

ACKNOWLEDGEMENT

Greatest thanks to God as it is only by His unfailing grace that the writer can finish the thesis, starting from the writing of thesis proposal up to the end of the writing of thesis report. This thesis is entitled “OPTIMUM CONDITION FOR THE PRODUCTION OF N-ACETYLGLUCOSAMINE FROM *Penaeus monodon* SHELL USING CRUDE AND SEMI-PURE INTRACELLULAR CHITINASE ENZYME FROM *Mucor circinelloides* ” that is written as partial fulfillment of the academic requirements to obtain the degree of *Sarjana Teknologi Pertanian Strata Satu*, Universitas Pelita Harapan.

Without support, guidance, encouragement, and prayers during the thesis program from the following important people, this thesis would not be finished on time. Thus, the writer would like to express her utmost gratitude toward these following people:

1. Mr. Eric Jobiliong, Ph.D. as Dean of Faculty of Science and Technology.
2. Ms. Dela Rosa, M.M., M.Sc., Apt. as Vice Dean of Faculty of Science and Technology.
3. Mr. Laurence, M.T. as Director of Administration and Student Affair of Faculty of Science and Technology.
4. Mr. Ir. W. Donald R. Pokatong, M.Sc., Ph.D. as Head of Food Technology Study Program and academic supervisor who had provided guidance and knowledge to the writer throughout the academic program.
5. Ms. Ratna Handayani, MP. As Vice Head of Food Technology Study Program who had given advices and guidance regarding the glucosamine research.
6. Ms. Yuniwaty Halim, M.Sc. as thesis supervisor and Head of Quality Control and Research Laboratory who had supervised, provide advices, guidance, support during the thesis program and had given permission for the writer to conduct the research in the laboratorium.
7. Dr. Ir. Hardoko, MS. as lecturer of Food Technology Study Program who had given advices, knowledge and guidance regarding the glucosamine research.

8. Ms. Titri Sirantantri Mastuti, MSi. and Ms. Lucia Crysanthi Soedirga, M.Sc. who had given advices and guidance regarding the glucosamine research.
9. Dr. Adolf J. Parhusip as Head of Microbiology Laboratory who had given permission for the writer to conduct the research in the laboratorium.
10. Dr. Tagor M. Siregar and Ms. Natania, M.Eng. as Head of Chemistry and Food Processing Laboratory who had given permission to the writer to conduct the research in the laboratories.
11. Mr. Yosafat Rudju, Mr. Adhi, Mr. Adzie, and Mr. Darius Wulakada who had provided help and guidance during the research in the laboratory.
12. Christopher Imansantoso Rimba, STP as lecturer assistant who had provided support and guidance throughout the thesis program.
13. All lecturers of Food Technology Study Program of Universitas Pelita Harapan for the knowledge and support given throughout the academic program which were applied by the writer during the thesis program.
14. Mr. Asiu who had contributed in providing black tiger shrimp shell as the main raw ingredient needed for the completion of the research.
15. Beloved mother, father, and brother who provided never ending support, love, and encouragement throughout the thesis program.
16. Cynthia Saputra as partner during thesis program who had given help, support, and knowledge during the thesis program.
17. All of glucosamine family who had provided help and support during the thesis program.
18. Richelle Kwok, Gabriella Maureen, James Patrick, Dewiclara Putriawan, Eric Gerald, Karen Grace, Jennifer Kuswanto, Ivana Chandra Purnama, Gilbert Moniaga, Halim Priyatna, Michelle Monica, and William Muliawan for the help and support during the thesis program.
19. Lydia Senjaya Leo, Elvina Suteja, Natasha Collhins, Sherly, and Celine Angelia for the help and support during the thesis program.
20. All people who had given contribution and support to the writer during the thesis program that cannot be mentioned one by one.

The writer realizes that this thesis report is far from perfect. The writer would like to apologize for any mistake done during the thesis program until the completion of this report. Therefore, the writer welcomes any criticisms and suggestions given to this report. Lastly, the writer hopes that the thesis report can provide knowledge and be beneficial for the pertinent readers.

