DEVELOPMENT OF IMMOBILIZED KERATINASE AND INVESTIGATION OF ITS EFFICIENCY ON EDIBLE BIRD NEST CLEANING PROCESS

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ABSTRAK

Proses pembersihan sarang burung walet (EBN) menjadi salah satu proses yang penting dan merupakan bahagian yang paling merumitkan dalam penyediaan EBN. EBN yang diperbuat daripada struktur yang stabil kerana mempunyai banyak keratin protein yang kuat. Oleh kerana ini, imobilisasi enzim digunakan dalam proses pembersihan EBN. Oleh itu, tujuan kajian ini, untuk menentukan faktor-faktor yang mempengaruhi penyedian Keratinase-CLEA menggunakan parameter-parameter yang berbeza, menganalisa kecekapan hidrolisis Keratinase-CLEA dalam pengoptimuman keadaan tindak balas dan untuk mengkaji kesan Keratinase-CLEA terhadap kualiti EBN dalam proses rawatan pembersihan dan komposisinya. Dalam kajian semasa, keratinase imobilisasi diperbuat menggunakan parameter-parameter yang berbeza seperti jenis pemendak, kepekatan pemendak, kepekatan glutaraldehid, masa terpaut silang, jenis bahan tambahan dan kepekatan bahan tambahan. Kemudian, Keratinase-CLEA dicirikan menggunakan parameter-parameter yang berbeza seperti suhu optimum, kestabilan haba, pH optimum, kestabilan pH, kebolehgunaan semula, spektroskopi inframerah fourier transformasi (FTIR) dan pelepasan bidang mikroskop electron (FESEM). Untuk menganalisis prestasi keratin hidrolisis menggunakan Keratinase-CLEA, proses pemeriksaan dilakukan menggunakan OFAT dan FFD diikuti dengan pengoptimuman suhu dan enzim dan kepekatan substrat menggunakan CCD. Di samping itu, parameter kinetik Km, Vmax, Kcat and Kcat/Km juga dijalankan dalam kajian ini. Akhirnya, Keratinase-CLEA digunakan pada proses pembersihan EBN dan EBN yang dihasilkan dianalisis berdasarkan kualiti pembersihan EBN, kebolehgunaan semula dan analisis proksimat. Dari hasil kajian ini, parameter-parameter yang terbaik dalam penyediaan imobilisasi keratinase ialah ammonium sulfat sebagai pemendak dengan kepekatan 100%, glutaraldehid dengan kepekatan 100 mM dan 14 jam masa terpaut silang untuk membentuk Keratinase-CLEA dengan pemulihan aktiviti enzim yang baik (127.34%). Oleh itu, prestasi keratin hidrolisis menggunakan Keratinase-CLEA terus dikaji menggunakan OFAT, FFD dan CCD dan parameter yang paling penting adalah suhu (50°C), kepekatan enzim (180 mg/ml) dan kepekatan substrat (0.25%). Analisis kinetik melaporkan bahawa Keratinase-CLEA mempunyai kecekapan pemangkin yang tinggi berbanding dengan enzim bebas dengan menggunakan parameter seperti Km (0.088 mm-1), Vmax (9.766 mmol L-1 min-1), Kcat (0.037 s-1) dan Kcat/Km (0.416 mm s-1). Kemudian, untuk perbandingan analisis masa, didapati bahawa Keratinase-CLEA memerlukan 30 minit untuk menghasilkan EBN yang bersih manakala apabila menggunakan air perlukan 40 minit dan Keratinase-CLEA boleh digunakan sehingga 5 kitaran untuk proses pembersihan EBN. Akhirnya, analisis proksimat melaporkan bahawa komposisi protein mentah (57.6% w/w) dan karbohidrat (22.3% w/w) didalam EBN yang telah dibersihkan menggunakan Keratinase-CLEA mempunyai kandungan yang lebih tinggi berbanding dengan EBN yang dibersihkan menggunakan air. Oleh itu, Keratinase-CLEA terbukti boleh digunakan untuk menghidrolisi keratin dari bulu dan penyelesaian ini boleh digunakan untuk meningkatkan proses pembersihan EBN dengan mengurangkan penggunaan masa.

