A PRELIMINARY STUDY: PROTEIN EXTRACTION OF MIGRATORY LOCUST (Locusta migratoria) VIA ALKALINE ISOELECTRIC PRECIPITATION METHOD

By

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Sekian, terima kasih.

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DECLARATION BY AUTHOR

This dissertation is composed of my original work and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carries out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Caption
AA	Amino acid
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Collaboration
cm	Centimeter
DLM	Defatted migratory locust
EAA	Essential amino acid
ED	Ethanol defatting
FAO	Food and Agriculture Organization
FDPE	Freeze-dried locust protein extract
g	Gram
h	Hour
HCl	Hydrochloric acid
HD	Hexane defatting
IE	Isoelectric precipitation
Ig-E	Immunoglobulin E
ILF	Insoluble locust fraction
LM	Migratory locust
min	Minute
mL	Milliliter
NaOH	Sodium hydroxide
NFE	Nitrogen free extract
NPU	Net utilization protein
PER	Protein efficiency ratio
RSM	Response Surface Method

SD	Standard deviation
SDG	Sustainable Development Goals
SF	Supernatant fraction
SLF	Soluble locust fraction
spp	Species
TCA	Trichloroacetic acid
UN	United Nations
WHO	World Health Organizations
%	Percentage
<	Less Than
>	Greater Than
℃	Degree Celcius
±	Plus Minus

KAJIAN AWAL: PENGEKSTRAKAN PROTEIN DARIPADA BELALANG JUTA (Locusta migratoria) MENGGUNAKAN KAEDAH PENGEKSTRAKAN ALKALI BERSAMA PEMENDAKAN ISOELEKTRIK

ABSTRAK

Jumlah populasi manusia yang bakal meningkat dijangka tidak mampu untuk menampung permintaan yang tinggi terhadap makanan berasaskan haiwan ternakan. Peningkatan populasi akan mengehadkan kawasan ternak. Justeru, entomofagi atau praktis memakan serangga menjadi alternatif baharu yang dapat mengatasi isu ini dengan menggunapakai protein serangga di dalam makanan. Dalam kajian awal ini, pengekstrakan protein daripada belalang juta *(Locusta migratoria manilensis)* menggunakan kaedah alkali bersama pemendakan isoelektrik telah dikaji. Pertama, kesan kaedah penyingkiran lipid terhadap protein telah dikaji. Kedua, pH 9 ke 13 telah dikenakan kepada sampel belalang untuk mendapatkan hasil ekstrak protein dan kadar pengekstrakan yang tertinggi. Pada awalnya, belalang telah dikisar and dinyah-lemak menggunakan pelarut etanol. Belalang yang dinyah-lemakkan telah diteruskan dengan kaedah pelarutan alkali (60 min, 40°C) dengan penambahan 0.25 M NaOH dan penyesuaian pH jika perlu. Seterusnya, supernatan yang telah diempar dikenakan pemendakan isoelektrik pada pH 4 untuk menghasilkan pelet protein. Berdasarkan keputusan, etanol merupakan pelarut yang bagus untuk menyingkirkan lipid memandangkan terdapat pengurangan dalam konten lipid daripada 5 kepada 0.7% (asas kering) dan pada masa yang sama meningkatkan konten protein daripada 54.9 kepada 57.2% (asas kering). Manakala hasil ekstrak protein pada pH 9, 10, 12 dan 13 adalah masing-masing 20.4, 23.2, 27.8 dan 32.5%. Keputusan ini menunjukkan bahawa peningkatan alkali merupakan kondisi pengekstrakan yang baik, namun kajian yang lebih mendalam secara optimisasi mengunakan RSM

diperlukan, untuk kondisi alkali solubilisi dan pemendakan isoelektrik untuk mendapatkan hasil ekstrak yang terbaik.

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ABSTRACT

Unprecedented growth of future human population around the globe seems non capable to overcome high food demand of animal livestock. Increasing in population will coincidentally shrink more livestock farming land. Hence, entomophagy or known as eating insect practice is a promising alternative to circumvent the issue by utilizing the insect protein in worldwide food application. In this preliminary study, protein extraction of oriental migratory locust *(Locusta migratoria manilensis)* using alkaline isoelectric precipitation was investigated. Firstly, the effect of lipid removal technique to protein content was investigated. Secondly, pH 9 to 13 during solubilisation was applied to obtain the highest protein yield and extraction rate. Initially, the whole dried locust was ground and defatted using ethanol solvent. Defatted locust (DLM) was subjected to alkaline solubilisation for 60 min at 40℃ with addition of 0.25 M NaOH and pH adjustion if necessary. Subsequently, centrifuged supernatant underwent isoelectric precipitation at pH 4 to yield the target protein pellet. From the result, it is noteworthy that ethanol worked well in defatting the locust powder since there was reduction in lipid content from 5 to 0.7% (dry weight basis) and simultaneously increased in protein content from 54.9 to 57.2% (dry weight basis). Whereas protein extraction yield at pH 9, 10, 12 and 13 were 20.4, 23.2, 27.8 and 32.5% respectively. The results indicated that increased alkaline pH is a good protein extraction condition, however further study on optimization of alkaline solubilisation and isoelectric precipitation condition using

Response Surface Method is advocated to be conducted in order to obtain the best extraction protein yield.

