



ANTIHYPERTENSIVE, ANTIOXIDATIVE, ANTIDIABETIC PROPERTIES OF JACK BEAN PEPTIDES PRODUCED UNDER ENZYMATIC MEMBRANE REACTOR

ROSE ULI RUTH CECILIA



**FOOD SCIENCE STUDY PROGRAM
FACULTY OF ENGINEERING AND TECHNOLOGY
IPB UNIVERSITY
BOGOR
2026**

- Hak Cipta Dilindungi Undang-undang
1. Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan dan menyebutkan sumber :
 - a. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik atau tinjauan suatu masalah
 - b. Pengutipan tidak merugikan kepentingan yang wajar IPB University.
 2. Dilarang mengumumkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.



@Hak cipta milik IPB University

Hak Cipta Dilindungi Undang-undang

1. Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan dan menyebutkan sumber :
 - a. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik atau tinjauan suatu masalah
 - b. Pengutipan tidak merugikan kepentingan yang wajar IPB University.
2. Dilarang mengumumkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.



DECLARATIONS CONCERNING GRADUATE THESIS AND SOURCES OF INFORMATION AND TRANSFER OF COPYRIGHT

I hereby declare that the thesis with the title “Antihypertensive, Antioxidative, Antidiabetic Properties of Jack Bean Peptides Produced Under Enzymatic Membrane Reactor” is genuinely my work with the direction of my supervisor and has not been submitted in any form to any university. Sources of information derived or quoted from published or unpublished works from other authors have been mentioned in the text and listed in the Bibliography at the end of this thesis.

I hereby assign the copyright of my paper to the Bogor Agricultural University.

Bogor, April 2026

Rose Uli Ruth Cecilia
F2501241085



RINGKASAN

ROSE ULI RUTH CECILIA. Sifat Antihipertensi, Antioksidatif, dan Antidiabetik Peptida Kacang Koro Pedang Yang Diproduksi dengan Reaktor Membran Enzimatis. Dibimbing oleh AZIS BOING SITANGGANG dan ENDANG PRANGDIMURTI.

Peningkatan minat masyarakat terhadap pangan fungsional berakar dari pemahaman yang menguat terkait hubungan nutrisi dari makanan yang dikonsumsi terhadap efek kesehatan, sejalan dengan peningkatan minat terhadap peptida bioaktif karena bioaktivitasnya yang terbukti bermanfaat bagi kesehatan. Dalam penelitian ini, peptida bioaktif yang memiliki aktivitas antioksidan, aktivitas penghambatan DPP-IV, dan ACE diperoleh dari isolat protein kacang koro pedang (*Canavalia ensiformis*) menggunakan sistem proteolisis kontinu dengan kombinasi enzim alkalase dan neutrase. Penelitian ini terdiri dari dua tahap utama: (1) persiapan isolat protein kacang koro pedang dan (2) hidrolisis enzimatis kontinu yang berfokus pada pengaruh $[E]/[S]$, pH, dan waktu tinggal (τ). Membran PES 5 kDa digunakan karena kemampuannya dalam menahan molekul enzim sepenuhnya di dalam reaktor. Ketika reaksi penentuan parameter rasio $[E]/[S]$ optimal, aktivitas penghambatan DPP-IV yang diperoleh kecil dan tidak signifikan, sehingga tidak dilakukan analisis untuk parameter lainnya. Kondisi operasi optimal dari seluruh parameter, diperoleh pada perlakuan $[E]/[S] = 5\%$, pH = 7,5, dan $\tau = 12$ jam. Dalam kondisi ini, kadar peptida, kapasitas antioksidan, dan penghambatan ACE dalam permeat masing-masing adalah 0,6143 mg SE/mL, 0,0454 mg TEAC/mL, dan 92,18%. Jika dibandingkan dengan substrat yang tidak dihidrolisis dan disaring (SUF), terjadi peningkatan kadar peptida dan penghambatan ACE serta penurunan kapasitas antioksidan pada permeat. Tren peningkatan ini disebabkan oleh produksi peptida dengan bioaktivitas, sedangkan tren penurunan disebabkan oleh penolakan senyawa fenolik oleh membran. Selain itu, selama proses filtrasi kontinu jangka panjang selama 48 jam, kadar peptida dan kapasitas antioksidan meningkat sementara aktivitas penghambatan ACE menurun. Nilai IC_{50} yang diperoleh untuk kapasitas antioksidan dan penghambatan ACE masing-masing adalah 34,93 mg peptida/mL dan 36,62 μ g peptida/mL. Hasil ini menunjukkan bahwa isolat protein kacang koro pedang merupakan substrat yang potensial untuk menghasilkan peptida bioaktif. Penelitian ini dapat dijadikan panduan untuk produksi bahan pangan fungsional yang menjanjikan dan berkelanjutan dari kacang-kacangan yang masih belum dimanfaatkan secara maksimal menggunakan teknologi bioproses kontinu.

