis of the yoghurt that was carried out at the beginning and at the end of the storage period as followed:

- Measure the colors with Hunter (L*,a*,b*) (ColorQuest® XT, USA)

- Measure the viscosity with Brookfield viscometer (DV-II+Pro EXTRA, USA)

3.2.1.3 Chemical analysis

The chemical analysis of the yoghurt that was carried out at the beginning and during the storage period as followed:

- Measure pH value by pH meter (CONSORT C830, CE, Belgium)

3.2.1.4 Microorganism analysis

Microorganism enumeration of *S. thermophillus*, *L. bulgaricus, B. bifidum* and *L. acidophilus* at day 0, 3, 6, 9, 12, 15, 20 and 30 of storage period was carried out according to the method described in 1.2.1.4.

3.2.1.5 Statistical analysis

Experimental data were analyzed by SPSS program (SPSS version 11, SPSS Inc., Chicago, USA). Significant differences among means were compared by a Duncan's new multiple range test. The predetermined acceptable level of probability was 5% ($p<0.05$) for all the comparison (Montgomery, 2001).

3.2.2 Effect of drying methods on the properties of goat and cow milk yoghurt powder

3.2.2.1 Condition of the spray dryer

The spray dryer model SDE 50 from J.C. Machinery and Civil Work Co., Ltd. (Germany) was adjusted to an output temperature of 75± 2°C before the yoghurt was dried with this dryer. The type of atomizer used was a nozzle atomizer with length of 44 cm together with an atomizer pressure at 15 psi, a co-current air flow and an air inlet temperature at 180°C. The total soluble solid of yoghurt was adjusted to 25% (w/v), using 25% (w/v) maltodextrin solution (Desobry *et al.*, 1997 and Cai and Corke, 2000) to produce a good drying rate during the spray-drying process.

3.2.2.2 Condition of the freeze-dryer

The probiotic-added goat and cow milk yoghurt prepared according to 3.2.1.1 was stored at 4-5°C for 18 h. Next, the yoghurt was frozen at -18°C for 24 h and dried out using a freeze dryer (FFD-42-Ws Freeze-Dryer, USA) at 25°C for 72 h.

3.2.2.3 Physical analysis

The yoghurt powder was dissolved in 100ml fresh water, gentle stir and then the physical analysis of the yoghurt that was carried out at the beginning and at the end of the storage period as followed:

- Measure the colors with Hunter (L*, a*, b*) (ColorQuest® XT, USA)
- Measure the viscosity with Brookfield viscometer (DV-II+Pro EXTRA, USA)

3.2.2.4 Chemical analysis

Ten grams yoghurt powder was dissolved in 100 ml fresh water, gentle stirred and analyzed for its chemical characteristics including:

- Measure pH value by pH meter (CONSORT C830, CE, Belgium)
- Moisture content by an AOAC method no. 927.05 (AOAC, 2000)

3.2.2.5 Microorganism analysis

Microorganism enumeration of *S. thermophillus*, *L. bulgaricus, B. bifidum* and *L. acidophilus* at day 0, 15, 30, 40, 60 and 120 of storage period was done, according to the method described in 1.2.1.4

3.2.2.6 Statistical analysis

Experimental data were analyzed by SPSS program (SPSS version 11, SPSS Inc., Chicago, USA). Significance amongst means were compared by a Duncan's new multiple range test. The predetermined acceptable level of probability was 5% ($p<0.05$) for all the comparison (Montgomery, 2001).

3.2.3 Effect of packaging materials and storage temperatures on the properties of goat and cow milk yoghurt powder

The goat and cow milk yoghurt powder was prepared by different drying methods, *i.e*., spray and freeze drying and packed in two different packaging materials. The first one was laminated plastic and the second one was aluminium foil pouch. Both packages were sealed as a vacuum packaging and stored at $4^{o}C$ for 4 months. The qualities of the powder were monitored as followed:

3.2.3.1 Physical properties

- Color of the yoghurt as described in 3.2.1.2
- Moisture content by an AOAC method no. 927.05 (AOAC, 2000)

3.2.3.2 Chemical properties

- pH and total tritratable acidity as described in 3.2.1.3

3.2.3.3 Microbiological properties

- The number of probiotic microorganisms in yoghurt powder were enumerated as in 3.2.1.4 at day 0, 15, 30, 40, 60 and 120.

3.2.3.4 Statistical analysis

Collected data from the experiments was analyzed using 2^2 factorial designs in CRD. Data from three repeated experiments was analyzed by a SPSS program (SPSS version 11, SPSS Inc., Chicago, USA). If the significant differences between means were found, the mean comparation with Duncan's multiple range test would be applied. The predetermined acceptable level of probability was 5% ($p<0.05$) for all the comparison (Montgomery, 2001).

3.3 RESULT AND DISCUSSION

Tables 17 and 19 show that there were significant losses in the cell number of both yoghurt starter culture and probiotic bacteria during 30 days of storage. The results of the treatment one that probiotic bacteria were added at the same time with starter culture, at 0% concentration of probiotic bacteria, showed that the yoghurt starter culture decreased from $4.8x10^8$ to $3.2x10^4$ cfu/g. At 2% concentration, starter culture decreased from $2.6x10^8$ to $1.2x10^4$cfu/g *L. acidophilus* and *B. bifidum* decreased from $2.2x10^8$ to $2.1x10^5$cfu/g and $1.6x10^6$ to $6.4x10^3$cfu/g, respectively. The amount of *L. acidophilus* and *B. bifidum* remained at the beneficial level for health (10^6-10^7cfu/g) at day 20 and day 0, respectively. At 4% concentration, starter culture decreased from $3.3x10^8$ to $9.1x10^3$cfu/g, while probiotic bacteria also decreased but still in acceptable level ($\geq 10^7$cfu/g, IDF (1992)) at day 30 for *L. acidophilus* ($4.4x10^7$ cfu/g) and day 3 for *B. bifidum* ($2.1x10^6$ cfu/g). At 8% concentration, the result showed that yoghurt starter culture was initially at $2.2x10^8$ cfu/g and finished at $4.6x10^3$ cfu/g, *L. acidophilus* began at $3.2x10^{10}$ cfu/g and remained for beneficial health claim at day 30 ($7.7x10^7$ cfu/g), while *B. bifidum* began at $2.7x10^7$ cfu/g and remained at day 3 for a good level of health benefit.

