CHAPTER 1
INTRODUCTION

1.1 Background information

During the past twenty years there has been a tremendous increase in the worldwide sales of cultured products containing probiotic bacteria. Today, most probiotic strains are used in yogurts, fermentation milks, ice cream and pharmaceutical products for their anecdotal health effect. Increasing knowledge underlines the important role of the intestinal flora for maintaining health and in the prevention of disease. Probiotics offer dietary means to support the balance of intestinal flora. The microorganisms primarily associated with this balance are lactobacilli and bifidobacteria (Ostme *et al.*, 2005).

Among the dairy-fermented products, yoghurt is the most popular one. At the same times the highest consumption of probiotic products is associated with probiotic yoghurt (Lourens-Hattingh and Viljoen, 2001). During the manufacture probiotic fermented dairy product, three main aspects, which have been identified that can affect the quality of the product are: 1, loss of viability of probiotic microorganism during the fermentation period and refrigerated storage; 2, relatively long incubation time is required when compared to traditional yoghurt and 3, unsaticfactory organoleptic properties of the final product. To improve the fermentation time and organoleptic properties of probiotic yoghurt, co-culturing of traditional yoghurt bacteria with probiotic microorganism is a common practice and therefore various culture combinations are used, such as BY (*Bifidobacterium* spp. and yoghurt bacteria), AY (*Lactobacillus acidophilus* and yoghurt bacteria), ABT (*Lactobacilus acidophilus, Bifidobacterium* spp. and *Streptococcus thermophilus*) and ABY (*Lactobacilus acidophilus, Bifidobacterium* spp. and yoghurt bacteria) and are commercially available on the market (Mortazavain *et al.*, 2006).

Probiotic survival in products is affected by a range of factors including pH, post-acidification (during storage) in fermented products, hydrogen peroxide production, oxygen toxicity (oxygen permeation through packaging), storage temperatures, stability in dried or frozen form, poorly growth in milk, lack of proteases to breakdown milk

Chandramouli, V., Kailasapathy, K., Peiris, P. and Jones, M. (2004) An improved method of microencapsulation and its evaluation to protect Lactobacillus spp. in simulated gastric conditions. . Journal of Microbiology Methods, 56, 27-35.
Grosso, C. R. F. and Favaro-Trindade, C. S. (2004) Stability of free and immobilized Lactobacillus acidophilus and bifidobacterium lactis in acidified milk and of immobilized B. lactis in yoghurt. Journal of Applied Microbiology, 84, 342-348.
Hansen Treulstrup, L., Allan-Wojtas, P. M., Jin, Y. L. and Paulson, A. T. (2002) Survival of Ca-alginate microencapsulated Bifidobacterium spp. in milk and simulated gastrointestinal conditions. Journal of Food Microbiology, 62, 47-55.
Iyer, C. and Kailasapathy, K. (2005) Effect of co-encapsulation of Probiotics with Prebiotics on increasing the viability of encapsulated bacteria under in vitro acidic and bile salt condition and in yoghurt. Journal of Food Science 70, M18-M23.
Kraseakoopt, W., Bhandari, B. and Deeth, H. (2003) Evaluation of encapsulation techniques of probiotics for yoghurt. International Dairy Journal 13, 3-13.
Kumar, P. and Mishra, H. N. (2004) Storage stability of mango soy fortified yoghurt powder in two different packaging material: HDPP and ALP. Journal of Food Engineering, 65, 569-576.
Lourens-Hattingh, A. and Viljoen, B. C. (2001) Yoghurt as probiotic carrier food. International Dairy Journal, 11, 1-17.
Miltz, J. and Ulitzur, S. (1980) A bioluminescence method for the determination of oxygen transmission rates through plastic films. Journal Food Technology, 15, 389-396.
Mortazavain, A. M., Ensani, M. R., Mousavi, S. M., Reinheimer, J. A., Emamdjomeh, Z., Sohrabvandi, S. and Rezaei, K. (2006) Prelimanary investigation of the combined effect of heat treatment and inocubation temperature on the viability of the probiotic micro-organisms in freshly made yoghurt. International Journal of Dairy Technology, 59, 8-11.
N.P., S. (2000) Symposium: Probiotic bacteria
Probiotic Bacteria: Selective Enumeration and Survival in Dairy foods. Journal of Dairy Science, 83, 894-907.
Ostme, H. M., Tremo, J. and Narvhus, J. A. (2005) Effect of temperature on growth and metabolism of probiotic bacteria in milk. International Dairy Journal, 15, 989-997.
Rybka, S. and Kailasapathy, K. (1996) Media for the enumeration of yoghrut bacteria. International Dairy Journal, 6, 839-850.
Selvamuthukumaran, M. and Shankarshukla, S. (2006) Optimization of Spray drying conditions for Production of Bifidus Milk Powder from cows milk. Journal of Food Quality, 26, 305-318.
Sheu, T. Y. and Marshall, R. T. (1993) MIcroentrapment of Lactobacilli in Calcium Alginate Gels. Journal of Food Science, 54, 557-561.
Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P. and Kailasapathy, K. (2000) Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. International Journal of Food Microbiology, 62, 47-55.

protein to simpler nitrogenous substances and compatibility with traditional starter culture during fermentation (Rybka and Kailasapathy, 1996; Shah , 2000).

