UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF TEXTURIZED VEGETABLE PROTEIN FROM MUNG BEAN PROTEIN ISOLATE AND EVALUATION OF ITS TECHNOFUNCTIONALITY, STRUCTURAL, RHEOLOGICAL AND QUALITY

FATEMA HOSSAIN BRISHTI

FSTM 2021 8

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By

FATEMA HOSSAIN BRISHTI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November 2020

To my beloved parents
Tofazzal Hossain Bhuiyan and Ayesha Begum and
sister Khodeja Begum
Without whom endless prayer none of my success would possible

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chair : Professor Nazamid Saari, PhD
Faculty : Food Science and Technology

In recent years, plant-based food proteins have surged in popularity as they are environmentally friendly and healthier compared to animal proteins. Mung bean protein also is an economic source of plant proteins with low greenhouse gas emission, thus revealing strong potential as a sustainable source to replace animal protein. In general, mung bean remains underutilized due to its hard-to-cook characteristics. Therefore, the objectives of this research were to prepare and characterize protein isolate from mung bean for the production of texturized mung bean protein using extrusion technology to raise its status from an underutilized food protein source while mitigating its hard-to-cook phenomenon in an attempt to attain global food security goals.

Mung bean protein isolate (MBPI) was produced in a lab-scale using alkaline extraction with subsequent isoelectric precipitation and compared with soy protein isolate (SPI) in terms of proximate composition, amino acid profile, techno-functional and thermal properties. The protein content, solubility profile, water and oil absorption capacities, and emulsion activity were found to be comparable with SPI. MBPI showed slightly better gelling capacity by exhibiting least gelation concentration at 12% than SPI 14%. Relatively, the denaturation temperature of MBPI (157.9ºC) was high while low denaturation enthalpy (41.6, J g^{-1}) was observed compared to SPI indicating the comparatively less compact structure of MBPI which may aid in protein unfolding and fibril structure formation during texturization. Following this, MBPI was produced in a pilot-scale using 100 L bioreactor and three different drying techniques evaluated i.e. freeze, spray, and oven drying techniques prior to analyzing the physicochemical, techno-functional, thermal, structural, and rheological properties of protein. Freeze-dried MBPI (FD) showed the highest protein solubility and oil absorption capacity when compared to spray-dried (SD) and oven-dried (OD) MBPI. All samples showed no dissociation of protein subunits in SDS-PAGE and were thermally stable with high denaturation temperature ranging from 157.9–158.1ºC. FD MBPI and SD MBPI formed