In the other treatment that probiotic bacteria was added after fermentation process (when pH dropped to or below 4.5), the result showed that at 0% concentration, the starter culture decreased from $9.6x10^8$ to $2.8x10^4$ cfu/g. At 2% concentration, starter culture decreased from $8.3x10^8$ to $8.7x10^5$ cfu/g, while *L. acidophilus* decreased from $3.8x10^8$ to $8.1x10^6$ cfu/g but it still remained within the regulation level throughout the period of storage. *B. bifidum* began at $3.8x10^6$ cfu/g and finished at$1.2x10^4$ cfu/g in day 15, the microorganism was non-detectable in day 30 and the level of health benefit of the probiotic was still shown on day 6. At 4% concentration, the starter cultures decreased from $8.8x10^8$ to $6.9x10^5$ cfu/g. *L. acidophilus* started at $5.5x10^{10}$ cfu/g and remained at beneficial health level throughout the storage period and showed a level of $6.6x10^7$ cfu/g on day 30. *B. bifidum* started at $4.3x10^7$ cfu/g and showed beneficial health effect ($\geq 10^7$ cfu/g, IDF (1992)) for only 6 days. At 8% concentration the amount of all bacteria was the highest. The yoghurt starter culture decreased from $9.0x10^8$ to $8.8x10^5$ cfu/g. *L. acidophilus* showed health benefit level throughout the storage period ($9.1x10^{10}$ to $9.3x10^7$ cfu/g) and *B. bifidum* decreased from $8.7x10^7$ to $3.0x10^4$ cfu/g and remained at health benefit level only for 9 days ($1.7x10^6$ cfu/g).

Table 17 The survival of yoghurt starter culture and encapsulated probiotic (cfu/g) that were added at the same time with the yoghurt culture during yoghurt storage at 4°C for 30 days

Storage period (day)	The inoculation of probiotic											
	0%			2%			4%			8%		
	S.t L.b[(1)]	L.a[(2)]	B.b[(3)]	S.t L.b[(1)]	L.a[(2)]	B.b[(3)]	S.t L.b[(1)]	L.a[(2)]	B.b[(3)]	S.t L.b[(1)]	L.a[(2)]	B.b[(3)]
0	$2.8\pm2.0\times10^{8aA}$ $2.0\pm1.3\times10^{8aA}$	-	-	$1.4\pm1.1\times10^{8aC}$ $1.2\pm1.3\times10^{8aC}$	$2.2\pm0.2\times10^{8aG}$	$1.6\pm1.2\times10^{6aK}$	$1.8\pm2.3\times10^{8aB}$ $1.5\pm2.1\times10^{8aB}$	$6.6\pm2.0\times10^{9aF}$	$6.3\pm1.0\times10^{6aJ}$	$1.2\pm2.0\times10^{8aD}$ $1.0\pm2.1\times10^{8aD}$	$3.2\pm0.9\times10^{10aE}$	$2.7\pm1.2\times10^{7aI}$
3	$2.6\pm2.0\times10^{8bA}$ $1.7\pm2.0\times10^{8bA}$	-	-	$2.2\pm2.1\times10^{7bC}$ $1.8\pm2.0\times10^{7bC}$	$1.1\pm0.5\times10^{8bG}$	$6.6\pm2.4\times10^{5bK}$	$2.5\pm1.9\times10^{7bB}$ $2.2\pm2.1\times10^{7bB}$	$4.3\pm1.2\times10^{9bF}$	$2.1\pm1.2\times10^{6bJ}$	$1.6\pm1.3\times10^{7bD}$ $1.1\pm1.0\times10^{7bD}$	$1.1\pm0.4\times10^{10bE}$	$7.9\pm1.4\times10^{6bI}$
6	$2.1\pm1.2\times10^{7cA}$ $1.5\pm1.2\times10^{7cA}$	-	-	$2.1\pm1.6\times10^{6cC}$ $1.9\pm1.4\times10^{6cC}$	$1.7\pm1.0\times10^{8cG}$	$2.20\pm1.0\times10^{5cK}$	$1.9\pm1.2\times10^{6cD}$ $1.4\pm1.0\times10^{6cD}$	$2.1\pm1.0\times10^{9cF}$	$6.7\pm3.0\times10^{5cJ}$	$3.1\pm1.2\times10^{6cB}$ $3.5\pm1.3\times10^{6cB}$	$8.4\pm1.2\times10^{9cE}$	$5.3\pm2.0\times10^{6cI}$
9	$2.5\pm2.0\times10^{6dA}$ $2.0\pm2.1\times10^{6dA}$	-	-	$4.5\pm1.1\times10^{5dB}$ $4.1\pm1.3\times10^{5dB}$	$9.7\pm3.0\times10^{7dG}$	$7.30\pm2.3\times10^{4dK}$	$3.4\pm2.3\times10^{5dC}$ $3.2\pm1.3\times10^{5dC}$	$7.8\pm2.2\times10^{8dF}$	$3.8\pm1.4\times10^{5dJ}$	$3.2\pm1.9\times10^{5dD}$ $2.2\pm1.5\times10^{5dD}$	$6.1\pm1.0\times10^{9dE}$	$6.1\pm2.7\times10^{5dI}$
12	$2.8\pm1.1\times10^{6eA}$ $2.5\pm1.3\times10^{6eA}$	-	-	$0.9\pm0.5\times10^{5eC}$ $0.7\pm0.7\times10^{5eC}$	$6.1\pm3.0\times10^{7eG}$	$3.20\pm2.0\times10^{4eK}$	$2.2\pm1.6\times10^{5eB}$ $1.1\pm1.5\times10^{5eB}$	$2.3\pm1.7\times10^{8eF}$	$1.0\pm0.4\times10^{5eJ}$	$0.1\pm1.7\times10^{5eD}$ $0.1\pm1.5\times10^{5eD}$	$2.8\pm1.2\times10^{9eE}$	$3.0\pm1.0\times10^{5eI}$
15	$3.6\pm1.7\times10^{6fA}$ $3.5\pm1.4\times10^{6fA}$	-	-	$3.2\pm0.3\times10^{5fB}$ $2.2\pm0.2\times10^{5fB}$	$1.2\pm2.0\times10^{7fG}$	$6.40\pm1.4\times10^{3fK}$	$2.4\pm1.6\times10^{4fC}$ $2.3\pm1.2\times10^{4fC}$	$1.4\pm1.0\times10^{8fF}$	$5.3\pm2.4\times10^{4fJ}$	$2.2\pm0.9\times10^{4fD}$ $1.7\pm0.8\times10^{4fD}$	$8.4\pm1.0\times10^{8fE}$	$8.7\pm2.2\times10^{4fI}$
20	$2.5\pm1.1\times10^{6gA}$ $2.4\pm1.0\times10^{6gA}$	-	-	$3.1\pm1.1\times10^{4gB}$ $2.9\pm1.1\times10^{4gB}$	$2.7\pm1.0\times10^{6gG}$	ND[(4)]	$2.1\pm1.6\times10^{4gC}$ $1.0\pm1.5\times10^{4gC}$	$6.1\pm2.4\times10^{7gF}$	$8.2\pm1.0\times10^{3gJ}$	$1.2\pm1.2\times10^{4gD}$ $1.0\pm1.4\times10^{4gD}$	$4.7\pm2.8\times10^{8gE}$	$3.9\pm2.1\times10^{4gI}$
30	$2.2\pm1.0\times10^{4hA}$ $1.0\pm1.2\times10^{4hA}$	-	-	$0.6\pm1.4\times10^{4hC}$ $0.6\pm1.3\times10^{4hA}$	$2.1\pm1.0\times10^{5hG}$	ND[(4)]	$4.8\pm1.2\times10^{3hC}$ $4.3\pm1.1\times10^{4hA}$	$4.4\pm2.0\times10^{7hF}$	ND[(4)]	$2.4\pm2.0\times10^{3hD}$ $2.2\pm1.1\times10^{4hA}$	$7.0\pm1.0\times10^{7hE}$	$8.8\pm2.2\times10^{3hI}$