Tangerang, February 4th, 2019

(Fransiska)

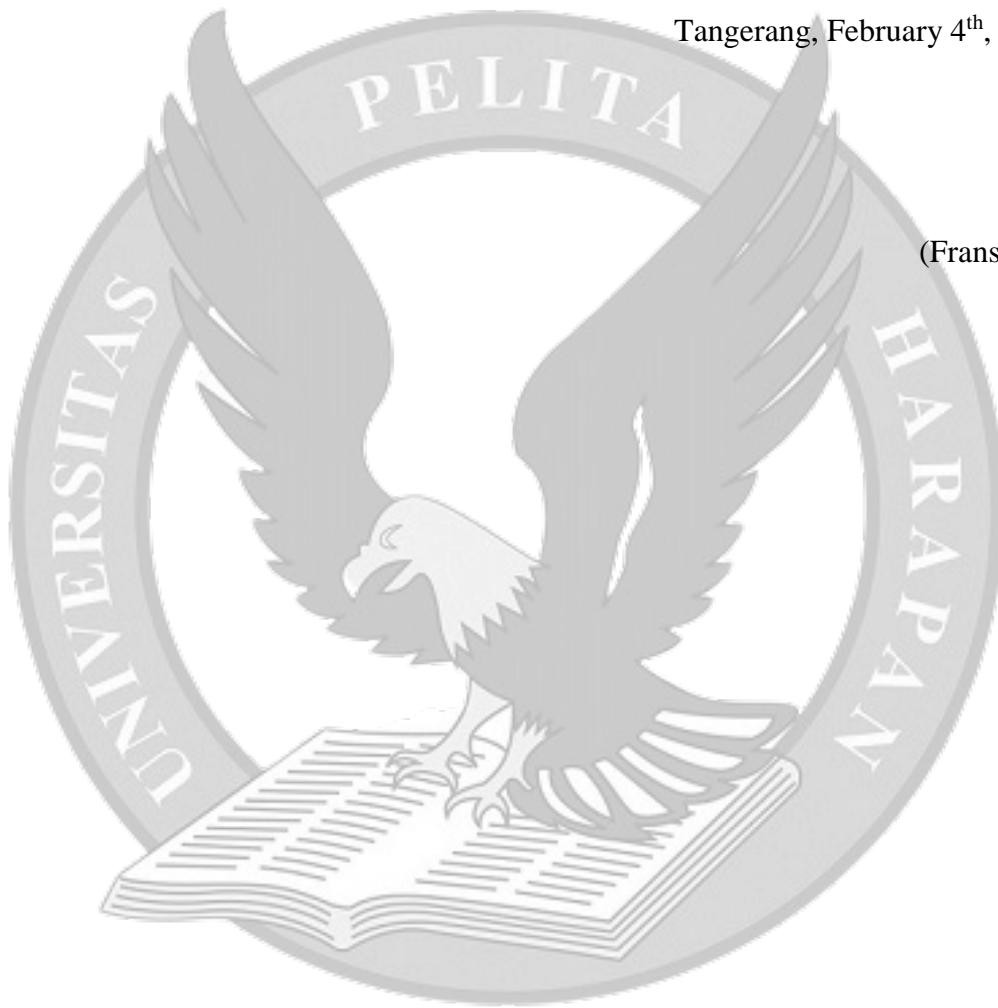


TABLE OF CONTENTS

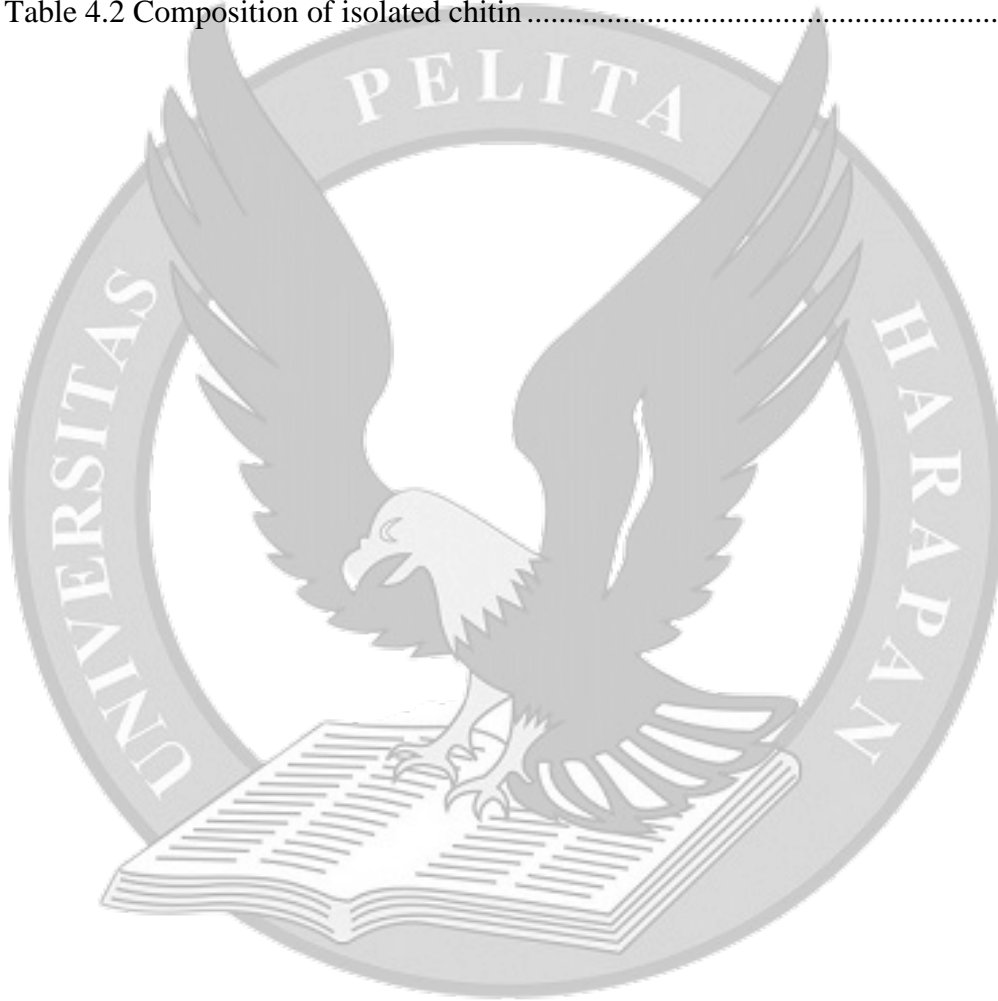
	page
COVER	
STATEMENT OF THESIS AUTHENTICITY	
APPROVAL BY THESIS SUPERVISOR	
APPROVAL BY EXAMINATION COMMITTEE	
ABSTRACT.....v	v
<i>ABSTRAK</i>vi	vi
ACKNOWLEDGEMENT.....vii	vii
TABLE OF CONTENTS.....x	x
LIST OF TABLES.....xiii	xiii
LIST OF FIGURES.....xiv	xiv
LIST OF APPENDICES.....xv	xv
CHAPTER I INTRODUCTION.....1	1
1.1 Background.....1	1
1.2 Research Problem.....3	3
1.3 Objective.....4	4
1.3.1 General Objective.....4	4
1.3.2 Specific Objective.....4	4
CHAPTER II LITERATURE REVIEW.....5	5
2.1 Tiger Shrimp (<i>Penaeus monodon</i>).....5	5
2.2 Chitin.....6	6
2.3 Glucosamine.....11	11
2.4 Enzyme.....12	12
2.4.1 Extracellular Enzyme.....13	13
2.4.2 Intracellular Enzyme.....13	13
2.4.3 Purification of Enzyme.....14	14
2.5 Chitinase Enzyme.....14	14
2.5.1 Fungal Chitinase.....16	16
2.6 <i>Mucor circinelloides</i>17	17
2.7 Degree of Deacetylation.....18	18