elastic gels with better gelling capacity than OD MBPI which formed aggregated gel. Current work validated the different final properties achieved for MBPI produced under different drying techniques that would allow tailoring for different food systems, whereby FD MBPI would be ideal for meat extender. Thus, FD MBPI having the best techno-functional properties was then used to produce texturized mung bean protein (TMBP) using HTST (High-temperature, short-time) extrusion processing. TMBP with desirable physical properties was produced through optimization of extrusion processing parameters of feed moisture (30–60%), screw speed (70–100 rpm), and barrel temperature (120–170 ºC) using response surface methodology. The optimum processing parameters were 49% feed moisture, 81 rpm screw speed, and 145 ºC barrel temperature. Under these conditions, microstructure analysis revealed fibrous structure in TMBP while SDS-PAGE showed partial protein unfolding that was crucial for protein fibril formation during texturization. Feed moisture, at both low (19-30%) and high ends (60–70%), caused complete protein denaturation, irrespective of barrel temperature and screw speed, as illustrated by the disappearance of the majority of the protein gel bands on SDS-PAGE. Therefore, MBPI was then texturized at different feed moisture contents (30, 49, and 60%) and at constant barrel temperature (145 ºC) to evaluate the changes in protein profile, solubility, thermal, structural and rheological properties. Extrusion at intermediate (49%) feed moisture produced TMBP with favourable partial denaturation, the formation of small aggregates, improved solubility, and digestibility with strong gel-forming behaviour. In contrast, low (30%) and high (60%) moisture content resulted in complete protein denaturation, the undesirable formation of large aggregates and weak gels. This work established that protein denaturation and formation of aggregates could be controlled by critically controlling feed moisture content and 49% feed moisture produced TMBP with desirable qualities, fostering its use as plant-based meat extender. Finally, the techno-functionality, anti-nutrient, *in vivo* protein quality, and toxicity of texturized mung bean protein (TMBP) were evaluated. The findings showed that extrusion successfully produced TMBP with improved techno-functionalities that are crucial for meat-based food product application, credited to retained juiciness and fat-binding ability. Alkaline protein extraction and extrusion significantly reduced trypsin inhibitor, phytic acid, and tannin content in TMBP. *In vivo* study revealed true protein digestibility of TMBP was 99.3% resembling casein (99.4%, control protein). Lean muscle weight gain and reduced cholesterol and triglyceride had reflected TMBP's potential as protein meal replacer and supplement diet. Serum biochemical analysis showed no remarkable deviation from casein while microanatomy study revealed healthy heart, liver, kidney, lung, and testes in TMBP-fed group. This study ascertained the safety of alkaline extraction and extrusion to produce TMBP with improved techno-functionalities, and reduced anti-nutritional factors. Conclusively, current study successfully demonstrated the optimized production of TMBP from mung bean protein and its potential use as plant-based meat extender to serve as a healthier, safe, and sustainable protein source.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PEMBANGUNAN PROTEIN SAYURAN BERTEKSTUR DARIPADA ISOLAT PROTEIN KACANG HIJAU DAN PENILAIAN TERHADAP KEFUNGSIAN TEKNO, STRUKTUR, REOLOGI, DAN KUALITINYA

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Dalam beberapa tahun kebelakangan ini, protein makanan berasaskan tumbuhan meningkat popular kerana sumber protein tersebut mesra alam dan lebih sihat berbanding dengan protein daripada sumber haiwan. Protein kacang hijau juga merupakan sumber protein tumbuhan yang ekonomik dengan pelepasan gas rumah hijau yang rendah, justeru berpotensi besar sebagai sumber yang lestari untuk menggantikan protein makanan berasaskan haiwan. Umumnya, kacang hijau masih kekal sebagai sumber protein tumbuhan yang kurang digunakan kerana sifatnya yang sukar dimasak. Oleh itu, objektif kajian ini adalah untuk menyedia dan mencirikan isolat protein daripada kacang hijau untuk penghasilan protein kacang hijau bertekstur melalui teknologi penyemperitan bagi meningkatkan statusnya daripada suatu sumber protein yang kurang digunakan disamping mengurangkan fenomena ianya sukar dimasak dalam usaha untuk menyokong sasaran keselamatan makanan global.

Isolat protein kacang hijau (MBPI) dihasilkan dalam skala makmal dengan menggunakan pengekstrakan beralkali diikuti dengan pemendapan isoelektrik dan seterusnya isolat protein tersebut dibandingkan dengan isolat protein soya (SPI) dari segi komposisi proksimat, profil asid amino, sifat tekno-fungsian dan sifat terma. Kandungan protein, profil keterlarutan, kapasiti penyerapan air dan minyak serta aktiviti emulsi didapati setanding dengan SPI. MBPI menunjukan kapasiti penggelan yang sedikit lebih baik dengan kepekatan gel minima pada 12% berbanding dengan 14% untuk SPI. Secara relatifnya, suhu denaturasi MBPI (157.9 ^{o}C) adalah tinggi manakala entalpi denaturasi (41.6 J g^{-1}) adalah rendah berbanding dengan SPI. Ini menggambarkan bahawa struktur MBPI kurang padat dan mungkin memudahkan struktur protein terungkai serta pembentukan struktur fibril semasa penteksturan. Berikutan itu, MBPI telah dihasilkan dalam skala pilot menggunakan bioreaktor 100 L dan tiga kaedah pengeringan dinilai; kaedah pengeringan sejukbeku, kering-semburan, dan kering-ketuhar sebelum menganalisis sifat fisikokimia, tekno-fungsian, terma, struktur, dan sifat reologi protein.

