Chapter 1

Introduction

1.1 Principle and Rationale

Currently, probiotics have been supplemented to diverse food products to create "functional foods or nutraceuticals" in global markets (Hou *et al.*, 2003). Probiotic containing foods can be categorized as functional foods. The consumption of these products has increased in Europe, Asia Pacific and American countries. More than 90% of these products contain *Lactobacillus acidophilus* (*L. acidophilus*) or bifidobacteria (Dave and Shah, 1998). The market of probiotics containing food categories continues to expand in parallel with growing consumer awareness of the role of diet in health maintenance (Ross *et al.*, 2005).

The idea of using microbes to promote a good health and to prevent disease is not new. Initially, several microbes have been used unintentionally in food production such as dairy products and fermented vegetables. In recent years, there has been a renewed interest in microbial uses due to, apart from improving food flavour, their beneficial aspect in health restoration and disease treatment. Several microorganisms, under the name of "probiotics", have been proposed and used in a wide range of clinical trials, ranging from diarrheal disease to cancer prevention (Fuller, 1994; Kaur *et al.*, 2001). The term "probiotics" was originally used by Lilley and Stillwell (1965) to mean a substance(s) that stimulates growth of other microorganisms. The meaning of this term has now been redefined and restricted to "a viable microbial agent(s) which, when used in animal or man, beneficially affects the host possibly by improving the balance of the indigenous microflora (Fuller, 1991: Salminen *et al.*, 1999). Based on this meaning, several terms such as "friendly", "beneficial" or "healthy" bacteria are also commonly known for probiotics. The first study regarding beneficial effect of probiotics was carried out by Metchnikoff in the early 1900's. He reported the favourable effects of soured milk in human and suggested that consumption of live microbes (possibly LAB) in such fermented milk may help improve the balance of the gut microflora. Since then, microbial probiotics have gained an increasing interest and their use is now widely accepted (Chukeatirote, 2003)

Viability and functional activity of probiotics are major concerns in the functional foods, as a sufficient number of bacteria must resist the gastric juice and bile salts in the gastrointestinal tract before reaching the colon. To provide functional properties of probiotic products, the minimum level of viable probiotic bacteria is approximately 10^6 CFU/ml at the expired date of a product. However it is suggested that a therapeutic dose of probiotic bacteria is at the level of 10^8-10^9 viable cells per day (Hou *et al.*, 2003). Studies indicated that most probiotic products have a short shelf life, even when stored at low temperature (Lee and Salminen, 1995) and they may not survive in sufficient number. A number of studies have indicated that the quantity of viable bacteria in some commercially available products or in the host gastrointestinal tract is actually below the desired level (Shah *et al.*, 1995). To overcome this problem several approaches have being explored to increase the viability of these probiotic bacteria in commercial and experimented products.

including microencapsulation and incorporation of micronutrients such as peptides and amino acids (Hou *et al.*, 2003). To date, encapsulation methods have been widely applied to enhance the viability of probiotic bacteria in commercial products. In some studies, it was shown that 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 (Grosso and Favaro-Trindade, 2004).

Beans are a good source of B vitamins (thiamin, pyridoxine (B6) and niacin) and folic acid. Beans also provide the minerals iron, potassium, selenium, magnesium, zinc and even some calcium. Dried beans and their cousins also are a good source of insoluble fiber, which promotes digestive health and relieves constipation. Beans also provide soluble fiber, which can help reduce fat levels in the blood, non-starch polysaccharides and oligosaccharides. Beans provide little fat and absolutely no cholesterol. Beans have many potentially protective components for example, bioactive microconstituents such as protease inhibitors, saponins, phytosterols, lectins and phytates (Anonymous, 2007a).

At present bean milk has been recognized as a food commodity that supports a good health condition due to their nutritional composition and also as an inexpensive source of energy. At the same time, one of the probiotic bacteria, *L. acidophilus*, can be provided to consumers in a non-fermented acidophilus milk. This acidophilus milk product is prepared by adding *L. acidophilus* cells in a frozen concentrated culture to pasteurized low-fat milk, followed by storage at a refrigeration temperature (Hou *et al.*, 2003).

To provide adequate numbers of lactobacilli in functional foods, the cells must survive the frozen condition and also the subsequent refrigerated storage. Therefore, this research studied about the survival of *L. acidophilus* during chilled storage in non-fermented pasteurized bean milk and the viability of the probiotic bacterium under simulated gastrointestinal conditions. An encapsulation technique using an extrusion method was evaluated to understand its effect on improving the survival of *L. acidophilus* in the bean milk. In addition, the effects of pH value of bean milk and an addition of casein were also investigated to understand whether these factors had a significant contribution to the survival of *L. acidophilus* in the bean milk or not.

1.2 Study objectives

1. To study the survival of *L. acidophilus* in different types of pasteurized bean milk during storage at 4°C.
2. To investigate the effects of pH values, cysteine concentrations, initial concentrations of probiotic bacterium and an encapsulation technique (an extrusion method) on improving the survival of *L. acidophilus* during storage at 4°C in pasteurized bean milk.
3. To understand the survival of *L. acidophilus* in simulated gastrointestinal conditions, mainly high-acid gastric and bile-salt conditions, during the storage shelf-life of the *L. acidophilus* in bean milk at 4°C for 2 weeks.

1.3 Usefulness of the project

1. To increase the commercial value of bean products by incorporating a beneficial bacterium.
2. To develop a new probiotic food product using an agricultural material that is well-known by Thai people.
3. To provide Thai consumers and beyond a functional food product with a high viability of probiotic microorganism during its storage life and subsequent processes in the gastrointestinal tract.
4. To provide more variation of healthy food products for Thai market and beyond.

1.4 Scope of the project

This project was aimed to combine the probiotic bacterium of *L. acidophilus* with pasteurized bean milk to increase the economical value of The bean milk, while continue providing the consumers with a good nutrient composition of the beans. Since the viability of probiotic bacteria is one of the concern in functional foods, this study was not only investigate the survival of *L. acidophilus* during storage in non-fermented pasteurized bean milk, but it also carried out some experiments under simulated gastrointestinal conditions. An encapsulation technique, mainly an extrusion method was also evaluated to understand its effect on improving the survival of *L. acidophilus* in pasteurized bean milk.