

**EXTRACTION AND IDENTIFICATION OF  
ELASTASE INHIBITORY PEPTIDES FROM  
DIGESTED OVALBUMIN AS SKIN ANTI-AGING  
THERAPEUTICS AGENT**

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Sekian, terima kasih.

  
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by

**CHOK WEN XIN**

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of  
Bachelor of Technology (B. Tech) in the field of Bioprocess Technology  
School of Industrial Technology  
Universiti Sains Malaysia

July 2020

## **DECLARATION BY AUTHOR**

This dissertation is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.



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## List Of Symbol

<b>Symbol</b>	<b>Caption</b>
<	Smaller than
>	More than
≥	Larger than or equal to
/	Or or And
%	Percentage
±	Plus minus
α	Alpha
β	Beta
°C	Degree Celsius
D	Aspartic acid
E	Glutamic acid
F	Phenylalanine
K	Lysine
Q	Glutamine
R	Arginine
W	Tryptophan
Y	Tyrosine

<b>Abbreviation</b>	<b>Caption</b>
μg	Microgram
μL	Microliter
μm	Micrometre
3D	Three-dimensional

A	Alanine
B	Asparagine or aspartic acid
bonts	Botulinum neurotoxins
BT	Botulinum toxins
C	Cysteine
C-terminal	Carbon terminal
C1	Carbon number 1
Col-G	Collagenase G
CPD	Cyclobutane pyrimidine dimers
DNA	Deoxyribonucleic Acid
DOPA	Dihydroxyphenylalanine
e.g.	Exempli gratia (for example)
ECM	Extracellular matrix
g	Gram
G	Glycine
GAG	Glycosaminoglycan
GHK	Glycyl-L-histidyl-L-lysine
h	Hour
H	Hydrogen
H	Histidine
HA	Hyaluronan
HAF	Hyaluronan fragment
HCL	Hydrochloric acid
HSF	Human skin fibroblast
Hyals	Hyaluronidase

I	Isoleucine
i.e.	That is
IC50	Half maximal inhibitory concentration
kDA	Kilodalton
L	Litter
L	Leucine
LC/MS	Liquid Chromatography- mass spectrometer
M	Molar
M	Methionine
m/z	Mass-to-charge ratio
MAA	Mycosporine-like amino acid
MDA	Malondialdehyde
mg	Milligram
min	Minute
mL	Millilitre
mM	Millimolar
mm	Millimetre
MMP	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
MS/MS	Tandem mass spectrometer
mU	Microunit
N	Nitrogen
N	Asparagine
nM	Nanomolar
p	P-value

P	Proline
PC	Protein carbonylation
pH	Potential of Hydrogen
PKC	Protein kinase C
ppm	Part per million
R2	Regression
RA	Retanoic acid
ROS	Reactive oxygen species
S	Serine
SC	Stratum cronium
SNAP-25	Synaptosomal-associated protein of 25 kda
SOD	Superoxide dismutase
SPSS	Statistical Package for the Social Sciences
T	Threonine
TEWL	Transepidermal water loss
TGF	Transforming growth factor
UV	Ultraviolet
UV-A	Long-wave ultraviolet light
UV-B	Short-wave ultraviolet light
UVR	Ultraviolet radiation
V	Valine
w/w	Weight/weight

# **EKSTRASI DAN IDENTIFIKASI PEPTIDA PERENCAT ELASTASE DARIPADA OVALBUMIN TERHADAM SEBAGAI EJEN TERAPI ANTI- PENUAAN KULIT**

