



CONTINUOUS PRODUCTION OF CASEIN-BASED BIOACTIVE PEPTIDES USING DUAL ENZYMATIC PROTEOLYSIS

RUDANG IRENE CELICA



**DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY
FACULTY OF AGRICULTURAL TECHNOLOGY
IPB UNIVERSITY
BOGOR
2022**

STATEMENT LETTER OF MANUSCRIPT, SOURCES OF INFORMATION, AND DELEGATION OF COPYRIGHTS

I hereby declare that the manuscript with title of “Continuous Production of Casein-based Bioactive Peptides using Dual Enzymatic Proteolysis” is truthfully my works under the guidance of supervision committee and has never been published on any academic or institutional platform in any form. Sources of information taken or cited from another published and/or unpublished articles has been stated in the text and bibliography at the end of the manuscript.

With this, I bestow the copyright of this manuscript to IPB University.

Bogor, July 2022

Rudang Irene Celica
F24188010

© Hak Cipta Dilindungi Undang-undang
IPB University

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.

ABSTRACT

RUDANG IRENE CELICA. Continuous Production of Casein-based Bioactive Peptide using Dual Enzymatic Proteolysis. Supervised by AZIS BOING SITANGGANG and ENDANG PRANGDIMURTI.

Sodium Caseinate is a derivative form of Casein Protein that is commonly found in milk proteins and has the potential as a parent protein for producing bioactive peptides. The functionality of bioactive peptides can be increased by dual enzyme hydrolysis with alcalase and neutrase enzymes. Several parameters were considered in conducting continuous dual enzyme hydrolysis, namely; enzyme-to-substrate concentration ratio (E/S), pH value, and residence time (τ). This research aimed to produce bioactive peptides from sodium caseinate continuously using an enzymatic membrane reactor and study the optimum parameters that can enhance the functionalities of the hydrolysates. Permeate with an E/S of 7.5%, pH value of 7.0, and $\tau = 9$ h had the highest values for the antioxidant capacity of 19.65 ± 1.81 mgAEAC/mL for DPPH Method, 22.47 ± 6.50 mgAEAC/mL for FRAP method, and $91.39 \pm 3.13\%$ inhibition for ABTS method. The value of IC_{50} antioxidant with the DPPH method was $0,18 \pm 0,03$ g/mL obtained by filtering permeate with a PES (Polyethersulfone) membrane MWCO of 4 kDa.

Keywords: Alcalase, bioactive peptides, casein, enzymatic membrane reactor, neutrase

@Hak cipta milik IPB University

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.

Perpustakaan IPB University





CONTINUOUS PRODUCTION OF CASEIN-BASED BIOACTIVE PEPTIDE USING DUAL ENZYMATIC PROTEOLYSIS

A Manuscript
As Partial Fulfillment of the Requirements for the Attainment of the Degree of Sarjana in
Program Studi Ilmu dan Teknologi Pangan

RUDANG IRENE CELICA

**DEPARTEMEN ILMU DAN TEKNOLOGI PANGAN
FAKULTAS TEKNOLOGI PERTANIAN
INSTITUT PERTANIAN BOGOR
BOGOR
2022**

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 menggunakan 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.

Examiner during Manuscript Exam:

1 Dr. –Ing. Azis Boing Sitanggang, S. TP, M. Sc.

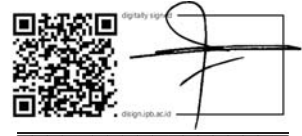
2 Dr. Ir. Endang Prangdimurti, M. Si

3 Prof. Dr. Ir. Lilis Nuraida, M. Sc

Manuscript Title : Continuous Production of Casein-based Bioactive Peptide
using Dual Enzymatic Proteolysis
Nama : Rudang Irene Celica
Student ID : F24188010

Approved by

Supervisor 1:
Dr. -Ing. Azis Boing Sitanggang, S.TP, M.Sc



Supervisor 2:
Dr. Ir. Endang Prangdimurti, M. Si



Approved by

Head of Food Science and Technology Department:
Dr. Ir. Eko Hari Purnomo, M.Sc
NIP 19760412 199903 1 004



Date of Examination:
18 July 2022

Date of Graduation:



“let the wise listen and add to their learning, and let the discerning get guidance—for understanding proverbs and parables, the sayings and riddles of the wise.” Proverbs 1:5-6

“baiklah orang bijak mendengar dan menambah ilmu dan baiklah orang yang berpengertian memperoleh bahan pertimbangan untuk mengerti amsal dan ibarat, perkataan dan teka-teki orang bijak.” Amsal 1:5-6

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.

PREFACE

All Praise and Gratitude I raise to the Lord Jesus Christ, because of His blessings I was able to get to this point, where I was finally able to complete my bachelor's degree for four years at the Department of Food Science and Technology, IPB University. I would like to express my gratitude to my parents, Mr. Benjani Saragih and Mrs. Rohni Mutiara Sipayung, because for their never-ending prayers and endless support, I can completed this thesis. Of course, to my brother, Obed Exaudi Nathanael, who has always been one of the places for me to tell stories, thank you very much, and congratulations on your success in getting into the same university as me. I hope you can fight to finish your study as much as I did.