[(1)] S.t is *S. thermophillus* and L.a is *L. bulgaricus*, [(2)] L.a is *L. acidophilus*, [(3)] B.b is *B. bifidum*, [(4)] ND is not detected, ***** Means in the same column with different small letter superscripts are significantly different; means in the same row for particular strain level with different capital letter superscripts are significantly different, A-D for yoghurt starter culture amount (*S. thermophillus* and *L. bulgaricus*), E-H for L.a (*L. acidophilus*) and I-K for B.b (*B. bifidum*)

Table 18 Physical and chemical characteristics of probiotic added goat and cow milk yoghurt in which the probiotic was added at the same time as the yoghurt culture during storage at 4°C for 30 days.

Yoghurt Characteristics	Inoculation level of probiotic							
	0%		2%		4%		8%	
	Start	End	Start	End	start	End	Start	End
L* Color value (Brightness)	70.71±0.7[a]	79.71±0.50[h]	70.55±0.5[b]	84.58±0.55[f]	70.01±0.5[d]	84.16±0.62[g]	70.45±0.6[c]	84.56±0.70[e]
a* Color value (Red-Green)	-1.61±0.10[d]	-1.07±0.12[h]	-1.71±0.18[c]	-1.12±0.11[g]	-1.90±0.14[b]	-1.18±0.13[e]	-1.99±0.15[a]	-1.15±0.11[f]
b* Color value (Yellow - Blue)	9.44±0.5[c]	9.77±0.6[h]	9.23±0.6[b]	9.84±0.5[g]	9.07±0.3[d]	9.90±0.4[f]	9.32±0.3[a]	9.92±0.5[e]
Viscosity (cp)	4203±125[b]	4368±130[h]	4238±142[a]	4460±132[g]	4230±151[a]	4704±230[f]	4220±160[b]	5120±280[e]
pH value	4.84±0.06[a]	4.12±0.01[e]	4.76±0.02[b]	3.91±0.01[g]	4.74±0.01[b]	4.01±0.03[f]	4.68±0.02[c]	3.86±0.03[h]
Titratable acidity (%w/w)	1.85±0.03[c]	1.96± 0.01[h]	1.91±0.03[b]	2.08±0.02[g]	1.93±0.02[b]	2.14±0.01[f]	1.98±0.01[a]	2.24±0.03[e]

**** Means in the same row with different small letter a-d superscripts for the start time of analysis are significantly different; e-h superscripts for the end time of analysis are significantly different.

Table 19 The survival of yoghurt starter culture and encapsulated probiotic (cfu/g) that were added at the end of fermentation process during yoghurt storage at 4°C for 30 days