## **ABSTRAK**

Kulit merupakan bahagian badan manusia yang paling jelasnya menunjukkan penuaan seseorang. Penuaan kulit boleh dibahagikan kepada 2 jenis, iaitu proses dalaman dan proses luaran. Penuaan proses dalaman diakibatkan oleh (a) pengoksidaan, dan (b) aktiviti enzim penuaan seperti kolagenase, elastase, hyaluronidase dan tyrosinase, manakala penuaan kulit luaran diakibatkan oleh cahaya matahari. Secara tradisional, telur putih digunakan sebagai masker muka untuk meningkatkan kemudaan kulit. Oleh demikian, kajian ini bertujuan untuk menghasilkan peptida perencat elastase daripada ovalbumin (protein utama telur putih) dengan menggunakan pendekatan hidrolisis enzim (pepsin, trypsin, dan chymotrypsin), mengidentifikasikan peptida perencat dengan menggunakan kromatografi cecair spektrometer jisim tandem bersepadu dengan pendekatan bioinformatik, dan mengaji hubungan aktiviti struktur antara peptida perencat terpilih dengan elastase. Hidrolisat yang dihasilkan oleh pepsin menunjukkan aktiviti perencatan yang paling tinggi dengan  $IC_{50}$  4.93 mg/mL, manakala 5.75 mg/mL dan 9.54 mg/mL telah ditunjuk oleh hidrolisat yang dihasilkan oleh trypsin dan chymotrypsin masing-masing. Hidrolisat-hidrolisat ini mempunyai 56, 43 dan 43 jujukan peptida dengan skor PeptideRanker lebih daripada 0.5 masing-masing. Kesemua peptida ini dengan nilai signifikan Pepsite 2 ( $p < 0.05$ ) telah didapati mengikat 2 tapak utama pada elastase, iaitu W27, Y137, Q157, Y159, H200, dan Y207 (dikenali sebagai tapak pengikatan alosterik) dan Y91, Y93, Y101, dan W237 (dikenali sebagai tapak pengikatan halangan seterik). Terdapat sejumlah 5 peptida (DKLPGPF, KILELPFASGTM, FFGRCVS, KILELPF and QAM) mempunyai potensi menjadikan

perencat elastase. Justeru, ovalbumin telah dibuktikan bahawa ia mempunyai sifat aktiviti perencatan elastase selepas dihidrolisis oleh enzim.

# **EXTRACTION AND IDENTIFICATION OF ELASTASE INHIBITORY PEPTIDES FROM DIGESTED OVALBUMIN AS SKIN ANTI-AGING THERAPEUTICS AGENT**

## **ABSTRACT**

Skin is the most obvious part of human body to show aging sign of an individual. Skin aging can be divided into 2 types which are intrinsic and extrinsic process. Intrinsic skin aging is caused by (a) oxidation, and (b) activity of aging enzyme such as collagenase, elastase, hyaluronidase and tyrosinase, while extrinsic skin aging is caused by sunlight. Traditionally, egg white was used as face mask to enhance youthful skin. Thus, this research was aimed to produce elastase inhibitor peptides from ovalbumin (main protein in egg white) using enzymatic (i.e. pepsin, trypsin and chymotrypsin) hydrolysis approach, to identify the inhibitor peptides using liquid chromatography tandem mass spectrometry integrated with Bioinformatics approach, and to investigate the structural activity relationship between the selected inhibitor peptides and elastase. Hydrolysate produced by pepsin showed higher elastase inhibitory activity with  $IC_{50}$  of 4.93 mg/mL, while 5.75 mg/mL and 9.54 mg/mL were obtained in the hydrolysates produced by trypsin and chymotrypsin, respectively. These hydrolysates consisted of 56, 43, and 43 peptide sequences with PeptideRanker score more than 0.5, respectively. All these peptides with Pepsite 2 significance value ( $p < 0.05$ ) were observed binding towards 2 major sites of elastase, which are W27, Y137, Q157, Y159, H200, and Y207 (known as the allosteric binding sites) as well as Y91, Y93, Y101, and W237 (known as the steric hindrance binding sites). There are a total of 5 peptides (DKLPGPF, KILELPFASGTM, FFGRCVS, KILELPF and QAM) that have most potential to be elastase inhibitor. Therefore, ovalbumin was proved to have anti-aging properties by inhibiting elastase activity after hydrolysed by the enzymes.