NUTRITIONAL ANALYSIS AND EFFECTS OF VARIABLES ON PROTEIN EXTRACTION FROM PERAH SEED

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To my beloved mother, Zainon Mamat and my supported brothers.

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ABSTRACT

The nutritional content of fermented and non-fermented Perah seed (*Elateriospermum tapos*) was investigated. The proximate analyses such as moisture, fat, fiber, protein and ash contents from fermented seed were lower compared to non-fermented seed. However, only the fat content of fermented seed was higher than non-fermented seed. From that, maximum protein extractions from fermented and non-fermented Perah seed were further investigated using response surface methodology. A central composite design with three independent variables i.e. NaOH concentration (6, 8 and 10 %), extraction time (10, 20 and 30 minute) and solvent/meal ratio (50:1, 100:1 and 150:1, v/w) was used to study the response of protein yield. The experimental values of protein yield ranged between 13.8 and 0.05 g/100 g seed meal. A second-degree equation for independent and response variables was produced from simulation to obtain the contour plot graphs. From the graph and mathematical solutions, the best protein extraction protocol from both fermented and non-fermented Perah seed was obtained at 5.5 percent of solvent extraction, 40:1 ratio of solvent/meal and 32 minutes of reaction time. Surface concentration and ratio of solvent/meal were found to influence the protein yield from fermented seed; meanwhile only solvent concentration influenced protein yield from non-fermented seed. The maximum protein yields for both fermented and non-fermented perah seed were 18.0 g/100g and 5.0 g/100g seed meal respectively.

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ABSTRAK

Kandungan nutrisi dalam biji buah perah (*Elateriospermum tapos*) yang diperam dan segar tanpa peraman telah dikaji. Analisis hampiran seperti kandungan kelembapan, lemak, serat, protin dan abu daripada biji diperam adalah kurang berbanding dengan biji segar. Walau bagaimanapun, hanya kandungan lemak daripada biji diperam adalah lebih tinggi berbanding biji segar. Selain itu, pengekstrakan maksimum protin daripada biji perah diperam dan biji segar dikaji menggunakan metodologi sambutan permukaan. Eksperimen penggabungan dengan tiga pembolehubah tak bersandar iaitu kepekatan NaOH (6, 8 and 10 %); masa pengekstrakan (10, 20 and 30 minit) dan nisbah larutan/tepung (50:1, 100:1 and 150:1, v/w) telah digunakan untuk mengkaji jumlah sambutan protin. Julat jumlah sambutan protin bagi ujikaji yang dijalankan adalah antara 13.8 dan 0.05 g/100 g tepung biji. Persamaan tertib kedua bagi sambutan pembolehubah tak bersandar akan diperolehi menerusi simulasi bagi mendapatkan plot bentuk graf. Menerusi graf dan penyelesaian matematik, tahap terbaik bagi pengekstrakan protin daripada biji perah akan diperolehi pada 5.5 peratus kepekatan pelarut, 40:1 ratio larutan per tepung dan 32 minit masa pengekstrakan. Kepekatan larutan dan nisbah larutan per tepung mempengaruhi jumlah protin daripada biji diperam manakala hanya kepekatan larutan mempengaruhi jumlah protin bagi biji segar. Jumlah protin maksimum bagi kedua-dua biji diperam dan segar adalah masing-masing 18.0 g/100g and 5.0 g/100g kisaran biji.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
RSM	response surface methodology

LIST OF SYMBOLS

k	number of independent variable
n_0	number of replication
N	number of test
p	probability
X	process variable (independent variable)
Y	response
Z	coded value of the independent variable
α	significance level
β	regression coefficient
R^2	coefficient of determination
w/v	weight to volume ratio

LIST OF APPENDICES

CHAPTER I

INTRODUCTION

1.1 Background

Through generations, protein has become widely used in many sectors such as pharmaceutical, cosmetics and many more. Protein is one of the important nutrients in a human diet and many studies have been developed in order to get a good, high value and loads of protein extract. These studies have given many advantages to human lives and one of them has been to help the third world countries to get a sufficient diet. Extracted protein also helps human get faster nutrients during disasters or troubles. In addition, extracted proteins are very useful for medical teams and to help people.

Many studies have also been carried out to obtain materials that give a good and high volume of protein extracts. Perah seed or scientifically called *elateriospermum tapos* has been introduced as a protein source. Perah is a local wild tree that is large and has red colored young leaves that can be easily spotted. In Malaysia, it can be found in Sabah and Sarawak and also in the east peninsular states such as Kelantan and Terengganu. It is used by locals as a dish where the seed is cooked and fermented. However, not everyone knows that Perah seed actually has a higher protein nutrient compared to soya bean and other seeds such as watermelon seed. There is scarce literature on Perah seed except for its oil content.