Storage period (day)	Concentration encapsulated probiotic											
	0%			2%			4%			8%		
	S.t L.b[(1)]	L.a[(2)]	B.b[(3)]	S.t L.b[(1)]	L.a[(2)]	B.b[(3)]	S.t L.b[(1)]	L.a[(2)]	B.b[(3)]	S.t L.b[(1)]	L.a[(2)]	B.b[(3)]
0	$5.6\pm2.2\times10^{8aA}$ $4.0\pm1.2\times10^{8aA}$	-	-	$5.1\pm2.1\times10^{8aD}$ $4.2\pm2.3\times10^{8aD}$	$9.1\pm1.7\times10^{8aG}$	$3.8\pm1.2\times10^{6aK}$	$4.6\pm1.4\times10^{8aC}$ $4.2\pm1.2\times10^{8aC}$	$5.5\pm0.8\times10^{10aF}$	$4.3\pm0.8\times10^{7aJ}$	$5.0\pm0.5\times10^{8aB}$ $4.0\pm0.4\times10^{8aB}$	$9.1\pm2.0\times10^{10aE}$	$8.7\pm1.7\times10^{7aI}$
3	$2.1\pm1.3\times10^{8bD}$ $2.6\pm1.2\times10^{8bD}$	-	-	$2.9\pm1.2\times10^{8bC}$ $2.6\pm1.1\times10^{8bC}$	$4.3\pm2.2\times10^{8bG}$	$1.1\pm0.8\times10^{6bK}$	$3.1\pm2.0\times10^{8bB}$ $2.9\pm2.1\times10^{8bB}$	$1.3\pm0.4\times10^{10Bf}$	$8.3\pm1.0\times10^{6bJ}$	$4.1\pm1.1\times10^{8bA}$ $3.1\pm1.2\times10^{8bA}$	$4.6\pm1.7\times10^{10Be}$	$4.2\pm1.2\times10^{7bI}$
6	$4.2\pm1.7\times10^{7cB}$ $3.1\pm1.4\times10^{7cB}$	-	-	$3.6\pm2.2\times10^{7cD}$ $2.7\pm2.6\times10^{7cD}$	$2.7\pm1.0\times10^{8cG}$	$5.1\pm0.9\times10^{5Ck}$	$4.6\pm1.8\times10^{7cA}$ $3.1\pm1.9\times10^{7cA}$	$7.1\pm1.0\times10^{9cF}$	$1.1\pm0.8\times10^{6cJ}$	$3.9\pm0.8\times10^{7cC}$ $2.7\pm0.2\times10^{7cC}$	$8.8\pm1.2\times10^{9cE}$	$6.1\pm1.2\times10^{6cI}$
9	$0.9\pm15\times10^{7dD}$ $0.8\pm1.4\times10^{7dD}$	-	-	$1.2\pm1.8\times10^{7dC}$ $1.2\pm1.3\times10^{7dC}$	$8.6\pm2.2\times10^{7dG}$	$1.7\pm0.8\times10^{5dK}$	$2.3\pm1.1\times10^{7dB}$ $2.0\pm1.2\times10^{7dB}$	$3.4\pm1.2\times10^{9dF}$	$5.3\pm0.9\times10^{5dJ}$	$3.1\pm1.1\times10^{7dA}$ $2.1\pm1.0\times10^{7dA}$	$6.6\pm1.0\times10^{9dE}$	$1.7\pm1.0\times10^{6dI}$
12	$3.3\pm1.1\times10^{6eD}$ $2.2\pm1.3\times10^{6eD}$	-	-	$4.6\pm1.1\times10^{6eB}$ $3.1\pm2.1\times10^{6eB}$	$6.6\pm2.7\times10^{7eG}$	$8.1\pm0.8\times10^{4eK}$	$4.5\pm1.6\times10^{6eA}$ $4.0\pm1.7\times10^{6eA}$	$7.3\pm1.0\times10^{8eF}$	$2.2\pm1.2\times10^{5eJ}$	$4.1\pm1.7\times10^{6eC}$ $3.0\pm1.9\times10^{6eC}$	$2.3\pm0.2\times10^{9eE}$	$4.0\pm0.9\times10^{5eI}$
15	$2.0\pm0.8\times10^{6fD}$ $2.0\pm0.8\times10^{6fD}$	-	-	$2.2\pm1.2\times10^{6fC}$ $2.0\pm1.9\times10^{6fC}$	$3.5\pm0.7\times10^{7fG}$	$1.2\pm0.9\times10^{4fK}$	$3.6\pm1.4\times10^{6fB}$ $1.8\pm1.2\times10^{6fB}$	$2.4\pm0.8\times10^{8fF}$	$1.0\pm1.1\times10^{5fJ}$	$3.2\pm1.1\times10^{6fA}$ $3.2\pm1.3\times10^{6fA}$	$7.1\pm1.2\times10^{8fE}$	$1.9\pm0.8\times10^{5fI}$
20	$3.9\pm1.4\times10^{5gD}$ $2.8\pm1.3\times10^{5gD}$	-	-	$0.8\pm0.3\times10^{6gC}$ $0.7\pm0.1\times10^{6gC}$	$1.1\pm1.7\times10^{7gG}$	ND[(4)]	$1.4\pm1.6\times10^{6gB}$ $1.0\pm1.3\times10^{6gB}$	$8.3\pm1.2\times10^{7gF}$	$8.8\pm0.9\times10^{4gJ}$	$2.1\pm1.7\times10^{6gJ}$ $1.2\pm1.6\times10^{6gJ}$	$2.8\pm1.6\times10^{8gE}$	$8.3\pm1.2\times10^{4gI}$
30	$1.4\pm0.4\times10^{4hD}$ $1.4\pm0.4\times10^{4hD}$	-	-	$4.4\pm1.4\times10^{5hB}$ $4.3\pm1.4\times10^{5hB}$	$8.1\pm1.2\times10^{6hG}$	ND[(4)]	$3.6\pm0.8\times10^{5hC}$ $3.3\pm0.8\times10^{5hC}$	$6.6\pm1.0\times10^{7hF}$	$2.1\pm1.0\times10^{4hJ}$	$4.8\pm1.2\times10^{5hA}$ $4.0\pm1.2\times10^{5hA}$	$9.3\pm1.4\times10^{8hE}$	$3.0\pm0.8\times10^{4hI}$

[(1)] S.t is *S. thermophillus* and L.a is *L. bulgaricus*, [(2)] L.a is *L. acidophilus*, [(3)] B.b is *B. bifidum*, [(4)] ND is not detected, ***** Means in the same column with different small letter superscripts are significantly different; means in the same row for particular strain level with different capital letter superscripts are significantly different, A-D for yoghurt starter culture amount (*S. thermophillus* and *L. bulgaricus*), E-H for L.a (*L. acidophilus*) and I-K for B.b (*B. bifidum*)

Table 20 Physical and chemical characteristics of probiotic added goat and cow milk yoghurt in which the probiotic was added at the end of fermentation process during storage at 4ºC for 30 days.

Yoghurt Characteristics	Inoculation level of probiotic							
	0%		2%		4%		8%	
	Start	End	Start	End	Start	End	Start	End
L^* Color value (Brightness)	73.71±0.6 [b]	85.66±0.8 [g]	117.17±1.2 [a]	145±2.2 [f]	119.66±1.4 [a]	166±0.2 [e]	119.46±1.0 [a]	169±1.2 [e]
a^* Color value (Red-Green)	-1.70±0.2 [a]	-1.11±0.2 [g]	-1.73±0. [a]	-1.25±0.1 [f]	-1.73±0.1 [a]	-1.30±0.3 [e]	-1.47±.03 [b]	-1.31±0.2 [e]
b^* Color value (Yellow - Blue)	9.55±0.6 [d]	9.80±0.5 [g]	9.79±0.5 [c]	12.00±0.4 [f]	9.88±0.8 [b]	12.24±0.4 [e]	9.97±0.2 [a]	12.36±0.2 [e]
Viscosity (cp)	4540±225 [b]	4660±230 [g]	4508±212 [b]	4920±232 [f]	4530±201 [b]	5004±210 [f]	4670±260 [a]	5327±245 [e]
pH value	4.74±0.03 [a]	4.10±0.02 [g]	4.61±0.01 [b]	4.02±0.02 [f]	4.59±0.04 [b]	3.88±0.04 [e]	4.45±0.03 [c]	3.64±0.02 [h]
Titratable acidity (%w/w)	1.85±0.03 [ec]	1.98± 0.02 [h]	1.94±0.03 [b]	2.14±0.03 [g]	1.99±0.01 [b]	2.25±0.01 [f]	2.06±0.02 [a]	2.38±0.01 [e]

**** Means in the same row with different small letter a-d superscripts for the start time of analysis are significantly different; e-h superscripts for the end time of analysis are significantly different.