ABSTRACT

The edible Bird Nest (EBN) cleaning process is one of the important processes in the production of EBN and the most tedious part in the production of EBN because it requires a long time. Feathers are the most impurities found in EBN that are made up of stable structures because of the large abundance of the rigid protein keratin. For this reason, immobilized keratinase is utilized in the cleaning process of EBN. Therefore, this study aims to investigate factors affecting keratinase-CLEA preparation using different parameters, to analyze the hydrolysis efficiency of the Keratinase-CLEA in optimization of reaction conditions and to study the effect of Keratinase-CLEA on EBN quality in cleaning treatment process and the compositions. In the present study, keratinase immobilization was performed using different parameters such as type of precipitants, precipitant concentration, glutaraldehyde concentration, cross-linking time, type of additive and additive concentration. Then, keratinase-CLEA was characterized using different parameters such as temperature optimum, thermal stability, pH optimum, pH stability, reusability, Field Emission Scanning Electron Microscopy (FESEM) and Fourier Transform Infrared (FTIR) Spectroscopy. In order to analyze the performance of keratin hydrolysis using keratinase-CLEA, the screening process was done using OFAT and FFD, followed by optimization of temperature and enzyme and substrate concentration using CCD. In addition, the kinetic parameters of Km, Vmax, Kcat and Kcat/Km were also conducted in this study. Finally, keratinase-CLEA was applied to the cleaning process of EBN and the resulted EBN was analyzed based on their cleaning quality, reusability and proximate analysis. From the finding, the best parameters for the keratinase immobilization were found at 100% ammonium sulfate, 100 mM glutaralaldehyde and 14 hours cross-linking time to form keratinase-CLEA with the best enzyme relative activity (127.34 %). The reusability analysis found that keratinase-CLEA retained more than 40 % keratinase activity after 5 cycles of cleaning process. Therefore, the performance of keratin hydrolysis using keratinase-CLEA was further investigated using OFAT, FFD and CCD and the most significant parameters are temperature (50 °C), enzyme concentration (180 mg/ml) and substrate concentration (0.25 %). The kinetic analysis reported that keratinase-CLEA have high catalytic efficiency compared against the free enzyme by using parameters such as Km (0.088 mM-1), Vmax (9.766 mmol L-1 min-1), Kcat (0.037 s-1) and Kcat/Km (0.416 mM s-1). Then, for the time analysis comparison, it was found that keratinase-CLEA required 30 minutes to produce cleaned EBN while water required 40 minutes and the keratinase-CLEA can be used up to 5 cycles for the cleaning process of EBN compared to water only. Finally, the proximate analysis reported that the composition of crude protein (57.6 % w/w) and carbohydrate (22.3 % w/w) of EBN cleaned using keratinase-CLEA have higher content than EBN cleaned using water. Hence, keratinase-CLEA was proven to be used to hydrolyze keratin from feathers and this solution can be used to improve the cleaning process of EBN by reducing time consumption.

TABLE OF CONTENT

DECLARATION	
TITLE PAGE	
ACKNOWLEDGEMENTS	**i**
ABSTRAK	**ii**
ABSTRACT	**iii**
TABLE OF CONTENT	**iv**
LIST OF TABLES	**ix**
LIST OF FIGURES	**x**
LIST OF SYMBOLS	**xiii**
LIST OF ABBREVIATIONS	**xiv**
CHAPTER 1 INTRODUCTION	**1**
1.1 Research Background	1
1.2 Statement of Problem	3
1.3 Research Objectives	4
1.4 Scope of Research	4
CHAPTER 2 LITERATURE REVIEW	**5**
2.1 Edible Bird Nest (EBN)	5
2.1.1 EBN Industry	6
2.1.2 Quality of EBN	7
2.1.3 Benefits of EBN	11
2.1.4 Application of EBN	13