CHAPTER III RESEARCH METHODOLOGY	19
3.1 Materials and Equipment	19
3.2 Research Method.....	20
3.2.1 First Stage Research.....	20
3.2.1.1 Preparation of Isolated Chitin (Dompeipen <i>et al.</i> , 2016)	21
3.2.1.2 Preparation of Stock and Working Culture (Saputra <i>et al.</i> , 2017)	22
3.2.1.3 Morphology of <i>Mucor circinelloides</i> (Coonrod <i>et al.</i> , 2012)	23
3.2.1.4 Counting of Fungal Spores (Bergmans <i>et al.</i> , 2005)	24
3.2.1.5 Extraction of Intracellular Chitinase Enzyme of <i>Mucor circinelloides</i> (Struszczyk <i>et al.</i> , 2008)	25
3.2.1.6 Determination of Optimum pH (Annamalai <i>et al.</i> , 2011)	27
3.2.1.7 Determination of Optimum Temperature (Annamalai <i>et al.</i> , 2011)	27
3.2.2 Second Stage Research	28
3.2.2.1 Determination of Optimum Substrate Concentration and Incubation Period (Annamalai <i>et al.</i> , 2011)	28
3.3 Experimental Design	29
3.3.1 First Stage Research.....	29
3.3.2 Second Stage Research	31
3.4 Method of Analysis	33
3.4.1 Moisture Content Analysis (AOAC, 2000).....	33
3.4.2 Determination of Ash Content (AOAC, 2000)	33
3.4.3 Protein Content Analysis (Ernst and Zor, 2010).....	34
3.4.4 Chitin Isolate Analysis	35
3.4.4.1 Degree of Deacetylation (Benhabiles <i>et al.</i> , 2012)	35
3.4.4.2 Yield of Chitin and Shrimp Shells (Demir <i>et al.</i> , 2016)	35
3.4.5 Measurement of Chitinase Activity (Jha and Modi, 2017)	36
3.4.6 Analysis of <i>N</i> -acetylglucosamine.....	36

3.4.6.1 Preparation of <i>N</i> -acetylglucosamine Standard Curve (Rahmansyah and Sudiana, 2003).....	36
3.4.6.2 Quantification of <i>N</i> -acetylglucosamine (Rahmansyah and Sudiana, 2013).....	37
CHAPTER IV RESULTS AND DISCUSSIONS	38
4.1 Characteristics of Shrimp Shells Powder	38
4.2 Characteristics of Isolated Chitin	40
4.3 Identification of <i>Mucor circinelloides</i>	44
4.3.1 Morphology of <i>Mucor circinelloides</i>	44
4.3.2 Spore Counting of <i>Mucor circinelloides</i>	46
4.4 First Stage Research	47
4.4.1 Effect of pH on Chitinase Activity.....	47
4.4.1.1 Crude Chitinase Enzyme.....	47
4.4.1.2 Semi-pure Chitinase Enzyme.....	49
4.4.2 Effect of Temperature on Chitinase Activity	51
4.4.2.1 Crude Chitinase Enzyme.....	51
4.4.2.2 Semi-pure Chitinase Enzyme.....	53
4.5 Second Stage Research.....	55
4.5.1 Effect of Substrate Concentration and Incubation Period on <i>N</i> -acetylglucosamine Production using Crude Chitinase Enzyme	56
4.5.2 Effect of Substrate Concentration and Incubation Period on <i>N</i> -acetylglucosamine Production using Semi-pure Chitinase Enzyme.....	59
CHAPTER V CONCLUSIONS AND SUGGESTIONS.....	62
5.1 Conclusions	62
5.2 Suggestions.....	63
BIBLIOGRAPHY	64
APPENDICES	75

