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E41100



PURIFICATION AND CHARACTERIZATION OF  
AMYLASE IN MON THONG DURIAN  
(*Durio zibeithinus*) Murr. cv. Mon Thong)

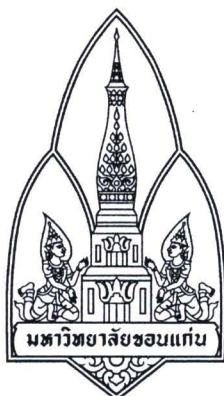
MISS SAIJAI POSOONGNOEN

A THESIS FOR THE DEGREE OF MASTER OF SCIENCE  
KHON KAEN UNIVERSITY

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**MISS SAIJAI POSOONGNOEN**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF MASTER  
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(*Durio zibethinus* Murr. cv. Mon Thong)

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สายใจ ปอสูงเนิน. 2554. การแยกบริสุทธิ์และศึกษาสมบัติของเอนไซม์อะไมเลส (amylase) ใน  
ทุเรียนหมอนทอง (*Durio zibethinus* Murr. cv. Mon Thong). วิทยานิพนธ์ปริญญาวิทยาศา  
สตรมหาบัณฑิต สาขาวิชาชีวเคมี บัณฑิตวิทยาลัย มหาวิทยาลัยขอนแก่น.

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### บทคัดย่อ

**E41100**

ทุเรียน (*Durio zibethinus* Murr.) เป็นผลไม้เศรษฐกิจที่สำคัญของประเทศไทย มีกลิ่นหอมเฉพาะตัว รสหวานจัดเมื่อสุก เนื่องจากมีการสะสมแป้งในปริมาณสูง และเมื่อสุกจะถูกเปลี่ยนเป็นน้ำตาล คาดว่าจะเป็นผลจากการทำงานของเอนไซม์อะไมเลส ดังนั้นงานวิจัยนี้จึงสนใจแยกบริสุทธิ์และศึกษาสมบัติของเอนไซม์อะไมเลสในทุเรียนหมอนทอง เริ่มจากการประมาณระยะสุกด้วยการวัดปริมาณแป้งในเนื้อทุเรียนโดยวิธี Iodine test พบปริมาณแป้ง 1.10% ของน้ำหนักสด และวัดความเข้มข้นของน้ำตาลรีดิวซ์ในสารสกัดทุเรียนด้วย DNS method พบน้ำตาล 0.17% ของน้ำหนักสด จากนั้นวัดปริมาณโปรตีนด้วยเทคนิค Bradford พบปริมาณโปรตีน 0.97 mg ต่อกรัมเนื้อทุเรียนสด จากนั้นศึกษาแบบแผนโปรตีนด้วยเทคนิค SDS-PAGE พบแถบโปรตีนในช่วง 28-97 kDa และมีแถบโปรตีนหลักในช่วง 38-55 kDa เมื่อตรวจหาแอกติวิตีของเอนไซม์อะไมเลสด้วย Zymographic method พบกิจกรรมการทำงานของอะไมเลสที่ขนาดโมเลกุล 45 kDa จากนั้นจึงได้ศึกษาลำดับกรดอะมิโนบางส่วนด้วยเทคนิค 2D-PAGE และ LC-MS/MS สามารถระบุชนิดของโปรตีนในสารสกัดทุเรียนได้ 27 spots จากทั้งหมด 40 spots ซึ่งสามารถจัดกลุ่มโปรตีนได้ 9 กลุ่ม คือ carbohydrate, protein, lipid และ secondary metabolism และกลุ่มของ protein folding, ripening process, antioxidant enzyme, cell wall hydrolysis และอื่นๆ (not identified) โดยพบโปรตีนที่น่าสนใจ คือ Glutathione reductase, Isoflavone reductase และพบเอนไซม์แอลฟาอะไมเลส มีขนาด 45 kDa และ pI 6.51 มีลำดับกรดอะมิโนบางส่วน คือ IATVLPDK จากนั้นแยกบริสุทธิ์เอนไซม์อะไมเลสโดยการตกตะกอนด้วยเกลือแอมโมเนียมซัลเฟตอิ่มตัว 70%, affinity chromatography (epoxy-activated sepharose 6B ligated with  $\beta$ -cyclodextrin) และ DEAE Toyopearl Anionic Exchange Chromatography พบเอนไซม์มีความบริสุทธิ์ขึ้น 340.36 เท่า, specific activity 234.85 units/mg และพบกิจกรรมการทำงานของอะไมเลสที่ขนาดโมเลกุล 45 kDa จากนั้นนำเอนไซม์ที่ผ่านการแยกบริสุทธิ์ มาศึกษาสมบัติต่างๆ พบว่ามี Optimum pH 7.0 และ Optimum temperature 40 °C และยังสามารถทำงานได้ที่อุณหภูมิสูง 90 และ 100 °C มีความเสถียรที่ pH 6-10 และอุณหภูมิ 30-60 °C และเมื่อศึกษาผลของไอออนโลหะชนิดต่างๆ คือ  $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Na}^+$  และ  $\text{K}^+$  พบว่าไม่มีผลต่อการทำงานของเอนไซม์อะไมเลส แต่ EDTA ส่งผลให้เอนไซม์สูญเสียการทำงาน แสดงให้เห็นว่าเอนไซม์อะไมเลสจากทุเรียนหมอนทองเป็นชนิด แอลฟา ( $\alpha$ -type) จากสมบัติดังกล่าว จะสามารถนำไปศึกษาและพัฒนาเพื่อนำไปประยุกต์ใช้ในอุตสาหกรรมต่อไป

Saijai Posoongnoen. 2011. **Purification and characterization of amylase in Mon Thong durian (*Durio zibethinus* Murr. cv. Mon Thong).** Master of Science Thesis in Biochemistry, Graduate school, Khon Kaen University.