2.2 Cleaning Process of EBN 16

2.2.1 Impurities of EBN 16

2.2.2 Conventional Method of Cleaning Process 18

2.2.3 Enzyme Technology Method for EBN Cleaning Process 19

2.3 Keratinase 21

2.3.1 Application of Keratinase 22

2.3.2 Immobilization of keratinase 23

2.4 Immobilization Enzyme 24

2.4.1 Types of Enzyme Immobilization 24

2.4.2 Application of Enzyme Immobilization 26

2.5 Cross-Linked Enzyme Aggregates (CLEAs) 28

2.5.1 Components in CLEA 30

2.6 Keratinase-CLEA 32

2.6.1 Formation of Keratinase-CLEA 32

2.6.2 Mechanism of Keratinase-CLEA 33

2.6.3 Application of Keratinase-CLEA 34

CHAPTER 3 METHODOLOGY 36

3.1 Introduction 36

3.2 Outline of Methodology 37

3.3 Chemicals 38

3.4 Collection and Preparation of Sample 38

3.5 Preparation of Keratin as Enzyme Substrate 38

3.5.1 Pre-treatment of Feathers 38

3.5.2 Extraction of Keratin 39

3.6 Protein Assay 39

3.7 Keratinase Assay 40
3.8 Enzyme Immobilization via CLEA 40
3.8.1 Preparation of Keratinase-CLEA 41
3.8.2 Effect of Different Precipitants 41
3.8.3 Effect of Precipitant Concentration 42
3.8.4 Effect of Glutaraldehyde Concentration 42
3.8.5 Effect of Cross-linking Time 42
3.8.6 Effect of Different Additive 43
3.8.7 Effect of Additive Concentration 44
3.9 Characterization of Keratinase-CLEA 44
3.9.1 Temperature Optimum 45
3.9.2 Thermal Stability 45
3.9.3 pH Optimum 46
3.9.4 pH Stability 46
3.9.5 Reusability 47
3.9.6 Field Emission Scanning Electron Microscopy (FESEM) 47
3.9.7 Fourier Transform Infrared (FTIR) Spectroscopy 47
3.10 Keratin Hydrolysis Analysis of Keratinase-CLEA 47
3.10.1 DTNB Assay 48
3.10.2 Screening of the Parameters Ranges on the Keratin Hydrolysis by using One Factor at One Time Method (OFAT) 48
3.10.3 Parameter Screening by Factorial Design 50
3.11 Optimization by experimental design 51
3.11.1 Optimization by Central Composite Design – RSM 51
3.12 Kinetic study 52
3.13 EBN quality analysis 53

3.13.1 Cleaning Process of EBN 53

3.13.2 Proximate analysis 53

CHAPTER 4 RESULTS AND DISCUSSION 55

4.1 Introduction 55

4.2 Analysis of Keratin from Swiftlet Feather 55

4.3 Preparation of Cross-Linked Enzyme Aggregates 56

4.3.1 Effect of Precipitants 57

4.3.2 Effect of Cross-linker 61

4.3.3 Effect of Additives 66

4.4 Characterization of Keratinase, Keratinase-CLEA and Keratinase-CLEA-Starch 69

4.4.1 Effect of Temperature on Keratinase Free Enzyme, Keratinase-CLEA and Keratinase-CLEA-Starch 70

4.4.2 Temperature Stability of Free Keratinase, Keratinase-CLEA and Keratinase-CLEA-Starch 71

4.4.3 Effect of pH on Keratinase Free Enzyme, Keratinase-CLEA and Keratinase-CLEA-Starch 73

4.4.4 pH Stability of Free Keratinase, Keratinase-CLEA and Keratinase-CLEA-Starch 75