In this study, the nutritional compositions for both the non- fermented and the fermented Perah seed were determined. These nutritional compositions were then compared to other seed in terms of the volume. The nutritional content can be determined according to the previous experimental methods such as moisture content by Ezeagu *et al.* (2002), crude fat and ash by Chopra and Kanwar (1976) and crude fiber by CEPP (UTM), (2007).

The study is also carried out to get the best condition of protein extraction. Many extraction methods have been considered, such as ultrasonic extraction and supercritical extraction but liquid-solid extraction was chosen due to its less expensive method and convenience for a lab scale. Liquid-solid extraction is also less hazardous and minimal effect to the nutrient components after several cycles of process.

Protein extraction by using solvents is very important in order to get the best variable conditions. The variables used are the solvent/seed ratio, reaction time and solvent concentration. At best, the solvent concentration gives a high quality of protein extract. The Perah seed is prepared in powder form to remove the moisture content first, before the protein can be extracted. Different reaction times give different values of protein extracted. Sufficient reaction time is a must to make sure all the protein content can be extracted successfully by the solvent. The high volume of protein extraction is also proportional to the volume of the solvent per seed. It is believed that the highest protein extracted comes from a sufficient volume of solvent per seed supply.

When it comes to optimization experiments, the study must consider influential variables and how they can relate to or influence the results. The use of Response Surface Methodology (RSM) is one of the experimental strategies to obtain optimization experiments. The RSM is widely used as it can account for interactions in several influential variables by using central composite design. In RSM use, there are three main stages involved in this study, which are designing the experiments, building the

mathematical models and determining the optimum conditions according to the variables that have been chosen.

1.2 Problem Statement

The insufficient supply of protein in human diet, especially in the growth of human population and also due to malnutrition in third world countries, has resulted in continuous efforts in exploring new resources such as plants that have higher protein contents. Perah seed has been chosen in this study since it is plentiful and easily found in the east coast of Malaysia, and easily grown. The problem of this study is whether Perah seed has the desired nutritional content. The problems are also in regard to which potential variables must be considered and what are the best conditions in order to get higher protein extraction.

1.3 Objective

i) To determine physical characteristics of fermented and non-fermented Perah seed.
ii) To determine potential nutritional contents of fermented and non-fermented Perah seed.
iii) To investigate the effect of independent variables (solvent concentration, reaction time and solvent/meal ratio) on the production of protein from fermented and non-fermented Perah seed by using solid liquid extraction.
iv) To determine the best condition of process variables for both fermented and non-fermented seed.

1.4 Significance of Study

The potentials of nutrition from Perah seed can be used as a new source for human needs. Protein extracted from Perah seed can be an alternative source as compared to animal protein, as it is less expensive and safe to use.

1.5 Scope of Study

The scope of this study is based on the experimental works and the analysis of both fermented and non-fermented Perah seed. There are several experimental works that need to be developed and done to achieve the objective of this study. The scope is divided into several sections:

i) Preparation of the fermentation and powder seed.

Perah seed was being fermented, dried at certain temperature and grinded until at desired and homogenized size.

ii) Nutritional content of Perah seed

Analyses of nutritional contents consist of moisture, crude fat, crude protein, ash, crude protein and crude fiber content. Powdered Perah seed has followed by referred methodologies in order to get desired proximate analysis.

iii) Protein extraction from fermented and non-fermented Perah seed.

Liquid-solid extraction method was used to extract protein from both fermented and non-fermented powdered Perah seed.

iv) Analysis of Perah seed.

The powdered Perah seed from the protein extraction process was analyzed by the protein contents or protein yield. A spectrophotometer was used to determine the protein yield as being compared to a standard curve.

v) Optimization of protein extraction

Response Surface Methodology (RSM) is used to get the relationship between the responses of each independent variable by the contour plots. An analysis of variance showed the adequacy of the model that has been developed and Pareto Chart showed the most significant independent variables.

1.6 Project Outline

This report contains five chapters. First chapter outlines the background of the research, problem statement, objectives, significant results and scope of the study. Second chapter discusses the Perah seed background, literatures of process extraction and variables involved and response surface methodology. Next is the third chapter, which covers the experimental methods for nutritional content determination, protein extraction and obtaining best condition by using response surface methodology. The fourth chapter covers the results and discussion from this study. Finally, the fifth chapter presents the conclusion and recommendations.

1.7 Summary

Perah seed nutritional content is determined by biological processes. Protein extraction from Perah seed is also determined by considering several variables such as solvent concentration, liquid-solid ratio from the seed soluble and reaction time. This study optimizes condition of extraction process by using response surface methodology.

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