CHAPTER 1
INTRODUCTION

1.1 Background information

Milk is food for young mammals and human babies during the first period of their lives. It is a complex fluid containing many components with high nutrients. At the moment, the trend of milk consumption is increased every year. This trend is conformed with the Thai's government policy to promote the usefulness of milk and the challenge to increase the consumption of milk among children. To accomplish this, the government has set up a school milk project for children. In this project, students will be the main target to receive the benefit of drinking milk. This project is done by supplying a budget to some schools to directly give pasteurized milks to their students during the school hours.

Thailand is a tropical country that has a hot and humid weather. This kind of weather gives a suitable condition for microorganisms to grow. At the same time, milk industries in Thailand are also expanded. Dairy farmers are assembled in cooperatives and raw milk is collected collectively from many farms and transferred to the dairy industries. During the collection and transportation, the quality of raw milk will be easily reduced if the temperature of the milk is not strictly controlled under a refrigeration temperature. The quality of raw milk will be worse if the location of the dairy farms is far from the factory and the collection of the milk is not done in a hygienic situation. All of these factors will increase the number of microorganisms in the raw milk and emphasize the importance of milk processing in factories to reduce, eliminate or destroy these organisms.

A heat treatment is the most common method to reduce, eliminate or destroy pathogenic and spoilage microorganisms. Pasteurization is a processing that heats milk at a specific temperature in a certain length of time to destroy pathogenic organisms and weaken the others (Kon, 1975). The shelf life of pasteurized milks depends on the number and type of microorganisms present in raw milk, the time and temperature of pasteurization, a Post Pasteurization Contamination (PPC) and upon the storage temperature. If the PPC is controlled, organisms survived after

pasteurization and growth at refrigeration temperatures become the limiting factor in the shelf life of pasteurized milks. These microorganisms will be dominated by Gram positive spore forming bacteria especially *Bacillus* genus (Harding, 1999). In fact, reports from researches have found *Bacillus* genus as a common spore forming bacterium that present in raw milk and the genus could cause a serious problem in milk industry. Their spores can survive pasteurization processes. Beside that, the vegetative cell of the organism can produce extra-cellular enzymes which cause deterioration in milk and milk products (Christiansson *et al.*, 1999). Among the *Bacillus* genus, *Bacillus licheniformis* was the greatest heat resistance bacterium as compared to the other species. The spore of this species can germinate after a heat treatment at 135°C (Janstova and Lukasova, 2001). For this reason the *Bacillus* genus can be a limiting factor for the shelf life of pasteurized milk and milk products. Therefore, applying a natural preservative will be a method to prevent a further problem from this surviving bacteria. Choosing the best preservative will not only include its safety measure but also its effectiveness against the Gram positive spore forming bacteria, especially *Bacillus* spp. One of the best choices is using a preservative called as nisin.

Nisin is an antimicrobial substance that is produced by *Lactococcus lactis* subsp. *lactis* strain. The organism was reported to be naturally presence in raw milk (Delves-Broughton, 1990). Nisin mainly has an activity against Gram positive bacteria. Several reviews have been published recording its antimicrobial potential (Henning *et al.*, 1986; Breukink and de Kruijff, 1999; Thomas *et al.*, 2000; Cleveland *et al.*, 2001; Wirjantoro *et al.*, 2001). The compound has been commercially produced since 1950's by fermenting its natural producer, *L. lactis* subsp. *lactis*. It is a non-toxic compound and can be digested by enzymes, which will inactivate the compound. Nisin has also been reported to have no effect on the bacterial microflora in the intestinal tract. A Joint FAO/WHO Committee on Food Additive in 1968 has recognized nisin as a safe and legal food preservative. The Scientific Committee for Food of the European Community decided the Acceptable Daily Intake of nisin is 0.1 mg/kg body weight based on a nisin concentration of 4.0×10^4 IU/mg. At the present time, nisin is permitted to be used as a preservative in more than 40 countries (De Vuyst and Vandamme, 1994; Thomas *et al.*, 2000).

A study that combined a heat treatment of milk at 117°C for 2 s and two nisin concentrations of 75 and 150 IU/ml found that nisin was able to inhibit microbial growth in the milk products during storage at 30°C. However, a low level of spoilage was found in some of the milk samples, suggesting the presence of one or more spore-forming bacteria that could resist the presence of nisin. One of these spore forming bacteria was identified as *B. licheniformis* (Wirjantoro *et al.*, 2001). The result of this study suggested that *Bacillus* spp. was an organism that had a higher heat resistant than the majority of indigenous microflora in milk and its presence in milk products should be expected for products that do not receive a sterilization process. Therefore, it is important to have a further understanding about the presence of *Bacillus* spp. in pasteurized milks, especially for the possibility to prevent the growth of this organism in the product.

The overall objectives of this study were to apply nisin to inhibit survival microflora in pasteurized milks at different storage temperatures and to investigate the effect of milk ingredients, the addition time of the antimicrobial compound and the pasteurization condition on the effectiveness of nisin to control the growth of *B. licheniformis*.

1.2 Research objectives

The objective of this research were:

1. To find the optimum level of nisin to control the survival microorganisms in pasteurized milk in order to extend the keeping quality of the product.

2. To study the effect of milk components on the effectiveness of nisin against *B. licheniformis* in imitated milk system.

3. To investigate the effect of nisin addition time on the activity of the antimicrobial compound to control the growth of *B. licheniformis* in imitated milk system.

4. To understand the effect of pasteurization condition on the effectiveness of nisin to inhibit *B. licheniformis* in imitated milk system.

1.3 Research location

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