MBPI yang dikeringkan secara sejukbeku (FD) menunjukkan keterlarutan protein dan kapasiti penyerapan minyak tertinggi berbanding MBPI kering-semburan (SD) dan kering-ketuhar (OD). Kesemua sampel tidak menunjukkan penceraian subunit protein pada SDS-PAGE dan mempamerkan kestabilan terma dengan suhu denaturasi diantara 157.9–158.1ºC. FD MBPI dan SD MBPI membentuk gel yang elastik dengan kapasiti penggelan yang lebih baik berbanding OD MBPI yang membentuk agregat gel. Kajian ini mengesahkan sifat akhir MBPI yang berbeza diperolehi apabila teknik pengeringan yang berbeza digunakan yang memungkinkan aplikasi kepada sistem makanan yang berbeza, dimana FD MBPI adalah sesuai sebagai bahan pengganti daging. Oleh itu, FD MBPI yang mempunyai sifat tekno-fungsian yang terbaik seterusnya digunakan untuk penghasilan protein kacang hijau bertekstur (TMBP) menggunakan proses penyemperitan HTST (Suhu tinggi, masa pendek). TMBP dengan sifat fizikal yang diinginkan telah dihasilkan melalui pengoptimuman parameter pemprosesan penyemperitan seperti kelembapan bahan suapan (30-60%), kelajuan skru (70-100 rpm) dan suhu barel (120–170ºC) dengan menggunakan kaedah gerak balas permukaan. Parameter pemprosesan optimum adalah kelembapan bahan suapan pada 49%, kelajuan skru pada 81 rpm, dan suhu barel pada 145ºC. Dalam keadaan penyemperitan yang optimum, analisis mikrostruktur menunjukkan struktur protein berserabut pada TMBP manakala SDS-PAGE menunjukkan protein telah separa terungkai yang merupakan faktor penting untuk pembentukan fibril protein semasa teksturisasi. Kelembapan bahan suapan pada tahap rendah (19-30%) dan tinggi (60-70%), menyebabkan denaturasi protein yang lengkap tanpa mengira suhu barel dan kelajuan skru, seperti yang digambarkan dengan kehilangan sebahagian besar jalur gel protein pada SDS-PAGE. Oleh yang demikian, MBPI kemudiannya ditekstur pada kandungan kelembapan bahan suapan yang berbeza (30, 49, dan 60%) dan pada suhu barel tetap (145ºC) untuk menilai perubahan pada profil protein, keterlarutan protein, sifat terma, struktur dan reologi. Penyemperitan pada kelembapan sederhana (49%) menghasilkan TMBP dengan separa denaturasi, membentuk agregat-agregat kecil, peningkatan pada keterlarutan dan kebolehcernaan beserta dengan sifat pembentukan gel yang kuat. Sebaliknya, kelembapan rendah (30%) dan tinggi (60%) mengakibatkan denaturasi protein sepenuhnya, pembentukan agregat besar dan gel lemah yang tidak diingini. Dapatan kajian ini membuktikan bahawa denaturasi protein dan pembentukan agregat dapat dikawal dengan mengamati kandungan lembapan bahan suapan dengan 49% kelembapan bahan suapan menghasilkan TMBP dengan kualiti yang dikehendaki, justeru mendorong penggunaannya sebagai bahan pengganti protein daging berasaskan tumbuhan. Akhir sekali, sifat tekno-fungsian, anti-nutrien, kualiti protein *in vivo,* dan ketoksikan TMBP telah dinilai. Dapatan kajian tersebut menunjukkan bahawa penyemperitan berjaya menghasilkan TMBP yang memiliki peningkatan pada sifat tekno-fungsian yang sangat penting untuk aplikasi pada produk makanan berasaskan daging yang akan menyumbang kepada pengekalan rasa berjus dan kemampuan mengikat lemak. Pengekstratan protein secara beralkali dan penyemperitan mengurangkan kandungan perencat trypsin, kandungan asid fitik dan kandungan tanin dalam MBPI. Kajian *in vivo* menunjukkan kebolehcernaan protein sebenar TMBP sebanyak 99.3% menyerupai kasein (99.4% sebagai protein kawalan). Peningkatan berat otot tanpa lemak dan penurunan kolesterol serta trigliserida menunjukkan potensi TMBP sebagai makanan gantian protein dan suplemen kepada diet. Analisis biokimia serum kumpulan tikus yang diberi makan TMBP tidak menunjukkan sebarang perbezaan berbanding dengan penggunaan protein kasein manakala kajian mikroanatomi menunjukkan jantung, hati, buah pinggang, paru-paru, dan testis yang sihat. Kajian ini membuktikan keselamatan pengekstratan protein beralkali dan penyemperitan untuk menghasilkan TMBP yang mempunyai tekno-fungsian yang lebih baik dan pengurangan faktor anti-nutrisi. Kesimpulannya, kajian ini berjaya menunjukkan penghasilan TMBP yang optimum daripada protein kacang hijau dan potensi penggunaannya sebagai bahan pengganti daging haiwan berasaskan tumbuhan untuk berfungsi sebagai sumber protein yang lebih sihat, selamat, dan mapan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ANF	Anti-nutritional factors
AOAC	Association Oils Chemist's Society
BD	Bulk density
CD	Circular dichroism
DSC	Differential scanning calorimetry
DT	Degree of texturization
ER	Expansion ratio
FD	Freeze-dried MBPI
FTIR	Fourier transform infra-red
G″	Loss modulus
G′	Storage modulus
H_0	Surface hydrophobicity
MBPI	Mung bean protein isolate
OD	Oven-dried MBPI
RR	Rehydration ratio
SD	Spray-dried MBPI
SEM	Scanning electron microscopy
-SH	Sulfhydryl group
SPI	Soy protein isolate
-s-s-	Disulfide bond
T_d	Denaturation temperature
T_e	Onset temperature
TMBP	Texturized mung bean protein
T_o	Endset temperature
TIA	Trypsin inhibitory activity
WAI	Water absorption index
δ	Loss tangent

