CHAPTER 1

INTRODUCTION

Proteolytic enzymes are important industrial enzymes and used in many areas of applications, such as in the food, textile, and medical industries. The use of proteases in the food industry such as cheese making, baking, preparation of soya hydrolyzates, and meat tenderization. The major application of proteases in the dairy industry is in the manufacture of cheese. In baking industry bacterial proteases are used to improve the extensibility and strength of the dough. Manufacture of soy products proteases have been used from ancient times to prepare soy sauce and other soy products. The alkaline and neutral proteases of fungal origin play an important role in the processing of soy sauce. Proteolytic modification of soy proteins helps to improve their functional properties. Modification of food protein can be carried out by chemical or enzymatic method. Enzymatic modification generally involves the use of proteolytic enzyme to hydrolyze specific peptide bonds. This has an advantage over chemical methods because it causes minimal undesirable side reaction. Enzymatic hydrolysis yields proteins that are smaller in molecular size, with secondary structure which may have improved functional properties including solubility, emulsifying and foaming properties.

Hydrolysis of protein foods has been investigated to produce value products, e.g. functional ingredients, dietary foods, and flavoring agents etc. (Ross and Bhatnagar, 1989). And most indigenous foods fermentation process produced by the primitive traditional methods so it takes a long time to produce a good quality products. Although chemicals methods are the most popular and convenient for modification of protein foods, enzymatic hydrolysis has advantages in its mild process conditions and relative specificity, which enable control the functionality of

end products. According, enzymatic methods are more attractive to the food processor and far more compliant to regulatory affairs than chemical treatments. Microbial enzymes are more useful than enzymes derived from plants and animals. This is because of the great variety of catalytic activities. The advantages using microorganisms to produce enzymes are microbial cell growth, which is characterized by short generation time and simple nutritional requirement.

The main problem associated with protein hydrolysis is the formation of bitter tasting peptides (Adler and Jens, 1984). The bitter peptide composed of low molecular weight and mainly hydrophbic amino acids. Methods for debittering of protein hydrolyzates include selective separation such as treatment with activated carbon, extraction with alcohol, isoelectric precipitation, chromatography on silica gel, hydrophobic interaction chromatography, and masking of bitter taste. Bio-based methods include further hydrolysis of bitter peptides with enzymes such as aminopeptidase, alkaline/neutral protease and carboxypeptidase, condensation reactions of bitter peptides using protease and use of *Lactobacillus* as a debittering starter adjunct (Saha and Hayashi, 2001).

Two objectives were studied. First, to isolate and characterize the protease from salt tolerant bacteria isolated from various sources in Thailand. Second, to study on purification, characterization and application of aminopeptidase from *Geobacillus thermoleovoran*s 47b for hydrolysis of bitter peptide.

Objectives

1. Isolation of salt tolerant bacteria from Thai fermented foods
2. Screening of salt tolerant bacteria for protease production
3. Optimization of condition for protease production of selected bacteria
4. To study of some characteristic of crude protease from selected bacteria
5. Optimum conditions of aminopeptidase production from *Geobacillus thermoleovorans* 47b
6. Purification and characterization of aminopeptidase from *Geobacillus thermoleovorans* 47b
7. Hydrolysis of bitter peptide by crude enzyme from *Geobacillus thermoleovorans* 47b