EXTRACTION OF PROTEASE FROM BILIMBI (*Averrhoa bilimbi L.)*

NUR 'AIN BT MOHAMAD KHAROE

BACHELOR OF SCIENCE (Hons)
FOOD SCIENCE AND TECHNOLOGY
FACULTY OF APLLIED SCIENCES
UNIVERSITI TEKNOLOGI MARA

JUNE 2013

ACKNOWLEDGMENTS

In the name of Allah, the Most Gracious and the Most Merciful. All praise to Allah SWT and blessings are upon His Prophet SAW. I would like to express my sincere gratitude and appreciation to my supervisor, Dr. Normah Ismail, the lecturer of Food Technology Department, who had given me a lot of guidance, encouragement and advice throughout the completion of this project.

I am very grateful to the laboratory staff that have helped me especially Cik Hairiyah Hashim, Puan Norahiza Mohd Soheh, Puan Siti Marhani Mardi, and Cik Nor Suhadah Mohammad Samri the laboratory assistants, for their assistants, guidance, cooperation, information in order to complete this final year project. Also thanks to postgraduate student Cik Siti Roha Ab. Mutalib, Cik Noorasma Mustakim and En. Mohd Suzairiff Zainal who helps me during the completion of this project. Thank you for your guidance and effort to make this project end successfully.

I would like to thank En. Muhammad Farid Hj. Shuib the biochemistry lab assistant who gives me permission to used instrument. My appreciation also goes to my friends for their helping during the preparation of this final year project report.

Last but not least, I am deeply grateful to my parents for their constant encouragement, advice and moral support.

Nur 'Ain Bt Mohmamad Kharoe

TABLE OF CONTENTS

ABSTRACT

EXTRACTION OF PROTEASE FROM BILIMBI (*Averrhoa bilimbi L.*)

Protease was extracted from bilimbi (*Averrhoa bilimbi L.*) and the juice obtained was purified by using two different concentrations of ammonium sulfate solution (40 and 60% (w/v)). The proteases were analysed for pH stability, temperature stability, storage stability, SDS-PAGE (molecular weight distribution), protein concentration and protein content. Protein content of bilimbi is 0.89 g. Protease activity at both ripening stage and 40 and 60% ammonium sulfate purification was optimum at pH 2 to 4 and at temperature 30 to 50ºC. As for storage stability at 4 ˚C, the protease activity decreased with storage time. Molecular weight distribution indicated that the proteases protein bands fall between 10 to 220 kDa with protein band at 50 kDa seen in both unripe and ripe bilimbi proteases purified with 40% ammonium sulfate. As the concentration of ammonium sulfate decreases, the protein concentration increase at both ripening stages. Purification using 40% ammonium sulfate precipitation could be a successful method to purify proteases from bilimbi especially from the unripe stage.

CHAPTER 1

INTRODUCTION

1.1 Background and problem statement

Enzymes have been used for food processing for as long as man processed food. Food enzymes have been obtained from a number of different sources for example by extraction from plant, microorganism and animal. In food industry, enzyme has been used to produce and to increase the quality and the diversity of food. The preparation of food products, for example cheese, bread, yogurt and soy sauce involved the degradation of proteins in the food as key reaction. The usage of enzyme and their action on several food led to not only the production of quality cheese, bread, yogurt and soy sauce but also the development of new usage and application for enzyme in the production of food and food ingredients. Enzyme can be extracted from a wide source such as plant, animal and microorganism and their property differ markedly from their sources. Each enzyme has its own mechanism and used in food industry to improve the quality of food in terms of flavour, aroma, colour and texture. Enzymes commonly used are pepsin, rennin, trypsin, papain, and bromelain (Salleh *et al.,* 2006). According to Global Industry Analysts, Inc. by 2015 the global market for industrial enzymes is forecast to reach US$3.74 billion and proteases constitutes the largest product segment in the global industrial enzymes market (Global Industry Analysts Inc, 2011).