CHAPTER 1

INTRODUCTION

Proteins are vital biomolecules for their crucial functions in sustaining life as well as for their multiple functions in food systems (Berg, Tymoczko, & Stryer, 2002; Li-chen & Lacroix, 2018). Majority of the protein-based food products are derived from the animal sources. The increase in world population as a result of the increased demand for animal-based proteins led to the substantial environmental impacts such as global warming and loss of biodiversity (Sanchez-Sabate, & Sabaté, 2019; Steinfeld et al., 2006). Moreover, animal livestock requires large amount of land, water and nitrogen, making them an unsustainable source of protein for this excessive population growth. Apart from environmental issues, high animal meat consumption has been reported to be responsible for severe health problems such as obesity, cardiovascular disease and cancer (Bouvard et al., 2015; Hu, 2011; Popkin & Gordon-Larsen, 2004). A 20-years epidemiological study deduced that evading animal products altogether will reduce or eliminate the chronic diseases i.e., obesity, type 2 diabetes, coronary heart disease, and cancers of breast, protest and bowel occurred by western-based diets such as animal products, sugars, refined fats and oils (Campbell & Campbell, 2005). Thus, there is an urgent need to shift toward a more sustainable source of protein diet such as plant-based protein for both environmental and health reasons.

The texturized vegetable protein (TVP) is the popular form of plant-based protein food, with high in protein, low in saturated fat, cholesterol-free, and economical compared to the animal-based protein. The demand for TVP is expected to rise at a significant rate due to the escalating trend of healthy food consumption worldwide. Typically, TVP is produced into two forms i.e. meat extenders and meat analogues using protein powder (50–90%) via extrusion processing technology (Riaz, 2011). Majority of the work reported on TVP used purchased protein powder in the form of spray-dried protein concentrate or isolate wherein the techno-functional properties of the raw ingredient is unknown (Chiang, Loveday, Hardacre, & Parker, 2019; Samard, Gu, & Ryu, 2019; Lin, Huff, & Hsieh, 2000). The protein extraction procedure and drying technique can significantly alter the protein structure, thus, can shift its techno-functional properties to a large extent (Vojdani, 2006). Therefore, it is crucial to determine the techno-functional properties of protein before application into food system. For instance, the successful production of TVP requires a protein-based raw material in the form either protein concentrate or isolate with good techno-functional properties such as high solubility, high water and oil absorption capacity, good gelling capacity along with a flexible protein structure to rearrange into fibril structure during extrusion cooking. No prior studies have examined the effects of protein extraction and drying technique on the techno-functional properties of protein ingredients to cater for the production of TVP with desired product qualities such as solubility, expansion ratio, fibril structure formation, emulsibility, water/oil holding capacity. Another important aspect for the development of TVP is the processing conditions of extrusion cooking. Even though extrusion is a widely used technology, however, it is still a complex and multivariable process which needs to be optimized for definite applications in the food systems based on the nature of raw materials and desired final product qualities. For instance, extruder type, screw speed, barrel