Microencapsulation seems to be the most promising technology to protect bacteria cells from adverse environment. Godward (2000) reported that microencapsulation improves the viability of probiotic bacteria in yoghurt and therefore, makes it a better probiotic food vehicle. There is increasing evidence that microencapsulation is helpful in protecting the probiotic cultures destined to be added in acidic foods such as yoghurt. Encapsulation facilitates the manufacture of fermented dairy products in which bacteria have constant characteristics and higher stability during storage.

According to Tables 18 and 20, over 30 days of storage period, the pH of yoghurt containing microencapsulated probiotic decreased. The number of cells was reduced over the same period. Between the two probiotics investigated in this study, *L. acidophilus* showed the best survival. It exhibited higher acid resistance and greater stability under the best condition present in yoghurt.

The number of probiotic bacteria remained above the recommended therapeutic minimum throughout the storage period except for *B. bifidum*, which decreased below the therapeutic minimum level in day 0 and after day 3, day 6 for 2%, 4% and 8% *B. bifidum* inoculation level, respectively**.**

The addition of encapsulated probiotic was done at two different stages of fermentation process. The first one was the probiotic and yoghurt culture were added at the same time at the beginning of the fermentation process. The second one was the probiotic was added after the fermentation process had finished. Both methods affected the survival number of probiotic with statistical significance. For the yoghurt which was fermented with yoghurt culture until the pH value became 4.5 and then encapsulated probiotic was added, it was found that the survival number of probiotic was higher than yoghurt which was fermented with both probiotic and yoghurt culture at the same time. The initial amount of *L. acidophilus* at all starting concentration levels was high enough and acceptable for the health, approximately $\geq 10^7$ cfu/g (IDF, 1992), except for *B. bifidum* which decreased below therapeutic minimum.

From Ishibashi and Shimamura (1993) results, it recommended that inoculation of *L. acidophilus* and bifidobacteria should be done simultaneously during fermentation since bifidobacteria are anaerobic and *S. thermophilus* has a high oxygen utilization ability, which results in the removal of dissolved oxygen in the product and

enhances the viability of bifidobacteria. Modler and Villa Garcia (1993) suggested that the cells of *Bifidobacterium spp*. should be added into the yoghurt after fermentations. Based on these studies, *B. bifidum* and *L. acidophilus,* both probiotic bacteria cells, were added into yoghurt after fermentation.

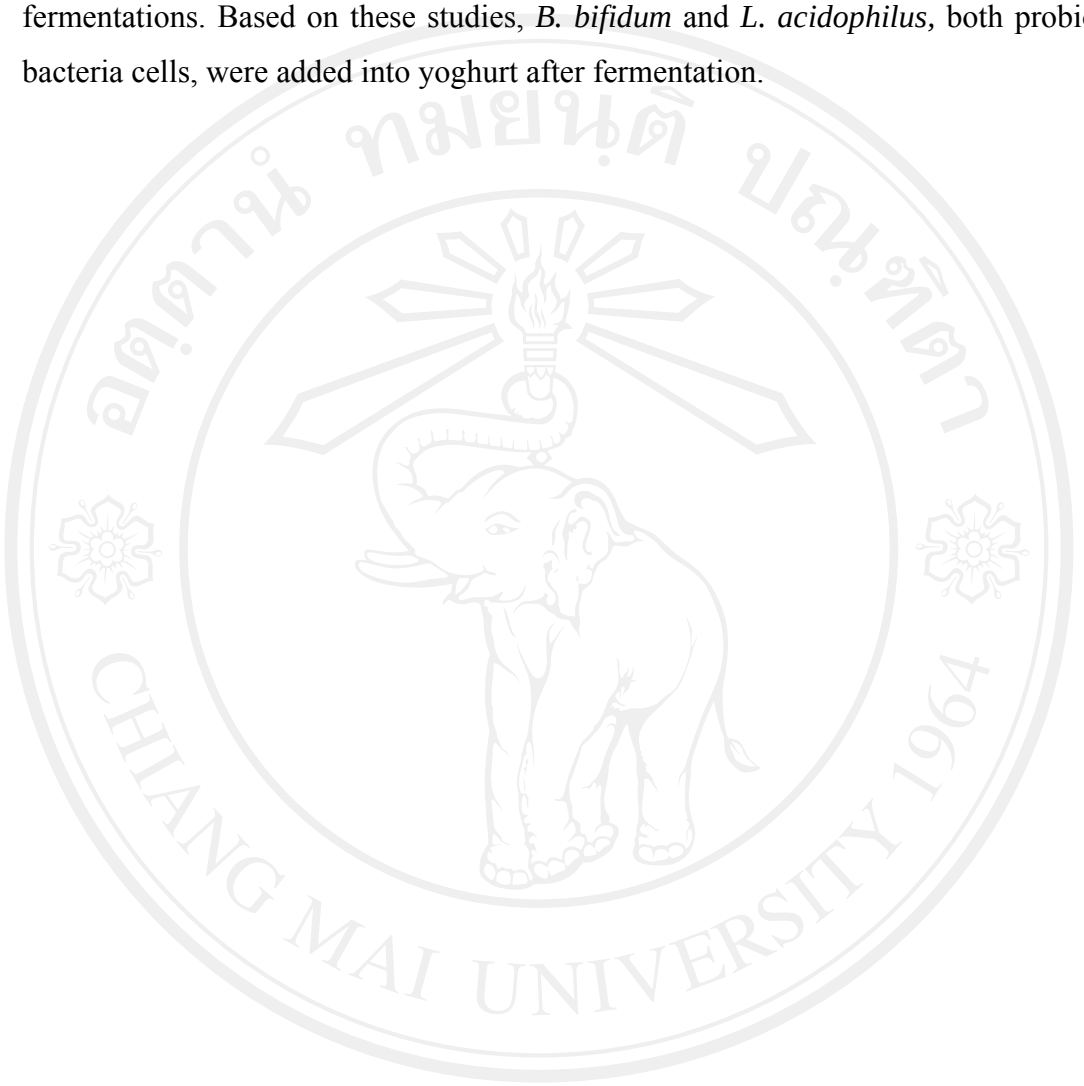

Table 21 The survival number of yoghurt starter culture and probiotic bacteria in goat and cow milk yoghurt powder processed by freeze drying and stored at 4°C for 120 days in different packaging materials

