

MOLECULAR AND BIOINFORMATICS
CHARACTERIZATION OF FRUIT BROMELAIN
FROM *ANANAS COMOSUS*

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Master of Science

UNIVERSITI MALAYSIA PAHANG



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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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Dedicated to my parents

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ABSTRAK

Nanas secara saintifik dikenali sebagai *Ananas comosus*, mempunyai beberapa kultivar yang terdapat di Malaysia, termasuk Morris cv, N36 cv, dan Sarawak cv. Bromelain telah dikenal pasti sebagai komponen aktif dan protease utama *A. comosus* dan telah diterima dan diakui secara meluas luas sebagai ubat phototherapeutic. Walaupun pelbagai penyelidikan telah dikhaskan untuk bromelain dari *A. comosus*, perhatian kurang diberikan kepada bromelain buah berbanding dengan bromelain empulur. Oleh itu, tujuan kajian ini adalah untuk mendedahkan maklumat mendalam tentang bromelain buah dari *A. comosus*. Sehingga baru-baru ini, struktur tiga dimensi (3D) bromelain kekal dapat dijelaskan. Maklumat komprehensif mengenai struktur struktur bromelain yang menyeluruh adalah penting untuk aplikasi terapeutik dan pemahaman tentang peranan mereka dalam sel dan dalam mekanisma molekul lain yang berkaitan. Dalam kajian ini, saringan bromelain buah dari kultivar nanas tempatan (Morris cv, N36 cv dan Sarawak cv) telah dilaksanakan, diikuti oleh pengasingan dan pengklonan bromelain buah dari kultivar terbaik dengan aktiviti proteolitik tertinggi untuk analisis urutan. Selain itu, perbandingan bromelain buah dan empulur dilakukan menggunakan alat bioinformatik, termasuk kedua-dua asid amino dan perbandingan struktur. Dari hasil pemeriksaan, aktiviti proteolitik tertinggi (0.8220 U / mL) diperhatikan dari bromelain buah Morris cv, diikuti oleh N36 cv (0.7695 U / mL) dan Sarawak cv (0.6942 U / mL). Pengekodan gen untuk bromelain buah nanas berjaya diasingkan dari Morris cv. menggunakan Reverse Transcription-Teknik Reaksi Rantai Polimerase (RT-PCR). Urutan asid amino dan analisis domain buah dan empulur bromelain menunjukkan beberapa perbezaan dan persamaan anggota keluarga protease cysteine. Selain itu, analisis bromelain buah (BAA21848) dan empulur (CAA08861) bromelain mendedahkan kehadiran sifat unik struktur yang diramalkan sebagai pemangkin Cys-148, His-281, Gln-174 dan Asn-275 oleh bromelain buah sementara itu bromelain empulur Cys-147, His-281, His-141 dan Asn-302. Ini memainkan peranan penting dalam pemangkinan kimia sebagai pemangkin asid / asas am. Analisis urutan dan ramalan struktur bromelain buah dan empulur dari *A. comosus*, bersama-sama dengan perbandingan kedua-dua struktur memberikan wawasan baru mengenai sifat-sifatnya yang berbeza untuk aplikasi perindustrian. Dari analisis, bromelain empulur lebih hidrofobik daripada bromelain buah. Pengetahuan struktur enzim proteolitik dari *A. comosus* dijangka dapat meningkatkan pemahaman fungsi dan mekanisma.

ABSTRACT

Pineapple scientifically known as *Ananas comosus*, has several available cultivars in Malaysia, including Morris cv, N36 cv, and Sarawak cv. Bromelain has been identified as an active component and a major protease of *A. comosus* and has gained wide acceptance and compliance as a phototherapeutic drug. Although a considerable level of research has been devoted to bromelain from *A. comosus*, less attention has been paid to the fruit bromelain compared to the stem bromelain. Therefore, the purpose of this research is to reveal an in-depth information regarding fruit bromelain from *A. comosus*. Until recently, the three-dimensional (3D) structure of bromelain remained to be elucidated. A comprehensive information on the thorough structural organisation of bromelain is vital for therapeutic application and in the understanding of their role in cells and in other related molecular mechanisms. In this study, the screening of fruit bromelain from the local pineapple cultivars (Morris cv, N36 cv. and Sarawak cv) was implemented, followed by the isolation and cloning of the fruit bromelain from the best cultivar with the highest proteolytic activity for sequence analysis. Additionally, a comparison of the fruit and stem bromelain was performed using bioinformatics tools, including both amino acids and structural comparisons. From the screening results, the highest proteolytic activity (0.8220 U/mL) was observed from the fruit bromelain of Morris cv, followed by N36 cv (0.7695 U/mL) and Sarawak cv (0.6942 U/mL). A gene encoding for pineapple fruit bromelain was successfully isolated from Morris cv. using Reverse Transcription - Polymerase Chain Reaction (RT-PCR) techniques. The amino acid sequence and domain analysis of the fruit and stem bromelains demonstrated several differences and similarities of the cysteine protease family members. Additionally, an analysis of the modelled fruit (BAA21848) and stem (CAA08861) bromelains revealed the presence of unique properties of the predicted structures Cys-148, His-281, Gln-174 and Asn-275 are the catalytic residues of fruit bromelain whereas stem bromelain Cys-147, His-281, His-141 and Asn-302. This play crucial roles in chemical catalysis as general acid/base catalysts. The sequence analysis and structural prediction of the stem and fruit bromelain from *A. comosus*, along with the comparison of both structures provided a new insight on their distinct properties for industrial application. From the analysis, stem bromelain is more hydrophobic than fruit bromelain. The knowledge of the structure of these proteolytic enzymes from *A. comosus* is expected to increase the understanding of their functions and mechanism.

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LIST OF SYMBOLS

R^2	Coefficient of Determination
Kg	Kilogram
kDa	kilo-Dalton
μmol	micromoles
μL	Microliter
μM	Micrometer
mg	milligram
mL	millilitre
nm	nanometer
Ng	Nanogram
%	Percentage
$^{\circ}\text{C}$	Celcius
α	Alpha
β	Beta
Å	Ångström
pI	Isoelectric point

LIST OF ABBREVIATIONS

A	Absorbance
<i>A. comosus</i>	<i>Ananas comosus</i>
BLAST	Basic Local Alignment Tool
cDNA	Complementary Deoxyribonucleic Acid
cv.	Cultivar
DEPC	Diethyl pyrocarbonate
DDBJ	DNA Data Bank of Japan
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide
ENA	European Nucleotide Archive
F-C	Folin Ciocalteu
GRAS	Generally Regarded As Safe
IDT	Integrated DNA Technology
IPTG	Isopropyl β -D-1-thiogalactopyranoside
LB	Luria Bertani
LPNM	Lembaga Perindustrian Nanas Malaysia
MPIB	Malaysian Pineapple Industrial Board
Min	Minutes
mRNA	Messsenger Ribonucleic Acid
NCBI	National Center for Biotechnology Information
NMR	Nuclear Magnetic Resonance
NSAID	Nonsteroidal Anti-inflammatory Drugs
PCR	Polymerase Chain Reaction
PDB	Protein Database Bank
pNA	p-nitroalanine
RIN	RNA Integrity Number
RMSD	root-mean-square deviation
RNA	Ribonucleic acid
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction

SPDBV	Swiss Protein Database Bank Viewer
S	Seconds
TA	Thymine Adenine
TAE	Tris-acetate
TCA	Trichloroacetic Acid
V	Volts
X-Gal	5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside

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