Chapter 1
Introduction

1.1 Rationale

Fermented soybeans are widely regarded as a healthy food and are consumed in several Asian countries, including Japan (*Natto*), India (*Kinema*), Korea (*Chungkukjang* and *Doenjang*), China (*Douchi*), and Thailand (*Thua Nao*). *Bacillus subtilis* and related bacilli (i.e., *B. licheniformis* and *B. pumilus*) are usually found to be dominant microflora of the fermented soybeans (Tamang and Nikkuni, 1996; Omafuvbe *et al*., 2002; Chukeatirote *et al*., 2006). A major activity in the fermentation is the hydrolysis of proteins to peptides and amino acids which are responsible for the typical 'umami' taste in fermented soybeans (Yoshida, 1998). Subsequently, the utilisation of amino acids by bacteria leads to ammonia formation and thus increases the pH value to alkaline; hence these products are then often known as alkaline soy-fermented foods (Steinkraus, 1996; Allagheny *et al*., 1996). Typical characteristics of traditional fermented soybeans are distinct ammoniacal smell, brownish colour, palatable taste and seed covered with mucous substance. Flavour volatile compounds of fermented soybeans are important to the quality of the product; for example, *Natto*-like odour and fruity/nutty odour are indicative of a good quality product, but ammonia-like and fishy aromas indicate food spoilage.

Isoflavones are phytoestrogens that are abundant in soy-fermented foods and have been related to several health benefits. Increasing evidence suggests that pharmacological and antioxidant properties of soy isoflavones are involved in prevention of breast cancer (Gotoh *et al*., 1998; Peterson *et al*., 1998; Jung *et al*., 2006), antihypertensive effect (Okamoto *et al*., 1995), reduced risk of cardiovascular diseases (Potter *et al*., 1998; Park *et al*., 2003), improvement of bone health (Ishimi *et al.,* 2002), reduced menopause symptoms (Eden, 1998), antimutagenic effects (Park *et al*., 2003) and antidiabetic effects (Liu *et al*., 2006). Additionally, the functionality of soy amino acids has also been reported as a source of antihypertensive peptides,

reduced blood cholesterol concentration, reduction in coronary heart disease risk and increased antioxidant potential (Potter, 1995; Anderson *et al.,* 1995; Saito *et al.,* 2003; Gibbs *et al*., 2004).

Thua Nao products are normally produced by artisanal techniques, which utilise natural *Bacillus* strains as inoculum. Like other traditional soy-fermented foods, soybeans are initially soaked with water over night, boiled for 3 - 4 h, packed in a bamboo basket, covered with banana leaves, and stored at ambient temperature for ~3 days. In contrast, *Natto* is prepared by inoculating autoclaved soybeans with pure starter culture of *Bacillus subtilis* and fermented under controlled conditions (Ohta, 1986; Wei *et al.*, 2001). Good quality *Natto* products are indicated by white coloured mucous substance, characteristic flavour, palatably soft texture, light yellow colour, and ability to generate silky and sticky mass (Wei *et al.*, 2001). *Natto* is widely consumed in Japan with approximately 80,000 tons produced per year, whereas *Thua Nao* is locally produced, of irregular quality and under risk of contaminantion with spoilage and pathogenic microorganisms during the artisanal process. In previous studies, the nutritional and sensory qualities of traditional soy-fermented foods such as *Natto* (Wei *et al*., 2008), *Kinema* (Sarkar and Tamang, 1995), *Chungkukjang* (Lee *et al*., 2005) and *Daddawa* (Omafuvbe *et al*., 2002) were successfully improved by using potential pure starter culture of *Bacillus* strains. However, only limited research has been done on Thai *Bacillus*-fermented soybean. There are still no reports on nutritional information of traditional *Thua Nao*. The production of *Thua Nao* is rarely fully developed to use pure inoculum along with a suitable soybean seed in a commercial process. Also, there is a lack of systematic procedures including temperature control during fermentation. These troubles lead to inconsistent quality and undesirable attributes of this product. Therefore, there is a need for scientific research to improve the fermentation process to ensure that a safe product with high quality, more acceptability, pleasant aroma and high nutritional components is formed.

This study aimed to investigate the physicochemical and microbiological qualities including their amino acid profile, total phenolic compounds and some biological activity of commercial *Thua Nao* products collected from various producers in Chiang Mai. The use of *Bacillus* spp. isolated from *Thua Nao* as pure culture starter in the

fermentation process of protein-rich soybean cultivar were attempted. Changes in physicochemical and microbiological qualities during fermentation process were analysed. Besides, nutritive values as previously described including volatile compounds and isoflavones were also investigated. Finally, shelf-life storage of fermented soybeans using normal- and vacuum packing was studied.

1.2 Research objectives

1.2.1 To study nutritional information of *Thua Nao* including physicochemical and microbiological qualities, antimicrobial assay, antioxidant activities, total phenolic compounds and amino acid profile.

1.2.2 To develop high nutritional *Thua Nao* from protein-rich soybean cultivar fermented by pure starter culture and to characterise physicochemical and microbiological qualities during fermentation process as well as its nutritional issue.

1.2.3 To evaluate shelf-life storage of normal and vacuum packaging of *Thua Nao* product.