CHAPTER 1
INTRODUCTION

1.1 Background information

Probiotics are defined as live microbial food ingredients that have beneficial effects on human health by improving the microbial balance in the gut. The commonly studied probiotic bacteria include members of the genera *Lactobacillus* and *Bifidobacterium* (Sanders, 1999; Siuta-Cruce and Goulet, 2001). *Lactobacillus* is a facultative anaerobe bacterium, that survives in either low oxygen or anaerobic conditions. *Lactobacillus* can survive in fermented milk products. *Bifidobacterium* is a strict anaerobic bacterium that is intolerant to high acidity. Therefore, *Bifidobacterium* generally has a limited capability to grow in the fermented milk products. Reported literature indicate that consumption of probiotic bacteria at high level of 10^9-10^{11} viable cells per day could decrease the incidence, duration, and severity of some intestinal illnesses. The low quantity of *Bifidobacterium* in fermented milk causes the bacterium to be less effective to promote human health benefits (Sanders, 1999). Using cell immobilization technique to entrap bifidobacteria within the supporting material, and then coating the supporting material with edible bilayer films should increase the survival and delivery of bifidobacteria through the gastrointestinal tract.

An immobilized cell is defined as a cell that is prevented from moving independently or the cell that the freedom of movement is restricted in supporting material (Tampion and Tampion,1987). There are two common methods of cells immobilization that has been reported: adsorption and encapsulation. Adsorption is based on the physical adsorption of cell proteins on a surface of the carriers. Adsorption of cells onto the solid surface is probably the mildest and cheapest of cell immobilization techniques. Adsorption has major disadvantage that the adsorbed cells may be leaked from the carrier during usage due to a weak binding force between the cells and the carrier (Tampion and Tampion, 1987). Encapsulation is the method to incorporate cell into the lattices of a semi-permeable gel or enclosing the cell in a semi-permeable polymer membrane. The protection provide by encapsulation prevent cell degradation due to exposure to light or oxygen (Risch and Reineccius, 1995).

Edible film is defined as an edible coating material on food components. The film is used to inhibit migration of moisture, oxygen, and carbon dioxide between the commodity and the external atmosphere. Edible films have been used to promote the anaerobic condition within the coated materials. The edible films are effectively coated on foods by dipping, spraying, or panning (Krochta and Mulder-Johnston, 1997). Components of edible films are divided into four categories: polysaccharides, proteins, lipids, and composites. Lipids are waxes, acylglycerols or fatty acids. The composite edible films contain both lipid and polysaccharide or protein components in the form of bilayer or emulsion of the two components. The combination of the composite films can be formulated to gain the advantages of the two components and, besides, to reduce the limitation of each individual component (Donhowe and Fennema, 1994). The barrier property that prevents the migration of moisture and gas, of the bilayer films is more effective than the emulsion films (Wong *et al.*, 1992; as cited by Wong *et al.*, 1994).

Tapioca starch beads (TSB) or tapioca starch pearls are recognized as food ingredient in Thailand including many countries in Asia and South-East Asia. Tapioca starch beads are made from the tapioca (*Manihot esculenta* Crantz) starch. The commercial production of TSB is as follows: adjusting the moisture content of tapioca starch powder to the level that the starch powder could be attached to each other, forming the bead in the rolling bowl, drying, and size-screening, respectively (Thaiwa Co. Ltd., Bangkok, Thailand, unpublished data). The diameter of the beads is between 1.4 and 2.8 mm for the small size and between 4.8 and 6.7 mm for the large size (Thai Standard for Industrial Products 1011-2533, 1991). The advantages of TSB are non-toxic in nature, very low cost (ca 12 baht or 0.30 US$ per kg), easy to handling, and commercial available as food ingredient. In order for bifidobacteria to exert beneficial health effects, it is important to ensure their safe delivery to the human colon. Sultana *et al.* (2000), Sun and Griffiths (2000), and Khalil and Mansour (1998) reported using immobilization techniques to increase the survival of bifidobacteria. However, insufficient quantities of the immobilized bifidobacteria (10^2-10^6 CFU/mL) survived after storage at 4°C for 8-16 weeks (Sultana *et al.*, 2000; Sun and Griffiths, 2000).

In this study, the freeze-dried-gelatinized-tapioca starch beads (FDTB) was used as a novel supporting material for immobilization of bifidobacteria. The FDTB with immobilized bacteria were then coated with the edible bilayer films of sodium caseinate and fat. According to our knowledge, there is no research report on applying an immobilization of cells on FDTB and edible bilayer films to increase the survival of bifidobacteria. This research is aimed to increase the survival of the immobilized *Bifidobacterium longum*, *Bifidobacterium bifidum* and *Bifidobacterium infantis* in yogurt and in the simulated gastrointestinal system of human. The outcome of this research should lead to increase the survival of bifidobacteria in food products that do not have favorable conditions for their survival.

1.2 Objectives of the research

The main purpose of this research is to investigate the effect of edible films and coatings on the survival of the immobilized *Bifidobacterium* in FDTB. The research outcome will lead to the technology that will improve the quantity and survival of probiotic bacteria in food products and provide the beneficially choice for the food manufacturers and consumers in health probiotic food product's market.

The specific objectives of the research were:

1. To investigate the moisture content and the physical properties including bulk volume, diameter, microstructure, porosity, specific surface area, water-holding capacity, adsorption capacity, adsorption behavior of freeze-dried-gelatinized tapioca starch beads (FDTB) from two freezing methods (quick vs. slow freezing) and three commercial brands (Golden Chef®, Special Sacoo®, and Thaiworld®).
2. To determine the capacity to load *Bifidobacterium longum*, *Bifidobacterium bifidum*, and *Bifidobacterium infantis* into FDTB produced from two freezing methods (quick vs. slow freezing) and three commercial brands (Golden Chef®, Special Sacoo®, and Thaiworld®).
3. To compare the effect of coating materials; edible fats (palmitic acid, PANODAN®, and beeswax) and sodium caseinate, on the enumeration of immobilized *B. longum*, *B. bifidum*, and *B. infantis*.
4. To evaluate the survival of free cells, non-coated, and coated-dried immobilized *B. longum*, *B. bifidum*, and *B. infantis* in simulated gastrointestinal fluids without enzyme at 37°C for 310 min.
5. To evaluate the survival of non coated and coated immobilized *B. longum*, *B. bifidum*, and *B. infantis* in pasteurized or sterilized yogurt, stored at 4-5°C for 4 wk.
6. To examine the change of three-dimensional structure of the immobilized beads in the simulated gastrointestinal fluids and yogurt stored at 4-5°C for 4 wk by Scanning Electron Microscope (SEM).