**Thesis Advisor:** Assoc.Prof. Dr. Sakda Daduang

## ABSTRACT

**E 41100**

Durian (*Durio zibethinus* Murr.) is an important commercial fruit of Thailand. This fruit has unique smell and sweet taste in ripening stage. Large storage of starch is converted into sugar by amylase activity. Therefore, the aim of this study is to purify and characterize amylase in Mon Thong durian. The study started from estimation of ripening stage by quantitation starch and sugar content using Iodine test and DNS method. Results showed 1.1% of starch in fresh durian pulp and 0.17% of reducing sugar in fresh weight durian pulp of reducing sugar in crude durian extract. Protein was 0.97 mg protein/g fresh weight by Bradford method measurement. SDS-PAGE technique showed protein pattern of crude durian with molecular weight ranging from 28 to 97 kDa, with major bands around 38-55 kDa. Zymographic detection showed clear transparent amylase band at 45 kDa. After that, 2D-PAGE and LC-MS/MS strategies were used consequently to determine partial amino acid sequence. The total of 40 excised spots were analyzed by LC-MS/MS, 27 spots were identified with high homologies to known proteins. They were organized into 9 groups, concerning in carbohydrate, protein, lipid and secondary metabolism. Some are predicted to be protein in folding process, ripening process, antioxidant enzyme, cell wall hydrolysis and other (not identified). Moreover, the most interested ones are glutathione reductase, isoflavone reductase and  $\alpha$ -amylase.  $\alpha$ -amylase in crude durian were identified with molecular weight about 45 kDa and pI 6.51, with IATVLPDK as partial amino acid sequence. Then, crude durian was precipitated and purified with at 70%  $(\text{NH}_4)_2\text{SO}_4$ , affinity chromatography (epoxy-activated sepharose 6B ligated with  $\beta$ -cyclodextrin) and further subjected to DEAE Toyopearl Anionic Exchange Chromatography, respectively, with 340.36 purification fold and 234.85 units/mg specific activity. Amylase showed high purity after SDS-PAGE analysis with molecular weight about 45 kDa. Furthermore, the activity of purified amylase was detected by Zymographic method exhibiting single clear transparent band. Optimum pH and temperature for durian amylase were 7.0 and 40 °C, respectively. Moreover, durian amylase still showed functional activity at 90 and 100 °C. Furthermore, its stability was over a broad range of pH 6 to 10 and temperature 30 °C to 60 °C. Many metal ions ( $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Na}^+$  and  $\text{K}^+$  ions) did not affect amylase activity, but activity almost completely abolished by 5 mM EDTA. This result implied that amylase of Mon Thong durian is a metalloenzyme and belongs to the member of the  $\alpha$ -type. Therefore,  $\alpha$ -amylase from crude Mon Thong durian could be applied to be a good choice for various applications in the future especially in industry.



**Goodness portion to the present thesis is dedicated for my parents,  
all of my teachers and all of my friends**

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## LIST OF ABBREVIATIONS

%	Percent
°C	Degree Celsius
$\alpha$	Alpha
$\beta$	Beta
$\gamma$	Gamma
$\mu\text{g}$	Microgram
$\mu\text{l}$	Microlitre
2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
$\text{Ag}^+$	Silver (I) ion
Asp	Aspartic acid
<i>B.</i>	<i>Bacillus</i>
$\text{Ca}^{2+}$	Calcium (II) ion
$\text{CaCl}_2$	Calcium chloride
$\text{Cd}^{2+}$	Cadmium (II) ion
cm	Centimetre
$\text{Co}^{2+}$	Cobalt (II) ion
$\text{Cu}^{2+}$	Copper (II) ion
DDW	Double-distilled water
DEAE	Diethyl amino ethyl
DNS	Dinitrosalicylic acid method
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
$\text{Fe}^{2+}$	Iron (II) ion
g	Gram
Glu	Glutamic acid
g/ml	Gram per millilitre
HCl	Hydrochloric Acid
$\text{Hg}^+$	Mercury (I) ion
His	Histidine

## LIST OF ABBREVIATIONS (Cont.)

hr	Hour
I <sub>2</sub>	Iodine
IAA	Iodoacetamide
IPG	Immobilized pH gradients
K <sup>+</sup>	Potassium (I) ion
kDa	Kilodalton
Kg	Kilogram
KI	Potassium iodide
K <sub>m</sub>	Michaelis constant
kVh	kilovolt hours
LC-MS/MS	Tandem Mass Spectrometry
Leu	leucine
Li <sup>2+</sup>	Lithium (II) ion
Lys	Lysine
M	Molar
MALDI-TOF	Matrix-assisted laser desorption ionization – Time of flight
mA	Milliampere
Met	Methionine
mg	Milligram
Mg <sup>2+</sup>	Magnesium (II) ion
mg/ml	Milligram per milliliter
ml	Millilitre
mM	Millimolar
Mn <sup>2+</sup>	Manganese (II) ion
N	Normal
Na <sup>+</sup>	Sodium (I) ion
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NL	Non-linear gradient



**LIST OF ABBREVIATIONS (Cont.)**

nm	Nanometre
Phe	Phenylalanine
pI	Isoelectric point
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
sec	Second
Trp	Tryptophan
Tyr	Tyrosine
U/ml	Unit per milliliter
V	Voltage
v/v	Volume per volume
Val	Valine
Zn <sup>2+</sup>	Zinc (II) ion