Kata kunci: alkalase, kacang koro pedang, neutrase, peptida bioaktif, reaktor membran enzimatis

SUMMARY

ROSE ULI RUTH CECILIA. Antihypertensive, Antioxidative, Antidiabetic Properties of Jack Bean Peptides Produced Under Enzymatic Membrane Reactor. Supervised by AZIS BOING SITANGGANG and ENDANG PRANGDIMURTI.

Rising consumer interest in functional foods stems from heightened understanding of nutrition-wellness connections, where bioactive peptides have gained considerable recognition due to their proven health-beneficial properties. This research focused on generating bioactive peptides with antioxidant, DPP-IV inhibitory, and ACE inhibitory activities from *Canavalia ensiformis* protein isolate through continuous proteolysis employing combined alcalase and neutrase enzymes. The investigation encompassed two primary phases: (1) jack bean protein isolate preparation and (2) sustained enzymatic hydrolysis examining [E]/[S] ratios, pH levels, and residence time (τ) impacts. A 5 kDa PES membrane was selected for its complete enzyme molecule retention capability within the reactor system. When the reaction to determine the optimal [E]/[S] ratio parameters was carried out, the DPP-IV inhibitory activity obtained was small and insignificant, so no analysis was carried out for other parameters. Optimal operational parameters were established at [E]/[S] = 5%, pH = 7.5, and τ = 12 h. These conditions yielded permeate results of 0.6143 mg SE/mL peptide content, 0.0454 mg TEAC/mL antioxidant capacity, and 92.18% ACE inhibition. Compared to unprocessed filtered substrate, peptide content and ACE inhibition showed enhancement while antioxidant capacity declined. This upward pattern resulted from bioactive peptide generation, whereas the downward trend occurred due to membrane rejection of phenolic compounds. Throughout 48-hour continuous filtration, peptide content and antioxidant capacity rose while ACE inhibition activity diminished. Antioxidant activity and ACE inhibition demonstrated IC₅₀ values of 34.93 mg peptide/mL and 36.62 μ g peptide/mL, respectively. These findings validate jack bean protein isolate as an effective source for producing multifunctional bioactive peptides. The research establishes groundwork for commercially viable and environmentally sustainable functional food ingredient manufacturing from lesser-utilized legumes via continuous bioprocessing technology.

Keywords: alcalase, bioactive peptides, enzymatic membrane reactor, jack bean, neutrase



Hak Cipta Dilindungi Undang-undang

1. Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan dan menyebutkan sumber :
 - a. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik atau tinjauan suatu masalah
 - b. Pengutipan tidak merugikan kepentingan yang wajar IPB University.
2. Dilarang mengumunkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.

© Copyright by IPB University, 2026
All Rights Reserved

It is prohibited to cite part or all of this paper without listing or mentioning the source. Citations are only for education, research, writing scientific papers, preparing reports, writing criticisms, or reviewing a problem, and such citations are not detrimental to the interests of IPB University.

It is prohibited to publish and reproduce part or all of this paper in any form without the permission of IPB University.

ANTIHYPERTENSIVE, ANTIOXIDATIVE, ANTIDIABETIC PROPERTIES OF JACK BEAN PEPTIDES PRODUCED UNDER ENZYMATIC MEMBRANE REACTOR

ROSE ULI RUTH CECILIA

Thesis
as one of the requirements to obtain a degree of
Master of Science
in Food Science

**FOOD SCIENCE STUDY PROGRAM
FACULTY OF ENGINEERING AND TECHNOLOGY
IPB UNIVERSITY
BOGOR
2026**



@Hak cipta milik IPB University

Hak Cipta Dilindungi Undang-undang

1. Dilarang mengutip sebagian atau seluruh karya tulis ini tanpa mencantumkan dan menyebutkan sumber :
 - a. Pengutipan hanya untuk kepentingan pendidikan, penelitian, penulisan karya ilmiah, penyusunan laporan, penulisan kritik atau tinjauan suatu masalah
 - b. Pengutipan tidak merugikan kepentingan yang wajar IPB University.
2. Dilarang mengumumkan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.

Research Title : Antihypertensive, Antioxidative, Antidiabetic Properties of Jack Bean Peptides Produced Under Enzymatic Membrane Reactor

Name : Rose Uli Ruth Cecilia

NIM : F2501241085

Approved by

Supervisor 1:

Prof. Dr.-Ing. Azis Boing Sitanggang, S.T.P., M.Sc.

