OPTIMIZATION, CHARACTERIZATION AND IMMOBILIZATION OF ALKALINE PROTEASE FROM CHICKEN FEATHER AND FISH FIN USING MIXED CULTURE

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Doctor of Philosophy

UNIVERSITI MALAYSIA PAHANG

SUPERVISOR'S DECLARATION

We hereby declare that We have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy in Biotechnology.

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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DEDICATION

I dedicate my dissertation work to my family and many friends.
A special feeling of gratitude to my loving parents, they let the words
of encouragement and push for tenacity ring in my ears. My sisters who have never
left my side and are very special. I also dedicate this dissertation to my many friends and who
have supported me throughout the process, I will always appreciate all they have done.
I dedicate this work and give special thanks to another half of mine; my lover
for being there for me throughout the entire doctorate program;
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LIST OF SYMBOLS

%	Percentage
±	Uncertainty
℃	Degree Celsius
$	U.S. Dollar
Ø	Unicode defined as U+2300 Ø Diameter sign
B	Beta

LIST OF ABBREVIATIONS

AL	Enzyme activity loss
AP	Alkaline protease
CF	Chicken feather
CFF	Cell-free filtrate
CLEA	These cross-linked enzyme aggregates
CLEC	Cross-linked Enzyme Crystals
Cm	Centimeter
Cont.	Control
DFP	Diisopropylfluorophosphate
DMA	N,N-dimethyl acrylamide
DTT	Dithiothreitol
e.g.	For example
EC	Enzyme code
EDTA	Ethylenediaminetetraacetic acid
et al	And others
FDR	False discovery rate
FF	Fish fin
Fig	Figure
g	Gram
GMA	Glycidyl methacrylate
h	Hour
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
i.e	That is
IAA	Iodoacetamide
IEAL	Immobilized enzyme activity loss
IN	Integrase
IU/mL	International Units Per Milliliter
kDa	Kilo Dalton
L	Liter
Lbs	Pound (pressure unit)
LC-MS	Liquid Chromatography–Mass Spectrometry
M	Molarity

Mg	Milligram
Mm	Millimeter
ND	Not detected
NIPAAm	N-isopropylacrylamide
Nm	Nanometer
OFAT	One-factor-at-a-time
PDMS	Poly(dimethylsiloxane)
PEI	Polyethyleneimine
PHEA	Poly(2-hydroxyethyl acrylate)
Phw	Protease from hair waste
p*K*a	Acid dissociation constant
PL	Protein loss
POS	Polysiloxane
PR	Genome protease
PVA	Polyvinyl alcohol
Rpm	Revolution Per Minute
RT	Room temperature
RT	Reverse transcriptase
SAP	Serine alkaline proteases
SBTI	Soybean trypsin inhibitor
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SmF	Submerged fermentation
SPE	Solid phase extraction
SSF	Solid state fermentation
TCA	Trichloroacetic acid
TLCK	Tosyl-L-lysine chloromethyl ketone
TPCK	Tosylamide-2-phenylethyl chloromethyl ketone
U	Units
U/mL	Units Per Milliliter
US	United States
UV	Ultraviolet
UV-Vis	Ultraviolet–visible
v/v	volume/volume
Vol	Volume
w/w	Weight/weight

X-TRA, REX, and OMO	Detergent brand types
Q-TOF	Quadrupole Time Of Flight
TM	Trademark