temperature, feed moisture, screw configuration can affect the product quality of TVP (Zhang et al., 2019; Ding et al., 2006). Furthermore, the nature of protein ingredient i.e., structure configuration and amino acid sequence can further affect the quality of TVP. Limited researches have reported the significance of these variables (Rehrah, Ahmedna, Goktepe & Yu, 2009; Basediya, Pandey, Shrivastava, Khan, & Nema, 2013; Guerrero, Beatty, Kerry, De la Caba, 2012; Garg & Singh, 2010). Besides, protein cross-linking, denaturation, and disulfide linkages vary depending on the extrusion processing techniques and protein composition (Akdogan, 1999). Traditionally, soy protein flour, concentrate or isolate have been extensively used to produce TVP (Fresán Mejia et al., 2019). Apart from soy, wheat, pea, and nut proteins have also been used to produce TVP. However, consumers tend to avoid specific TVP due to food allergies or intolerances i.e., soy and nut allergy or to follow a definite dietary pattern viz. gluten-free. Thus, it is of utmost importance to find an alternative to soy protein for the production of TVP to fulfil the dietary preference of the consumers.

Mung bean (*Vigna radiate* L.) is one of the important leguminous crops, cultivated in more than six million ha worldwide contributing to about 8.5% of global pulse area. Mung bean is consumed by most of the households in Asia. In South Asia, the demand for mung bean reached 5.4 million tons within 2015–2016 (Schreinemachers et al., 2019), credited to high protein content (25–28%), low fat content (1-2%) and easy digestibility (Hou et al., 2019; Khaket, Dhanda, Jodha, & Singh, 2015). As a protein-rich edible plant, mung bean demonstrates strong potential as a sustainable, economic, and environmentally-friendly source of “green protein” due to a lower carbon footprint compared to animal proteins like cattle and poultry, thus, attaining a wholesome balance between nutritional preferences and environmental protection. The low-fat content of mung bean provides an additional advantage by omitting sample defatting step prior to conversion into TVP via extrusion processing since fat (> 5 %) hinder the texturization process, thus reducing production cost. While mung bean has long been recognized as food, its protein is only starting to gain limelight in recent two years (the year 2018 and 2019) among food researchers, due to its potential acting as sustainable protein source, along with diverse applications in food manufacturing and processing. Apart from being an excellent source of protein, fibre, vitamins and minerals, mung bean also has been recognized to ameliorate hyperglycemia, hypertension, hyperlipidemia, and melanogenesis. Even though there are few reports on aqueous or solvent extracts of mung bean and its bioactivity, however, important knowledge gaps still exist concerning the effect of processing treatments i.e., heat treatment, mechanical treatment and biotechnological procedure on mung bean protein before consumption. Given, its nutritional and health benefit, mung bean protein-based food has the potent potentiality to be considered as an alternative to animal proteins in Asia as well as in other countries. Therefore, the goal of this research was to fully unravel the food properties of mung bean protein isolates in terms of physicochemical, techno-functional, thermal, and structural properties for the production of texturized vegetable protein as well as understand the protein structural changes during texturization and evaluate protein quality of texturized mung bean protein via *in vivo* study.