4.4.5 Reusability 77

4.4.6 Fourier Transform Infrared (FTIR) Spectroscopy 78

4.4.7 Field Emission Scanning Electron Microscopy (FESEM) 81

4.5 Keratinase-CLEA in Keratin Hydrolysis 83

4.5.1 Screening of the Parameters Ranges on the Keratin Hydrolysis by using One Factor at One Time Method (OFAT) 83

4.5.2 Screening of Reaction Conditions on Keratin Hydrolysis Using Design of Experiment (Two-Level Factorial Design) 91

4.5.3 Optimization of Reaction Conditions on Keratin Hydrolysis Using Central Composite Design (CCD) 95

4.5.4 Keratin Hydrolysis by Keratinase-CLEA at Optimized Reaction Conditions 101

4.6 Kinetic analysis 103

4.7 EBN quality analysis 105

4.7.1 Cleaning Process of EBN 105

4.7.2 Proximate analysis 110

CHAPTER 5 CONCLUSION 113

5.1 Conclusion 113

5.2 Recommendations 114

REFERENCES 116

APPENDIX A MATERIAL AND METHODS 135

APPENDIX B MATHEMATICAL CALCULATION 137

APPENDIX C GRAPH OF STANDARD CURVE 141

LIST OF TABLES

Table 3.1	Details of the low and high limits for each parameter used in the Factorial screening design.	51
Table 3.2	Details of the low and high limits for each parameter used in the Central Composite Design (CCD).	52
Table 4.1	Infrared absorption bands associated with free Keratinase, Keratinase-CLEA and Keratinase-CLEA-Starch.	80
Table 4.2	Experimental design and results of full factorial design.	91
Table 4.3	Analysis of variance of the factorial design for sulfhydryl production.	93
Table 4.4	Statistical analysis for sulfhydryl production in keratin hydrolysis by Keratinase-CLEA.	94
Table 4.5	Experimental design and results of the central composite design.	96
Table 4.6	ANOVA for response surface quadratic model sulfhydryl concentration.	97
Table 4.7	Statistical analysis for the sulfhydryl concentration of keratin hydrolysis using Keratinase-CLEA.	98
Table 4.8	Summary of the optimized reaction conditions for keratin hydrolysis using Keratinase-CLEA.	102
Table 4.9	Kinetic analysis of keratinase on feathers keratin. The kinetic parameters were calculated based on the Lineweaver-Burk plot.	103
Table 4.10	Grade of cleaned EBN using water and keratinase-CLEA for each different soaking time.	106
Table 4.11	Grade of cleaned EBN using keratinase-CLEA for a cycle of reusability.	108
Table 4.12	Proximate composition of the cleaned EBN using water and CLEA.	110

LIST OF FIGURES

Figure 2.1	Uncleaned white-nest swiflets.	5
Figure 2.2	The swiftlet 'Golden Triangle'(Babji et al., 2015).	6
Figure 2.3	Different shapes of EBNs (a) half-cup (b) stripe and (c) biscuit (Prime Bird Nest, 2020).	10
Figure 2.4	Some EBN products that are available in the food industry. (i) Instant Malaysian cubilose nourishing tonic, (ii) Bird's nest instant energy drink, (iii) Bird's nest pudding recipe, and (iv) Bird's nest granules for supplements. The image is adapted from (Yen, 2015).	14
Figure 2.5	Flowchart for EBN cleaning process using conventional approach (Jong et al., 2013).	19
Figure 2.6	Crystal structure of keratinases from different microorganisms (Li, 2021).	21
Figure 2.7	Formation of cross-linked enzyme aggregates (CLEAs) (Easa, Yusof, & Abd. Halim, 2017).	29
Figure 2.8	Comparison of type I structure (a) and type II structure (b) of lipase-CLEAs (Schoevaart et al., 2004).	33
Figure 2.9	Illustration mechanism of Keratinase-CLEA when reacted with disulphide bonds of keratin.	34
Figure 3.1	Flowchart of research methodology	37
Figure 4.1	Keratinase relative activity in Keratinase-CLEA preparation using different types of precipitants.	59
Figure 4.2	Keratinase relative activity in Keratinase-CLEA preparation using different concentration of precipitants.	61
Figure 4.3	Keratinase relative activity in Keratinase-CLEA preparation using different concentrations of cross-linker.	64
Figure 4.4	Keratinase relative activity in Keratinase-CLEA preparation at different cross-linking times.	65
Figure 4.5	Keratinase relative activity in Keratinase-CLEA preparation with different types of additives.	67
Figure 4.6	Keratinase relative activity in Keratinase-CLEA preparation with different starch concentrations as an additive.	69
Figure 4.7	Effect of temperature on enzyme activity of keratinase free enzyme, Keratinase-CLEA and Keratinase-CLEA-Starch. The activities are normalized relative to the highest activity value. Enzymes were incubated in Tris-HCl buffer (100mM, pH7) at different temperatures (30°C to 80°C) for 30 minutes, in accordance with keratinase assay conditions.	71

Figure 4.8 Temperature stabilities of free enzyme, Keratinase-CLEA and Keratinase-CLEA-Starch. Enzymes were incubated without substrate in Tris-HCl buffer (100mM, pH7) at (30–60°C) for 30 min. 73

Figure 4.9 Effect of pH on enzyme relative activity of free enzyme, Keratinase-CLEA and Keratinase-CLEA-Starch. The activities are normalized relative to the highest activity value. Enzymes were incubated in different pH buffers (pH4 to pH8) at 50°C for 30 min. 74

Figure 4.10 pH stabilities of free enzyme, Keratinase-CLEA and Keratinase-CLEA-Starch. Enzymes were incubated without substrate in varied pH buffer (pH4 to pH9) at 50°C for 30 min. 76

Figure 4.11 Reusability of Keratinase-CLEA and Keratinase-CLEA-Starch. Enzymes were incubated in a reaction medium (keratin solution suspended in Tris-HCl, 100mM, pH7) at 50°C. 78