Time of Preservation (Days)	The microorganism number (cfu/g)					
	Yoghurt starter culture* *S. thermophillus* *L. bulgaricus*		*L. acidophillus*		*B. bifidum*	
	Laminated plastic	Aluminium Foil	Laminated plastic	Aluminium Foil	Laminated plastic	Aluminium Foil
0	$3.4\pm0.2\times10^{10a}$ $3.3\pm0.6\times10^{10a}$	$3.7\pm0.3\times10^{10a}$ $3.4\pm0.5\times10^{10a}$	$5.9\pm0.4\times10^{10a}$	$6.7\pm0.8\times10^{10a}$	$6.8\pm0.4\times10^{7a}$	$7.2\pm0.6\times10^{7a}$
15	$0.6\pm0.3\times10^{9b}$ $0.5\pm0.8\times10^{9b}$	$2.7\pm0.7\times10^{9b}$ $1.4\pm0.3\times10^{9b}$	$1.1\pm0.2\times10^{8b}$	$4.4\pm0.4\times10^{8b}$	$8.8\pm0.2\times10^{5b}$	$6.2\pm0.2\times10^{6b}$
30	$1.4\pm0.1\times10^{8c}$ $1.3\pm0.2\times10^{8c}$	$3.6\pm0.3\times10^{8c}$ $3.0\pm0.1\times10^{8c}$	$3.4\pm0.8\times10^{7c}$	$9.6\pm0.3\times10^{7c}$	$3.2\pm0.6\times10^{4c}$	$3.4\pm0.4\times10^{5c}$
40	$3.2\pm0.4\times10^{7d}$ $2.9\pm0.3\times10^{7d}$	$1.8\pm0.6\times10^{8d}$ $1.0\pm0.2\times10^{8d}$	$9.1\pm0.6\times10^{5d}$	$1.2\pm0.2\times10^{6d}$	$7.6\pm0.2\times10^{2d}$	$8.1\pm0.4\times10^{3d}$
60	$2.9\pm0.2\times10^{6e}$ $2.5\pm0.1\times10^{6e}$	$3.1\pm0.1\times10^{7e}$ $2.6\pm0.3\times10^{7e}$	$1.3\pm0.2\times10^{5e}$	$6.1\pm0.4\times10^{5e}$	ND	$2.2\pm0.2\times10^{2e}$
120	$2.4\pm0.2\times10^{3f}$ $2.0\pm0.4\times10^{3f}$	$3.1\pm0.2\times10^{3f}$ $3.0\pm0.3\times10^{3f}$	ND	ND	ND	ND

* Yoghurt starter culture is amount of *S. thermophillus* and *L. bulgaricus*

** Means in the same row with different small letter a-d superscripts for the start time of analysis are significantly different; e-h superscripts for the end time of analysis are significantly different.

Table 22 The survival number of yoghurt starter culture and probiotic bacteria in goat and cow milk yoghurt powder processed by spray drying and stored at 4 ^{o}C for 120 days in different packaging materials

Time of Preservation (Days)	The microorganism number (cfu/g)					
	Yoghurt starter culture* *S. thermophillus* *L. bulgaricus*		*L. acidophillus*		*B. bifidum*	
	Laminated plastic	Aluminium Foil	Laminated plastic	Aluminium Foil	Laminated plastic	Aluminium Foil
0	$3.7\pm0.4\times10^{7a}$ $2.5\pm0.2\times10^{7a}$	$3.9\pm0.2\times10^{7a}$ $3.8\pm0.1\times10^{7a}$	$5.8\pm0.7\times10^{6a}$	$6.2\pm0.8\times10^{6a}$	$2.8\pm0.2\times10^{4a}$	$7.4\pm0.3\times10^{4a}$
15	$0.6\pm0.1\times10^{7b}$ $0.5\pm0.2\times10^{7b}$	$2.1\pm0.2\times10^{7b}$ $1.3\pm0.2\times10^{7b}$	$2.0\pm0.2\times10^{6b}$	$3.7\pm0.3\times10^{6b}$	$7.7\pm0.6\times10^{3b}$	$1.2\pm0.7\times10^{4b}$
30	$2.8\pm0.3\times10^{6c}$ $2.5\pm0.2\times10^{6c}$	$3.6\pm0.6\times10^{6c}$ $3.3\pm0.2\times10^{6c}$	$4.8\pm0.3\times10^{5c}$	$7.7\pm0.4\times10^{5c}$	$3.2\pm0.8\times10^{2c}$	$6.6\pm0.2\times10^{3c}$
40	$2.1\pm0.2\times10^{5d}$ $1.6\pm0.2\times10^{5d}$	$0.6\pm0.1\times10^{5d}$ $0.6\pm0.2\times10^{5d}$	$1.1\pm0.2\times10^{4d}$	$2.0\pm0.5\times10^{5d}$	ND	$7.2\pm0.4\times10^{2d}$
60	$1.6\pm0.1\times10^{3e}$ $1.0\pm0.2\times10^{3e}$	$2.5\pm0.2\times10^{4e}$ $2.3\pm0.4\times10^{4e}$	$5.6\pm0.4\times10^{3e}$	$4.8\pm0.4\times10^{4e}$	ND	ND
120	ND	ND	ND	ND	ND	ND

* Yoghurt starter culture is amount of *S. thermophillus* and *L. bulgaricus*

** Means in the same row with different small letter a-d superscripts for the start time of analysis are significantly different; e-h superscripts for the end time of analysis are significantly different.

The effect of freeze drying on the viability of probiotic organisms is shown in Table 21. The rate of the loss on cell viability depended on the microorganism types. In general, yoghurt starter culture had higher survival rate than *L. acidophilus* and *B. bifidum*. The highest amount of all microorganisms was shown on day 0 and then decreased during the storage period. Yoghurt starter culture, *L. acidophilus* and *B. bifidum* had the highest amount at 7.1×10^{10} , 6.7×10^{10} and 7.2×10^{7} cfu/g, respectively, and the lowest amount of them were 5.4×10^{6} , 1.3×10^{5} and 2.2×10^{2} cfu/g at day 60, respectively. However, the amount of probiotic, *L. acidophilus* and *B. bifidum* remained at the level of health benefit until day 30 and day 15, respectively.

Regarding the spray drying and probiotic survival (Table 22), the results obtained showed that the counts decreased during storage more than the products made from freeze-dried process. Initial cell count of yoghurt starter culture, *L. acidophilus* and *B. bifidum* ranged from 7.0 to 7.7×10^{7} , 5.8 to 6.2×10^{6} and 2.8 to 7.4×10^{4} cfu/g, while the final counts ranged from 2.6×10^{3} to 4.8×10^{4}, 5.6×10^{3} to 4.8×10^{4} and 7.2×10^{2} cfu/g, respectively. Tables 21 and 22 showed the decrease of yoghurt starter culture, *L. acidophilus* and *B. bifidum* cell viability in different packaging materials. In general, larger reductions were found in laminated plastic pouch packaging. *B. bifidum* were not detected in spray-dried yoghurt product and kept in the laminated plastic pouch after 30 days of storage.