1.2 Aim and outline of the thesis

The overall aim of this current study was to foresight the use of mung bean protein isolate as an alternative to animal meat protein. Therefore, the focus of the project was the production of mung bean protein isolate to be used as a raw material for the development of texturized vegetable protein to serve as a green alternative of animal-based protein.

Specifically, the objectives of the current study were:

1. To determine the techno-functional and thermal properties of mung bean protein isolate

2. To evaluate the effects of drying techniques on the physicochemical, techno-functional, thermal, structural and rheological properties of mung bean protein isolates

3. To determine the effects of extrusion conditions on the physical, textural, and protein quality of texturized mung bean protein

4. To evaluate the effects of extrusion feed moisture content on the structural and rheological properties of texturized mung bean protein.

5. To evaluate the effects of extrusion conditions on the techno-functionality, anti-nutrient, *in vivo* protein quality and toxicity of texturized mung bean protein.

The thesis is organized into eight chapters:

Chapter 1 lay the introduction and objectives of the study.

Chapter 2 represents the review of literature regarding protein structure, protein extraction and drying, extrusion and texturization.

Chapter 3 focuses on the extraction of mung bean protein isolate in the laboratory scale and compares its techno-functionality with reference protein (soy protein).

Chapter 4 centres on the extraction of mung bean protein isolate (MBPI) in pilot-scale wherein the effect of different drying techniques (freeze, spray and oven drying) on the physicochemical, techno-functional, structural and thermal properties are documented.

Chapter 5 here, the best mung bean isolate powder (high water and oil absorption capacity, gelling ability, porous structure) is selected to produce TVP using extrusion technology. The response surface methodology (RSM) is applied to get optimized extrusion processing conditions and best techno-functional properties for texturized mung bean protein.

Chapter 6 explores the structural changes of mung bean protein due to texturization at the molecular level.

Chapter 7 focuses on the effect of texturization on anti-nutritional properties of optimized texturized mung bean protein. *In vivo* study is conducted to determine the protein quality and toxicity of texturized mung bean protein.

Chapter 8 outlines summary of the research, conclusions and recommendations for future work.

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Fatema Hossain Brishti was born on 13 August, 1989 in Sylhet, Bangladesh. She attended secondary school in M.C. College, Sylhet, after which she was enrolled into Food Engineering and Tea Technology, Shahjalal University of Science and Technology, Sylhet, Bangladesh. She completed the Bachelor of Science with Honours, and then awarded with a MSC leading to PhD fellowship by the '*OWSD' (Organization for women in science for the developing world)*, Trieste, Italy. She, then, started to work as a MSC student in the Faculty of Food Science and Technology for one and half years and after fulfilling university requirements successfully (1 publication and viva-voce), converted into PhD program. While working in the PhD project, she attended workshop titled "Food Extrusion: Cereals, Pulses, Proteins & Other Ingredients" in Texas A&M University, College Station, USA.

After completion of PhD, she wishes to join the education line to become a lecturer and share her knowledge and experience in food science with the students. She also wishes to provide primary education to the underprivileged children in Bangladesh.

LIST OF PUBLICATIONS

Brishti, F. H., Chay, S. Y., Muhammad, K., Ismail-Fitry, M. R., Zarei, M., Karthikeyan, S., & Saari, N. (2020). Effects of drying techniques on the physicochemical, functional, thermal, structural and rheological properties of mung bean (Vigna radiata) protein isolate powder. *Food Research International*, 138, 109783.

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