Figure 4.12 FTIR spectra of a) Free Keratinase b) Keratinase-CLEA, and c) Keratinase-CLEA-Starch. 81

Figure 4.13 FESEM images of a) free Keratinase, b) Keratinase-CLEA and c) Keratinase-CLEA-Starch Magnification points were used at 5.0 kX to provide a comparative account of the differences in the structural appearance of the three different types of CLEA involved in the study. 82

Figure 4.14 Effect of pH on keratin hydrolysis and sulfhydryl concentration production. The highest sulfhydryl concentration was achieved at the neutral condition of pH7. 84

Figure 4.15 Effect of temperature on sulfhydryl concentration. The highest sulfhydryl concentration was achieved at 50°C. 85

Figure 4.16 Effect of reaction time on sulfhydryl concentration. The highest sulfhydryl concentration was achieved at 50 minutes of reaction time. 87

Figure 4.17 Effect of enzyme concentration on sulfhydryl concentration. The highest sulfhydryl concentration was achieved at 150mg/ml enzyme concentration. 89

Figure 4.18 Effect of reaction time on sulfhydryl concentration. The highest sulfhydryl concentration was achieved at 0.15% of substrate. 90

Figure 4.19 (a) Response surface of keratin hydrolysis on enzyme concentration vs. temperature with constant substrate concentration (0.23 w/v %) (b) Response surface of keratin hydrolysis on substrate concentration vs. temperature with constant enzyme concentration (180mg/ml) (c) Response surface of keratin hydrolysis on substrate concentration vs. enzyme concentration with constant substrate concentration (50℃). 100

Figure 4.20 EBN after washing using water for a) 10 minutes b) 20 minutes c) 30 minutes d) 40 minutes e) 50 minutes f) 60 minutes at 50°C. 107

Figure 4.21 EBN after washing using Keratinase-CLEA for a) 10 minutes b) 20 minutes c) 30 minutes d) 40 minutes e) 50 minutes f) 60 minutes at 50°C. 107

Figure 4.22 EBN quality after washing with Keratinase-CLEA solution f, a) 1st cleaning cycle b) 2nd cleaning cycle c) 3rd cleaning cycle d) 4th cleaning cycle and e) 5th cleaning. The cleaning process of EBN was performed at 50°C. 109

LIST OF SYMBOLS

%	percentage
°C	degree celcius
µl	microliter
kDa	kilodaltons
kg	kilogram
K_m	michealis-menten constant
M	molar
mg	miligram
mg/ml	milligram/mililiter
ml	milliliter
RM	Ringgit Malaysia
rpm	revolutions per minute
U	unit (enzyme activity)
V_{max}	maximum reaction rate
w/v	weight solute per volume
Mmol	micromole

LIST OF ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ANOVA	analysis of variance
BSA	bovine serum albumin
CCD	central composite design
CLEAs	cross-linked enzyme aggregates
CLECs	cross-linked enzyme crystals
CRP	C-reactive protein
DM	dried matter
DMSO	dimethyl sulfoxide
DTNB	5,5′-dithiobis-(2-nitrobenzoic acid)
EBN	edible bird nests
EDTA	ethylenediaminetetraacetic acid
ETP	Economic Transformation Programme
FDA	Food and Drug Administration
FESEM	field emission scanning electron microscope
FFC	full factorial design
FTIR	fourier-transform infrared spectroscopy
GNI	Gross National Income
HCI	hydrochloric acid
HPV	human papillomavirus
IL6	interleukin 6
KBr	potassium bromide
Lac-CLEA	Laccase-crosslinking enzyme aggregates
MDCK	Madin-Darby Canine Kidney Epithelialc
MNPs	magnetic nanoparticles
Na_2CO_3	sodium carbonate
NANA	N-acetylneuraminic acid
NiCar	nickel-carnosine complex
OD	optical density
OD	optical density
OFAT	one one-time factor method
OFAT	one-factor-at-a-time
OpdA	organophosphate-degrading enzyme
PEG	polyethylene glycol
PVA	polyvinyl alcohol

PVA-P	polyvinyl alcohol-pectin
RSM	response surface measurement
S	substrate
SARS	severe acute respiratory syndrome
SDS	sodium dodecyl sulfate
SDS	sodium dodecyl sulfate
TCA	trichloracetic acid
TME	2-mercaptoethanol
Tnf	tumour necrosis factor-alpha
UV-VIS	ultraviolet–visible
V	velocity
ω-TA	ω-transaminase

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