NIP. 198609112010121007

Supervisor 2:

Prof. Dr. Ir. Endang Prangdimurti, M.Si.

NIP. 196807231992032001

Acknowledged by

Head of The Study Program:

Dr. Nur Wulandari, S.T.P., M.Si

NIP. 197410032000032001

Dean of Faculty of Engineering and Technology:

Prof. Dr. Ir. Slamet Budijanto, M.Agr.

NIP. 196105021986031002

Examination Date:

April 17th, 2026

Graduate Date:

April 27th, 2026

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to all those who supported and guided me throughout my research journey. First and foremost, I extend my heartfelt thanks to my supervisor, Prof. Dr.-Ing. Azis Boing Sitanggang, S.T.P., M.Sc., for providing me with the opportunity, support, guidance, motivation, and insightful feedback. I am grateful for the trust you placed in me to participate in this research. I would also like to thank Prof. Dr. Ir. Endang Prangdimurti, M.Si. for assisting me in the process of writing this thesis. I appreciate all your input and advice. For MEASURE-Hub family, Kak Nadine, Kak Farah, Bang David, Bang Yohan, etc. Thank you for your guidance, motivation, and counsel with this research. Your care and concern for my work and my feelings as an individual are truly appreciated. I would like to extend my sincere gratitude to all my friends in AMFOODTER 57 and the Sinergi Group for their encouragement, companionship, and support. Your presence has been invaluable in providing moments of relief and emotional strength during challenging times.

I am also deeply grateful to my family for their constant love and encouragement. To my parents, Freddy Liong and Rosmaida Helena, and especially my beloved mother Yessy Betty Evalyna Sirait (+) your belief in me and your endless support provided me with the strength to persevere. Thank you for always trusting me every step of the way. I couldn't have done this without you. To my not so little brother, Michael Paulus Hasiholan, your support has meant the world to me. Thank you for the reels and jokes you always send me through DM to keep my mood up. To my Rocky, Yoseph Bramantyo, thank you for your unwavering support and for always accompanying me through this journey. With your astrophage, you kept me fueled with constant motivation and light, even in the most difficult moments. Lastly, I would like to express my gratitude to myself for surviving thus far and for staying resilient in the face of adversity. Thank you for holding on and for continuing to trust yourself along the way.

Above all, I am thankful to Jesus Christ, for His providence and plan have made all of this possible. "For I know the plans I have for you, says the LORD, plans to prosper you and not to harm you, plans to give you hope and a future" (Jeremiah 29:11).

Bogor, April 2026

Rose Uli Ruth Cecilia

TABLE OF CONTENT

| | |
|--|----|
| LIST OF TABLES | x |
| LIST OF FIGURES | x |
| LIST OF APPENDIXES | xi |
| I INTRODUCTION | 1 |
| 1.1 Background | 1 |
| 1.2 Problem Statement | 2 |
| 1.3 Research Objectives | 2 |
| 1.4 Research Outcomes | 2 |
| II METHODOLOGY | 3 |
| 2.1 Time and Place | 3 |
| 2.2 Equipment and Materials | 3 |
| 2.3 Research Procedure | 3 |
| 2.4 Analytical Methods | 7 |
| 2.5 Statistical Analysis | 9 |
| III RESULTS AND DISCUSSION | 10 |
| 3.1 Jack Bean Protein Isolate Characteristics | 10 |
| 3.2 Enzyme Rejection | 11 |
| 3.3 Influence of Enzyme-to-Substrate Ratio | 13 |
| 3.4 Influence of pH | 16 |
| 3.5 Influence of Residence Time | 19 |
| 3.6 In Long-Term Continuous Production | 22 |
| 3.7 IC ₅₀ Values of Peptide Fractions | 23 |
| IV CONCLUSION AND SUGGESTION | 25 |
| 4.1 Conclusion | 25 |
| 4.2 Suggestion | 25 |
| REFERENCES | 26 |
| APPENDIX | 32 |
| BIOGRAPHY | 36 |

LIST OF TABLES

| | | |
|---|---|----|
| 1 | Protein and moisture content of jack bean protein isolate | 11 |
| 2 | The activity of alcalase, neutrase, and the combination | 11 |
| 3 | Permeate characteristics from production of bioactive peptides with enzyme-to-substrate ratio parameter using alcalase and neutrase (1:1 (v/v)) | 13 |
| 4 | Permeate characteristics from production of bioactive peptides with pH parameter using alcalase and neutrase (1:1 (v/v)) | 17 |
| 5 | Permeate characteristics from production of bioactive peptides with residence time parameter using alcalase and neutrase (1:1 (v/v)) | 20 |