B. bifidum was a more sensitive probiotic bacterium, since higher values of cell decrease were found for it in both drying methods and packaging conditions. In every case, at the end of the storage, the counts of starter culture and probiotic microorganism were significantly different from the initial ones.

In general, freeze drying resulted in a higher survival percentage of all microorganisms than spray drying. For example, yoghurt starter culture in freeze-dried goat and cow milk yoghurt packed in laminated plastic pouch showed a survival rate of 5.4×10^{6} cfu/g on day 60 of storage while a significantly lower survival rate of only 2.6×10^{3} cfu/g was noted in the spray dried product. It was also noted that the survival rate of *L. acidophilus* and *B. bifidum* was similar to the above result, freeze drying enabled probiotic bacteria to exhibit a higher number of survival than spray drying. Furthermore, *L. acidophilus* survived better than *B. bifidum* in the goat and

cow milk yoghurt powder and was similar to yoghurt starter culture after freeze drying or spray drying**.**

Freeze drying had a less deleterious effect on the viability of probiotic bacteria than spray drying. Bacteria resistance to freeze drying depends on a variety of factors related to the microorganisms themselves and to the manufacturing condition. The species also influences bacterial resistance to freeze drying. Lactobacilli generally survive better than bifidobacteria due to difference in cell size and cell structure (Elena *et al*., 2006).

Damage to biological systems from freeze drying can be attributed to two primary causes: changes in the physical state of the membrane lipids and changes in the structure of sensitive protein. In general, the Gram positive cocci are the most resistant to freeze drying, and *Lactobacillus* is more resistant than bifidobacteria. During spray drying, lethal thermal injury is the main reason for reduced cell viability. Generally, it was found that the percentage of survival of lactobacilli strain was higher than bifidobacteria (Wen-Chian *et al.,* 2001). Johnson and Etzel (1995) reported that increasing outlet air temperature reduced the survival of microorganisms after spray drying.

Table 23 The physical and chemical characteristics of goat and cow milk yoghurt powder processed by spray drying and stored at 4°C for 120 days in laminated plastic packaging

Yoghurt Characteristics	Time of preservation (days)						
	0	5	15	30	45	60	120
L* Color value (Brightness)	115.64±2.6[a]	112.37±3.9[b]	103.71±2.5[c]	91.49±3.7[e]	87.12±2.1[f]	84.29±1.6[g]	80.19±2.1[h]
a* Color value (Red - Green)	-1.80±0.10[a]	-1.74±0.12[b]	-1.70±0.2[b]	-1.61±0.15[c]	-1.54±0.12[d]	-1.33±2.4[e]	-1.02±1.2[f]
b* Color value (Yellow - Blue)	9.56±0.71[a]	9.51±0.80[a]	9.46±0.62[b]	9.37±0.5[c]	9.11±0.46[d]	9.01±0.6[e]	8.81±2.0[f]
Viscosity (cp)	4260±120[a]	4290±158[b]	4300±133[b]	4417±110[c]	4460±163[c]	4501±178[d]	4523±120[e]
pH value (pH)	4.63±0.06[a]	4.57±0.02[b]	4.51±0.07[b]	4.46±0.02[c]	4.25±0.64[d]	4.23±0.02[d]	4.05±0.13[e]
Moisture (%)	11.35±0.41[a]	11.41±0.44[b]	11.63±0.56[c]	11.84±0.40[d]	11.91±0.21[e]	12.15±0.61[f]	12.35±0.35[g]

**** Means in the same row with different small letter superscripts are significantly different

Table 24 The physical and chemical characteristics of goat and cow milk yoghurt powder processed by spray drying and stored at 4°C for 120 days in aluminium foil packaging

Yoghurt Characteristics	Time of preservation (days)						
	0	5	15	30	45	60	120
L* Color value (Brightness)	120.69±1.2[a]	117.10±1.0[b]	112.0±3.0[c]	93.14±2.5[d]	89.80±3.1[e]	87.99±2.5[f]	84.55±1.1[g]
a* Color value (Red - Green)	-1.88±0.2[a]	-1.71±0.10[b]	-1.66±0.12[c]	-1.60±1.5[c]	-1.50±0.13[d]	-1.38±0.2[e]	-1.17±3.1[f]
b* Color value (Yellow - Blue)	9.67±0.42[a]	9.58±0.5[a]	9.49±0.17[b]	9.41±0.22[b]	9.26±0.20[c]	9.10±0.45[d]	8.91±2.0[e]
Viscosity (cp)	4347±160[a]	4361±144[b]	4380±180[b]	4402±160[c]	4439±147[d]	4498±121[e]	4512±120[f]
pH value (pH)	4.70±0.04[a]	4.61±0.03[b]	4.55±0.01[b]	4.47±0.04[c]	4.29±0.66[d]	4.20±0.01[d]	4.25±0.24[d]
Moisture (%)	11.32±0.43[a]	11.37±0.44[b]	11.46±0.71[c]	11.66±0.66[d]	11.71±0.37[e]	11.79±0.37[f]	11.88±0.35[g]

**** Means in the same row with different small letter superscripts are significantly different

Table 25 The physical and chemical characteristics of goat and cow milk yoghurt powder processed by freeze drying and stored at 4°C for 120 days in laminated plastic packaging.

Yoghurt Characteristics	Time of preservation (days)						
	0	**5**	**15**	**30**	**45**	**60**	**120**
L* Color value (Brightness)	118.70±2.1[a]	114.43±2.7[a]	109.0±2.5[b]	101.17±2.4[c]	96.46±1.8[d]	91.36±2.2[e]	87.19±1.1[f]
a* Color value (Red - Green)	-1.81±0.12[a]	-1.78±0.24[a]	-1.75±0.20[b]	-1.71±0.18[b]	-1.67±0.15[b]	-1.61±0.14[c]	-1.43±2.2[d]
b* Color value (Yellow - Blue)	9.61±0.66[a]	9.57±0.54[b]	9.50±0.50[c]	9.44±0.40[d]	9.39±0.67[e]	9.31±0.60[e]	9.11±1.2[f]
Viscosity (cp)	4256±105[a]	4283±110[b]	4310±122[c]	4436±170[d]	4450±112[d]	4490±107[e]	4500±120[f]
pH value (pH)	4.65±0.04[a]	4.62±0.02[a]	4.54±0.06[b]	4.50±0.03[b]	4.43±0.04[c]	4.40±0.06[c]	4.45±0.11[c]
Moisture (%)	11.74±0.33[a]	11.79±0.47[a]	11.84±0.36[b]	11.97±0.20[c]	12.19±0.12[d]	12.28±0.18[e]	12.34±0.31[f]

**** Means in the same row with different small letter superscripts are significantly different

Table 26 Physical and chemical characteristics of goat and cow milk yoghurt powder processed by freeze drying and stored at 4°C for 120 days in aluminium foil packaging.