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ABSTRAK

Pencemaran alam sekitar adalah masalah utama bagi setiap negara maju dan negara yang sedang membangun. Sirip ikan (FF) dan bulu ayam (CF) boleh menjadi sumber pencemaran sisa pepejal. Oleh itu, kedua-dua masalah ini dikaji untuk pengeluaran protease alkali (AP) melalui degradasi mikrob. Kajian ini bertujuan untuk mengekstrak dan menghasilkan keratin dan kolagen dari sisa haiwan dengan tindakan bakteria kultur campuran dan degradasi protein terpilih untuk menghasilkan enzim protease alkali yang bertujuan bagi melancarkan enzim dan menggunakannya untuk aplikasi industri. Protein diekstrak dari sisa dan dimasukkan ke dalam medium pengeluaran minimum M9 yang mengandungi bakteria terampai, dan diuji untuk pengeluaran AP. Parameter telah dioptimumkan melalui kaedah OFAT dengan optimum pH adalah pada pH 9.0 untuk CF dan FF, pada suhu 28 °C untuk CF dan 40 °C untuk FF, dengan tempoh inkubasi masing, selama 6 dan 10 hari,. Sumber karbon yang optimum adalah galaktosa dan glukosa manakala sumber nitrogen optimum adalah ammonium klorida dan ekstrak daging lembu bagi setiap CF dan FF. Didapati saiz inokulum bagi kedua-dua sampel adalah 1.5 mL manakala isipadu protein untuk CF dan FF telah direkodkan sebanyak 1.5 mL dan 2.0 mL. Protein tersebut ditulenkan secara pemendakan ammonium sulfat dalam parameter optimum 1L bagi setiap sisa. Kandungan protein bagi CF dan FF telah diuji dan dikenalpasti sebanyak 1.183 ± 0.035 dan 0.852 ± 0.050 mg/mL, manakala aktiviti enzim bagi CF dan FF telah dikenalpasti sebanyak 0.254 ± 0.001 dan 0.246 ± 0.014 U/ml. Selepas dialisis, terdapat 1.235 dan 2.110 peningkatan lipatan dalam aktiviti enzim CF dan FF. Protein tersebut dicerna dan peptida tersebut telah disusun menggunakan LC-MS. Daripada laporan LC-MS, terdapat 2 dan 4 daripada 11 dan 59 dipadankan dan peptid yang dikenalpasti bagi CF dan FF. Imobilisasi enzim telah dijalankan di dalam natrium alginat menggunakan 1.3g dan 0.688g larutan bebas enzim untuk membentuk manik-manik. Aktiviti imobilisasi enzim dalam 0.25g manik adalah 0.398 dan 0.411 (U/ml) untuk enzim yang diperolehi daripada CF dan FF. Kebolehgunaan semula enzim yang tidak aktif dalam degradasi protein telah diuji selepas kitaran keempat degradasi protein. Kandungan protein yang dibebaskan selepas kitaran keempat adalah 0.232 ± 0.001 dan 0.238 ± 0.006 mg/ml untuk enzim yang diperolehi daripada CF dan FF. Selepas kitaran keempat tersebut, pengurangan secara beransur-ansur (kira-kira 10% kehilangan aktiviti) telah diperhatikan seperti kandungan protein yang diperoleh berkurang sehingga 40% (0.168 ± 0.002 dan 0.152 ± 0.003 mg/ml) untuk enzim yang diperolehi daripada CF dan FF. Kestabilan penyimpanan manik yang tidak aktif ditentukan dengan mengekalkan enzim-enzim yang tidak aktif melalui silang silang dan penjerapan mudah pada 4℃ selama 5 hari. Enzim yang tidak aktif tersebut juga diperiksa untuk penyingkiran lumuran darah (sebuah ujian untuk memeriksa potensinya sebagai bahan tambahan detergen) dan didapati lumuran darah dari sehelai pakaian dapat disingkirkan selepas 6 rawatan yang berbeza dalam lingkungan 60 minit pada suhu bilik. Boleh disimpulkan bahawa kedua-dua enzim AP yang mentah dan tidak aktif boleh digunakan di industri sebagai bahan tambahan detergen.

ABSTRACT

Environmental pollution is a major problem in the developed and developing countries. Fish fins (FF) and chicken feathers (CF) can be sources of solid waste contamination; hence, both were investigated for alkaline protease (AP) production through microbial degradation. This study aims to extract and produce keratin and collagen from animal waste by the action of mixed culture bacteria and degrade the selected proteins to produce alkaline protease enzyme. The study further aims to immobilize the produced enzymes for industrial application. In this study, proteins were extracted from wastes and added to M9 minimal salt production medium containing the bacterial suspension and assayed for AP production. The process parameters were optimized via One-Factor-at-a-Time (OFAT) and the optimum conditions for CF and FF were determined as pH 9.0, the temperature of 28 ℃ for CF and 40 ℃ for FF, and an incubation period of 6 and 10 days, respectively. The optimum carbon source was determined as galactose and glucose while the optimum nitrogen source was ammonium chloride and beef extract for CF and FF, respectively. Inoculum size was found to be 1.5 mL for both samples, while a protein volume of 1.5 and 2.0 mL for CF and FF respectively, was recorded. The protein was partially purified using ammonium sulphate precipitation in 1L optimum parameters for each waste. Protein content was assayed and determined as 1.183 ± 0.035 and 0.852 ± 0.050 mg/ml respectively, for CF and FF while enzyme activity was determined as 0.254 ± 0.001 and 0.246 ± 0.014 U/ml respectively, for CF and FF. After dialysis, there was 1.235 and 2.110 folds increase in the enzyme activities of CF and FF respectively. The proteins were digested and the peptides were sequenced using LC-MS. From the LC-MS report, there were 2 and 4 out of 11 and 59 matched and identified peptides for CF and FF, respectively. Enzyme immobilization was performed in sodium alginate using 1.3 and 0.688g of the free enzyme solution to form beads. The activity of the enzymes immobilized in 0.25g beads was 0.398 and 0.411 (U/ml) for CF and FF-derived enzymes, respectively. The reusability of the immobilized enzymes in protein degradation was assayed for 4 protein degradation cycles. The liberated protein content after the 4^{th} cycle was found to be 0.232 ± 0.001 and 0.238 ± 0.006 mg/ml for CF and FF-derived enzymes, respectively. After the 4^{th} cycle, a gradual decrease in activity (approximately 10% activity loss) was noticed as the obtained protein contents decreased by up to 40% (0.168 ± 0.002 and 0.152 ± 0.003 mg/ml) for the CF and FF-derived proteins, respectively. The storage stability of the immobilized beads was determined by maintaining the immobilized enzymes via crosslinking and simple adsorption at 4^{o}C for 5 days. The immobilized enzymes were also checked for blood stain removal (a test to check for its potential as a detergent additive) and found to remove blood stains from a piece of cloth after 6 different treatments within 60 min at room temperature. It can be concluded that both the crude and immobilized AP enzymes can be industrially used as detergent additives.

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