The survival of *L. acidophilus* and *Bifidobacterium* spp. in yoghurt has been shown to be a problem, due to their intolerance of acid conditions and the presence of other cultures, such as *Lactobacillus delbrueckii* spp. *bulgaricus* (Grosso and Favaro-Trindade, 2004). However, microencapsulation or immobilization techniques could provide protection to acid sensitive bifidobacteria and thus, increase their survival rate during the shelf life of the yoghurt and during their passage through the gastrointestinal tract (Hansen Treulstrup *et al.*, 2002).

The method of immobilization by extrusion is the most common approach to make capsules with hydrocolloids. It simply involves preparing a hydrocolloid solution or setting bath (Kraseakoopt *et al.*, 2003). Calcium alginate has also been used widely for the immobilization of lactic acid bacteria (Sheu and Marshall, 1993) due to its ease of handling, its non-toxic nature and its low cost (Sultana *et al.*, 2000). Although calcium alginate encapsulation has been widely used for probiotic bacteria, there is no uniformity in the literature as to the protective nature of the capsule against adverse gastrointestinal conditions and in product shelf life (Chandramouli *et al.*, 2004).

Some sciencetists suggested that prebiotics may improve the survival of bacteria crossing the upper part of the gastrointestinal tract, thereby enhancing their effects in the large bowel. To further increase the viability of encapsulation bacteria, the effect of adding complementary prebiotics has been assessed especially starch (Iyer and Kailasapathy, 2005). When the supporting material of alginate is combined with starch, the probiotic bacteria can grow in the beads. It has been reported that a combination of alginate with hi-maize starch (prebiotic) prepared using an extrusion technique could support probiotic bacteria is survive in the acid condition of yoghurt and in the acid-bile condition inside gastrointestinal tract of human (Jankowski *et al.,* 1997). In the light of this report, the combination of alginate hi-maize starch was used in this study to support the survival of *L. acidophilus* in dried yoghurt.

The shelf life of yoghurt is very short for example 1 day at ambient temperature (25-35^{o}C) and about 4-5 days at 7^{o}C (Kumar and Mishra, 2004). Besides the huge acidity of yoghurt, the product is needed to be kept at low temperature (less than 5^{o}C) to prevent off-flavor from the continuous activity of lactic acid bacteria in the product. The need of low storage temperature causes a difficulty to sell yoghurt in the areas that are lacked with refrigerators, which further leads to a lower consumption of the product. One alternative to solve this problem is to dry the product. Spray drying is one of the most effective process in extending the shelf life of dairy products. This process has the advantages of long time preservation, convenience in handing, storage, marketing and consumption. Although a number of dried fermented milk products are available as pharmaceutical preparation or as healthy foods, their viable counts are low (Selvamuthukumaran and Shukla, 2006).

Kim *et al.* (1990) had reported that if the outlet temperature during drying is less than 100^{o}C, bacteria can have a chance to survive during a drying process. A higher survival rate of bacteria can be achieved when using a lower outlet temperature, such as at 60^{o}C. Beside that, the survival of a bacterium during a drying process is also affected by the type of food. Rehydration is a complex process aimed at the restoration of raw material properties when dried material is contacted with water. There is no consistency in procedures used, nor in nomenclature. The ratio between the dry material mass and water mass varied from 1:5 to 1:50 and the temperature of rehydrating water is from room temperature to boiling. During storage of yoghurt powder many chemical changes occur and starter culture counts decrease. Common effects of improper storage conditions on products such as yogurt powder are browning (Kumar and Mishra, 2004).

Application of plastic films and laminates in flexible packaging in the food industry is expanding very rapidly. Plastic pouches and bags are widely used for dried, frozen and baked products as well as coffee and spices, and recently for liquids. The advantages of these films and laminates over the packaging material previously used stem from the ease of their manufacture, excellent seal ability, variety of permeabilities of different gases and vapor and their low cost. Many foods undergo oxidation in the presence of atmospheric oxygen followed by off-flavor and off-taste formation which reduce their shelf life (Miltz and Ulitzur, 1980).

This work studied about the survival of *L. acidophilus* in spray dried drinking yoghurt. This topic was chosen because yoghurt powder is a healthy food product that can be stored at ambient condition. The high storage temperature to keep the product will make the product to be convenient to be transported, stored and consumed. It will also give a benefit for distribution of the product to the areas that are lacked with refrigerators. The product would be a new choice of healthy food products that would deliver beneficial microorganism to the end-customers.

1.2 Objectives of this research

The main purpose of this research was to investigate the effect of an immobilization process on the survival of *L. acidophilus* in dried yoghurt.

The specific objectives of this research were:

1. To find the beat concentration of supporting material (alginate and hi-maize starch) to immobilized L. acidophilus and increase its survival and recovery rates during storage in a dry product.
2. To study the best addition time and concentration of immobilized cell of *L. acidophilus* to produce a high recovery rate of *L. acidophilus* in yoghurt.
3. To know the best outlet temperature of a spray drier that produces the highest survival rate of *L. acidophilus* in yoghurt powder.
4. To investigate the optimum water temperature to rehydrate probiotic added yoghurt powder.
5. To understand the suitable packaging material to keep probiotic added yoghurt powder during storage at different storage temperatures.