CHAPTER 1 INTRODUCTION

1.1. Research Background

While economic shocks, conflicts and extreme weather are driving to global food insecurity, the unforeseen existence of COVID-19 pandemic had worsened the present food crisis. According to recent Global Report on Food Crises (GRFC) by Food Security Information Network, FSIN (2021), there are estimated 155 millions people in 55 countries or territories in the world, suffering acute hunger with an increase in approximately 20 million people from 2019, including Democratic Public of the Congo (21.8 millions), Yemen (13.5 millions), and Afghanistan (13.2 millions). Most of hungers are facing malnutrition or protein deficiency for their growth development and survival. Moreover, the major concern on the needs of nutritious food is not only as the current issue, yet also the prediction by United Nations (UN) on unprecedented rate of future human population exceeding 9 billion by 2050. Thus, more lands need to be sacrificed when the world relies solely on traditional plant crops and meats that require much more farming space.

To the attainment of Sustainable Development Goals (SDG) by UN, many researchers are in consensus that approximately 2,000 edible insect could be potential alternative proteins to traditional plants protein (Jongema, 2017; Rumpold and Schlüter, 2013; Purschke et al., 2018). Insects are ubiquitous and abundance in wild which has adequate amount of essential amino acid (EAA) required in human diet. Nine essential amino acids suggested by World Health Organization (WHO) are non capable to be synthesized by the body yet can be found in other sources including edible insects (Köhle et al., 2019). The developed countries might not being affected by this issue, however the hungers whom depending on plant proteins

alone that are still lack in EAA without meat, would be consequently prone to Kwashiorkor and marasmus diseases (Lopez and Mohiuddin, 2021).

Not to mention on current shrinking of available land for food production, edible insects are predominantly saving one hectare land at least 150 tons of insects than that of soybean which yielded less than one ton for the akin space (Zanolli, 2014). Moreover, Thailand already led the largest advanced insect farming with approximately 20 000 edible insect farms available (Hanboonsong et al., 2013). In addition, the insects' short life cycle undoubtedly contributes to rapid reproduction.

Migratory locust is an orthopteran which abundantly found in Tropical Africa, Australian, Oriental and Palearctic realms (Jongema, 2017) and synonymous to agricultural crop destructive culprit because of their ability to transform from solitarious to gregarious phase and forming swarm (Pflüger and Bräunig, 2021). Their protein content and protein quality are potentially high comparable to meat and plant (MItra et al., 2017). The consumption of insects are also permissible in most religions including Jewish and Christian (Thakur et al., 2017) and as in Islam ruling, locust is allowed to be consumed (Tarmizi, 2018).

Improved protein quality is obtained when the protein is extracted from the whole insect. Various extraction methods have been proposed and experimented including alkaline, acidic, aqueous and enzymatic extraction which each accompanies benefit and setbacks in order to yield the maximum protein for food application in industry.

1.2. Rationale of the Study

Recovery of protein from insects especially locust is widely practiced in present and for future food application. This is purposely to overcome future food insecurities and as emergency relief on malnutrition issues in developing and less developed countries or territories to the attainment of SDG 2 by United Nations. However, the appropriate and greater efficiency of extraction method is vital to be taken into account withal time consumption, availability of resources and cost spent to achieve the highest protein yield and protein quality.

Many studies have demonstrated that alkaline extraction method is highly potential to yield a good protein quality from edible insects including *Locusta migratoria* (Clarkson et al., 2018; Laroche et al., 2019; Purschke et al., 2018; Zhao et al., 2016; Zielińska et al., 2018). The other suggested method such as aqueous method are simple and short, yet the extraction protein yield was low (7.35%) in grasshopper protein extract (Chatsuwan et al., 2018).

The current studies on alkaline extraction from migratory locust by Purschke et al. (2018) and Clarkson et al. (2018) have a setback in terms of harmful organic solvent during lipid removal which is hexane. Thus, in order to opt for more environmental friendly organic solvent, ethanol is a better consideration which there was also no huge changes reported by Amarender et al. (2020) between ethanol and hexane defatted protein content with 73.72 and 72.08%, respectively. To the best of our knowledge, no study has ever been conducted yet to optimize alkaline extraction method using sodium hydroxide (NaOH) with ethanol as defatting solvent specifically on migratory locust.

1.3. Objective

The aim of this study is to investigate the protein extraction method using alkaline isoelectric precipitation from migratory locust for food application. In order to achieve the aim, the objectives are;

1.3.1. To analyse the effect of ethanol-defatting technique to fat and protein content in migratory locust.

1.3.2. To analyse the effect of the extraction condition (solubilisation pH) to the protein extraction yield and extraction rate from migratory locust.