I'm not forget to say thank you very much to my supervisor, Dr. –Ing. Azis Boing Sitanggang, because of his direction from the beginning until the end of the research, because of his four years of teaching, and because of him, I was interested in studying at the Department of Food Science and Technology. Thank you also, because of him I was able to get a lot of knowledge both in food science and life experiences. I also thank Dr. Ir. Endang Prangdimurti as my second supervisor, for providing a lot of knowledge and input, and being very patient and tender in dealing with me.

To my friends in Bandung, Yohana, Febi, Clara, thank you for always being my friend for more than a half of my age. Thank you for always being there and always ready to be a place for me to complain, not only during my college process but also about everything that happened in my life. I hope you all will be successful wherever Jesus places you in the future. Also to my unexpected partner, Deve, thank you for bring me joy, and your endless support and motivation for me to keeping up my spirit in writing this manuscript and also thank you for every effort that you made. Lastly, Jerem and Monic, thank you because even though we rarely see each other, you are very, very low-maintenance friends. Thank you very much because both of you have always been there since I was in high school.

To my friends in Bogor, “Cintar” (Carlsen, Chanson, Emerson, Marco, Sanjay, Valent, and Willy), and Kak Felia Prima Wefiani, thank you very much because you have been one of my supporters while studying at the campus, without you my grades might not have been saved lol. Thanks also to Emerson, Chanson and Matthew Kevin, because you guys have been willing to be the place where I go whenever I felt down and thank you for giving me a lot of advices and supports during my study. I wish both of you success in your life journey. For “Dastek Assistants” alias “ABS Team Siap Mati” (Nadine, Carlsen, Helena), thank you for helping me a lot through my research process. To Wildan, and Azka, thank you for being a place for me to discuss a lot of things and for being friends who are always entertaining. To my “Penghuni Starbucks Dramaga” (Ajeng, Arel, Ipeh, Ia, Evan, Wildan), thank you for wanting to work together to complete our studies together with me, I wish you all success in the future.

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.

Especially for the members of Himitepa-Satu Layang Cabinet, especially Selma as chairman and External Division (Lintang, Levina, Alin, Aldi, Febian, Evan, Alit, Willy, and Adel), thank you very much for the wonderful experience in working on all work projects during this term of office. Thank you also for being an entertaining friends, a place to discuss, to a place to complain together, and thank you because you all wanting to keep learning with me with every weakness I have. I wish you all success in achieving your respective dreams.

Finally, to the Astrofood ITP 55 family and International Class FATETA 55, to “Jangan Dibawa Keluar Yaaa” (Sheila Hanindra, Dinda Afifah, Hana Humaira), thank you very much because you taught me many things in life while I was studying at FST, a lot of memorable experiences, and taught me what solidarity means.

*“Boras i pinggan sapa
I babouni pinggan pasu,
Sonon ma na boi hu patupa
Andohar gabe pasu-pasu.”*

@Hak cipta milik IPB University

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.



TABLE OF CONTENTS

LIST OF TABLE	xii
LIST OF FIGURE	xii
LIST OF APPENDIX	xiii
I INTRODUCTION	1
1.1 Background	1
1.2 Problem Formulation	2
1.3 Objectives	2
1.4 Benefits	2
II LITERATURE REVIEW	3
2.1 Sodium Caseinate	3
2.2 Enzymatic Hydrolysis	3
2.3 Bioactive Peptides	4
2.4 Antioxidant	4
2.5 Enzymatic Membrane Reactor	4
III MATERIALS AND METHODS	6
3.1 Time and Places	6
3.2 Equipment and Materials	6
3.3 Methodology	6
IV RESULT AND DISCUSSION	15
4.1 Continuous Production of Casein-Based Bioactive Peptides	15
4.2 IC ₅₀ Value	34
V CONCLUSIONS AND RECOMMENDATIONS	38
BIBLIOGRAPHY	39
APPENDIX	44

LIST OF TABLES

1	Functional properties of hydrolysis permeate with E/S parameter to sodium caseinate substrate	19
2	Functional properties of hydrolysis permeate with pH parameter to sodium caseinate substrate	25
3	Functional properties of hydrolysis permeate with residence time parameter to sodium caseinate substrate	31
4	Antioxidant IC ₅₀ value from certain protein sources	37

LIST OF FIGURES

1	Research procedure	7
2	Membrane reactor design	8
3	The design view of LABVIEW programme	12
4	The Effect of E/S concentration to antioxidant capacity	17
5	Flux and pressure profile of E/S factor	21
6	The Effect of pH level to antioxidant capacity	23
7	Flux and pressure profile of pH factor	27
8	The Effect of residence time to antioxidant capacity	29
9	Flux and pressure profile of residence time factor	33
10	IC ₅₀ value with DPPH method on several sizes of membrane	35

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.

LIST OF APPENDICES

15	ANOVA Analysis of E/S Parameter	44
16	ANOVA Analysis of pH Parameter	46
17	ANOVA Analysis of Residence Time Parameter	47
18	ANOVA analysis of IC ₅₀	49
19	Tyrosine Standard Curve	50
20	Ascorbic Acid Standard Curve (DPPH)	50
21	Bovine Serum Albumin (BSA) Standard Curve	51
22	5kDa MWCO membrane	52



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 menggunakan dan memperbanyak sebagian atau seluruh karya tulis ini dalam bentuk apapun tanpa izin IPB University.



@Hak cipta milik IPB University

IPB University



IPB University
— Bogor — Indonesia —

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.