Yoghurt Characteristics	Time of preservation (days)						
	0	**5**	**15**	**30**	**45**	**60**	**120**
L* Color value (Brightness)	117.16±1.8[a]	116.20±2.0[a]	113.16±1.0[b]	108.20±2.4[c]	105.11±2.8[c]	101.32±2.7[d]	98.14±0.8[e]
a* Color value (Red - Green)	-1.88±0.10[a]	-1.84±0.12[a]	-1.81±0.27[a]	-1.78±0.20[b]	-1.72±0.18[b]	-1.64±0.10[c]	-1.53±1.4[d]
b* Color value (Yellow - Blue)	9.68±0.44[a]	9.61±0.38a	9.54±0.47[b]	9.50±0.55[b]	9.48±0.88[b]	9.41±0.20[c]	9.21±2.2[d]
Viscosity (cp)	4320±104[a]	4346±110[b]	4357±101[b]	4369±114[c]	4378±104[d]	4391±121[d]	4408±120[e]
pH value (pH)	4.62±0.01[a]	4.59±0.03[b]	4.56±0.02[b]	4.52±0.01[c]	4.50±0.02[c]	4.48±0.01[c]	4.51±0.32[c]
Moisture (%)	11.71±0.47[a]	11.74±0.27[a]	11.79±0.17[a]	11.86±0.20[b]	11.92±0.30[c]	11.98±0.21[d]	12.09±0.31[e]

**** Means in the same row with different small letter superscripts are significantly different

It is reported that oxygen, moisture and light are detrimental to probiotic culture (Miller *et al.,* 2002). Therefore, to maintain high viability with appropriate packaging material and storage condition is one of the important issues concerned in the yoghurt production.

In this study, dried probiotic-added goat and cow milk yoghurt was packed in laminated plastic pouch and aluminium foil and held at 4^{o}C for a period of 120 days. Tables 21 and 22 showed the survival of yoghurt starter culture, *L. acidophilus* and *B. bifidum* during the storage period. It was found that, regardless of packaging material and storage condition, the viable cells of all microorganisms were decreased as the storage time increased. However, a higher viable population of yoghurt starter culture and probiotic cell was found in the yoghurt powder packed in aluminium foil and a lower reduction rate of their survival number in this packaging was noted than when the yoghurt was kept in laminated plastic pouch.

Ishibashi and Shimamura (1993) observed that permeation of oxygen through packaging during storage affected the viability of bifidobacteria in milk or yoghurt. The higher oxygen permeability of the package, the lower the viability was. In the present study, both yoghurt starter culture and probiotic bacteria were found to survive better in goat and cow milk yoghurt powder kept in aluminium foil than in laminated pouch.

Considering the drying processes from the experiment results, goat and cow milk yoghurt added with encapsulated probiotic which passed the freeze drying process had a better result than encapsulated probiotic which passed the spray drying process. Freeze drying process could produce a higher initial microbe and probiotic survival because the drying temperature was lower compared to the spray drying process, so the organism number in the final product was higher than that from the spray drying process.

When the product from freeze drying process was kept in laminated plastic pouch or aluminium foil at the temperature of 4^{o}C for 120 days, it was found that the product in aluminium foil changed less than the product in laminated plastic pouch from the aspect of microbiological, chemical and physical properties. The color of the product in an aluminium foil changed less than the products in laminated plastic pouch because the aluminium foil has the properties to protect UV and O_2 that

stimulate the reaction in the product (Ishibashi and Shimamura, 1993). The lactose in the yoghurt is converted to lactic acid so that the sugar is less and the product could not change the color. The good property of aluminium foil is that it can resist the air quite well and then the humidity is not too high (Roy, 2005). It is shown that a food product that can retrieve the water back rapidly to the product from the drying process is crucial to use aluminium foil because it can resist light, water and air quite well (Talwalkar *et al.*, 2004). In general, the pH of the yoghurt powder was significantly reduced in the first and second month of storage, followed by a lower reduction for the rest of the storage period (Tables 23 – 26), both for yoghurt powder packed in aluminium foil and in laminated plastic pouch. In the product which was made from freeze drying and packed in aluminium foil packaging, the reduction of pH was the slowest compared with others. This might be due to chemical reaction that could occur at low water activity and in the presence of low oxygen content inside the aluminium foil packaging (Talwalkar and Kailasapathy, 2003). These results agreed with the finding reported by Jawalekar *et al*. (1993). As revealed from the paper data, pH decreased throughout the storage period of cow and buffalo milk yoghurt. The decrease of pH was due to the formation of lactic acid by certain bacteria of yoghurt (Abrahamsen, 1973). It was also reported earlier that pH decrease with an increase in the storage period.

Since the laminated plastic pouches used to pack the yoghurt product in this study were not an air-tight container, the moisture content of the yoghurt powder during storage significantly increased after 120 days of storage period, indicating the unsuitability of the packaging material to keep the product. The aluminium foil packaging material was shown to be better in maintaining the moisture content of the yoghurt powder, although it could not completely protect the food product. Changes in the yoghurt chemical properties, pH, moisture content could also affect the survival of probiotic bacteria in the yoghurt powder.

For microbiological quality, it was found that the number of yoghurt starter culture and probiotic decreased in both the laminated plastic pouch and aluminium foil but the decrease level in aluminium foil was lower than that in the laminated plastic. In terms of container technology, it is widely accepted that laminated plastic

pouch is one of the best materials to be used as a container, especially the food product which has microbe as one of the ingredients, because it can resist the air quite well. The microorganisms inside the container can use some air but for some probiotic such as strictly-anaerobic one does not need O_2 to survive. So laminated plastic pouch is not considered proper to be used as a container for those products. Since the circulation rate of air in aluminium foil is very low, it is appropriate to be used as a container for products with probiotic and the amount of survival of probiotic will be usually high, especially, *B. bifidum* which is very weak and more sensitive to O_2. If the microbe is kept in an aluminium foil, it should survive more as well (Wang *et al.*, 2004).