LIST OF TABLES

	page
Table 3.1 Statistical design of pH factor	30
Table 3.2 Statistical design of temperature factor.....	30
Table 3.3 Statistical design of the second stage research.....	32
Table 4.1 Composition of powdered shrimp shell	38
Table 4.2 Composition of isolated chitin	41



LIST OF FIGURES

	page
Figure 2.1 Morphology of <i>Penaeus monodon</i>	6
Figure 2.2 Chemical structure of chitin.....	10
Figure 2.3 Chemical structure of chitosan	10
Figure 2.4 Chemical structure of glucosamine	11
Figure 2.5 Chemical structure of <i>N</i> -acetylglucosamine.....	12
Figure 2.6 Cleaving mechanism of endochitinase (A), exochitinase (B) and <i>N</i> -acetylglucosaminidase (C)	16
Figure 2.7 Colonies of <i>Mucor circinelloides</i> grown on CYA.....	17
Figure 3.1 Flow diagram of substrate preparation from <i>Penaeus monodon</i> shell	22
Figure 3.2 Flow diagram of stock and working culture preparation.....	23
Figure 3.3 Five boxes counted in hemocytometer	24
Figure 3.4 Flow diagram of crude and semi-pure intracellular chitinase enzyme extraction.....	26
Figure 3.5 Flow diagram of optimum pH determination	27
Figure 3.6 Flow diagram of optimum temperature determination.....	28
Figure 3.7 Flow diagram of optimum substrate concentration and incubation period determination.....	29
Figure 4.1 Morphology of <i>Mucor circinelloides</i> at 10x magnification (a) sporangiospore, (b) sporangium, (c) sporangiophore, (d) aseptate (coenocytic) hyphae	45
Figure 4.2 Microscopic observation of <i>M. circinelloides</i> on PDA at 10x magnification (a) sporangiospore, (b) sporangium, (c) sporangiophore, (d) aseptate (coenocytic) hyphae.....	46
Figure 4.3 Effect of pH on crude chitinase activity	48
Figure 4.4 Effect of pH on semi-pure chitinase activity	49
Figure 4.5 Effect of temperature on crude chitinase activity	52
Figure 4.6 Effect of temperature on semi-pure chitinase activity	53
Figure 4.7 Effect of substrate concentration and incubation period on GlcNAc concentration using crude chitinase	57
Figure 4.8 Effect of substrate concentration and incubation period on GlcNAc concentration using semi-pure chitinase	60

LIST OF APPENDICES

	page
Appendix A.	
Identification Results of Black Tiger Shrimp (<i>Penaeus monodon</i>)	A-1
Appendix B. Shrimp Shell Powder Analysis Results	
B-1. Moisture Content	B-1
B-2. Ash Content	B-1
B-3. Protein Content	B-2
B-4. Yield.....	B-3
Appendix C.	
Documentation of Isolation Process of Chitin from Shrimp Shell	C-1
Appendix D. Isolated Chitin Analysis Results	
D-1. Moisture Content	D-1
D-2. Ash Content	D-1
D-3. Protein Content	D-2
D-4. Yield	D-2
D-5. Degree of Deacetylation	D-3
Appendix E.	
Counting of Fungal Spores Using Hemocytometer	E-1
Appendix F.	
Standard Curve of <i>N</i> -acetylglucosamine.....	F-1
Appendix G.	
Enzyme Activity of Crude and Semi-pure Intracellular Chitinase Enzyme	G-1
Appendix H. Second Stage Research	
H-1. <i>N</i> -acetylglucosamine Concentration Obtained from Crude Intracellular Chitinase Enzyme at Different Substrate Concentration and Incubation Period	H-1
H-2. <i>N</i> -acetylglucosamine Concentration Obtained from Semi-pure Intracellular Chitinase Enzyme at Different Substrate Concentration and Incubation Period	H-9