LIST OF FIGURES

| | | |
|---|---|----|
| 1 | Research flow chart. A = Proximate composition, B = Peptide content, C = Antioxidant capacity-DPPH method, D = DPP-IV inhibitory activity, E = ACE inhibitory activity | 4 |
| 2 | Automated enzymatic membrane reactor (EMR). 1 = Nitrogen (N), 2 = Pressure reducer, 3 = Proportional Pressure Regulator-MPPE, 4 = Substrate tank, 5 = Reactor, 6 = UF membrane, 7 = Analytical balance, 8 = Water bath system, 9 = PC with Lab VIEW program (Sitanggang <i>et al.</i> 2021) | 5 |
| 3 | Jack bean protein isolate | 10 |
| 4 | Profiles of transmembrane pressure (TMP), set flux, and real flux during alcalase, neutrase, and dual-enzyme filtration with 5-kDa PES membrane | 12 |
| 5 | Fouling rate during alcalase, neutrase, and dual-enzyme filtration with 5-kDa PES membrane | 12 |
| 6 | The influence of enzyme-to-substrate ratio on (a) peptide content, (b) antioxidant capacity, (c) DPP-IV inhibition and (d) ACE inhibition during continuous hydrolysis of jack bean protein isolate. Reacting conditions: [S] = 0.75% (w/v), residence time = 6 h (flux = 12.12 L/m.h), pH = 7.5, agitation speed = 300 rpm, T = 50 C, 5-kDa PES membrane | 13 |
| 7 | Profiles of transmembrane pressure (TMP), set flux, and real flux during continuous hydrolysis of jack bean protein isolate at different levels of [E]/[S]: (a) 5%, (b) 7.5%, and (c) 12% | 15 |
| 8 | Protein's charge distribution of alcalase and neutrase at varied physiological pH (The accession codes for calculating protein charge distribution of alcalase was P00781 and for neutrase was P06382. Accession codes were taken from www.brenda-enzymes.org) | 16 |
| 9 | The influence of pH on (a) peptide content, (b) antioxidant capacity, and (c) ACE inhibition during continuous hydrolysis of jack bean protein isolate. Reacting conditions: [S] = 0.75% (w/v), [E]/[S] = 5%, residence time = 6 h (flux = 12.12 L/m ² .h), agitation speed = 300 rpm, T = 50 °C, 5-kDa PES membrane | 17 |

- 10 Profiles of transmembrane pressure (TMP), set flux, and real flux during continuous hydrolysis of jack bean protein isolate at different levels of pH: (a) 7, (b) 7.5, and (c) 8.5 18
- 11 The influence of residence time on (a) peptide content, (b) antioxidant capacity, and (c) ACE inhibition during continuous hydrolysis of jack bean protein isolate. Reacting conditions: $[S] = 0.75\%$ (w/v), $[E]/[S] = 5\%$, pH = 7.5, agitation speed = 300 rpm, T = 50 °C, 5-kDa PES membrane 19
- 12 Profiles of transmembrane pressure (TMP), set flux, and real flux during continuous hydrolysis of jack bean protein isolate at different levels of residence time: (a) 4 h, (b) 6 h, (c) 10 h, and (d) 12 h 20
- 13 The influence of hydraulic residence time on fouling rate ($r_f = \frac{dP}{dt} \times \frac{1}{\eta \times J}$) during 8-h continuous hydrolysis of jack bean protein isolate. Reacting conditions: $[S] = 0.75\%$ (w/v), $[E]/[S] = 5\%$, pH = 7.5, residence time = 12 h (flux = 6.06 L/m².h), agitation speed = 300 rpm, T = 50 °C, 5-kDa PES membrane with dynamic viscosity assumed to be the same as water at T = 50 °C; 0.5471 mPa.s 21
- 14 Profile of (a) peptide content, (b) antioxidant capacity, and (c) ACE inhibition during continuous hydrolysis of jack bean protein isolate. Reacting conditions: $[S] = 0.75\%$ (w/v), $[E]/[S] = 5\%$, pH = 7.5, agitation speed = 300 rpm, T = 50 °C, 5-kDa PES membrane 22
- 15 Profiles of transmembrane pressure (TMP), set flux, and real flux during the long-term reaction (48 h) 23
- 16 IC₅₀ values of (a) antioxidant and (b) ACE inhibitory activity. Different superscript letters show significant differences with a confidence level of 95% 24

LIST OF APPENDIXES

- 1 Appendix 1 Tyrosine standard curve for enzyme activity assay 33
- 2 Appendix 2 Serine standard curve for peptide content assay 34
- 3 Appendix 3 Trolox standard curve for DPPH assay 35