UNIVERSITI PUTRA MALAYSIA

PURIFICATION AND CHARACTERIZATION OF GLUTAMATE DECARBOXYLASE FROM ASPERGILLUS ORYZAE NSK

AUDREY LEE YING YENG

FSTM 2014 11

PURIFICATION AND CHARACTERIZATION OF GLUTAMATE DECARBOXYLASE FROM *ASPERGILLUS ORYZAE* NSK

By

AUDREY LEE YING YENG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

May 2014

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Food Biotechnology

PURIFICATION AND CHARACTERIZATION OF GLUTMATE DECARBOXYLASE FROM *ASPERGILLUS ORYZAE* NSK

By

AUDREY LEE YING YENG

May 2014

Chairman : Prof. Nazamid Saari, PhD

Faculty : Food Science and Technology

γ-Amino butyric acid (GABA) is a major inhibitory neurotransmitter of the mammalian central nervous system that plays an important role in regulating vital neurological functions. The enzyme that is responsible for GABA production is glutamate decarboxylase (GAD), an intracellular enzyme that both food and pharmaceutical industries are currently used as the major catalyst in biotransformation process of GABA.

Recently, a novel strain of *Aspergillus oryzae* NSK with high GABA biosynthesizing capability was successfully isolated from a soy sauce koji in Malaysia. To gain a detailed insight of the GABA producing capability of the strain, an effective isolation and purification procedure of glutamate decarboxylase (GAD) were developed. Mechanical disruption by sonication, which yielded 1.99 U/mg of GAD, was by far the most effective cell disintegration method compared with the other extraction procedures examined, which include solvent permeabilization and enzymatic lysis. Further optimization of the sonication protocols successfully increased the yield of GAD by 176% from 1.99 U/mg to 3.50 U/mg.

After extraction of GAD, it was purified to 21-fold with a recovery of 11.60% using a combination of 30-70% ammonium sulphate precipitation followed by liquid chromatography techniques comprising reverse (Flow-through) mode ion exchange chromatography (DEAE- Sepharose FF) and twice gel filtration using Superdex 200 HR 10/30 column. An electrophoretic study showed that the purified GAD exists as a hexameric structure under native conditions with an estimated molecular weight of 240 kDa and 38 kDa of subunit molecular weight. It is thermally stable between 0-

40˚C and was found to be optimally active at 55˚C and pH 5.5. The K_m and V_{max} values calculated were 10.18 mM and 5.15 U/ml/min, respectively. Activity of the enzyme was significantly reduced by $FeCl_3$ (62.14%) and sulfate minerals in the following order: $MgSO_4$ (66.32%)> $(NH_4)_2SO_4$ (73.29%)> $NaSO_4$ (80.96%) > $CuSO_4$ (81.64%).

An excellent stability of GAD over a wide pH range offer high potential application in food industries to produce various GABA-enriched fermented foods using a GRAS (Generally Regarded as Safe) fungal source. Current study provided a detailed insight of the characteristics of GAD from *A. oryzae* NSK in order to achieve maximum catalytic capacity of the GAD during production of GABA-enriched food and also demonstrating a feasible alternative method of GAD purification.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Bioteknologi Makanan

PENULENAN DAN PENCIRIAN ENZIM GLUTAMAT DEKARBOSILASE DARIPADA *ASPERGILLUS ORYZAE* NSK

Oleh

AUDREY LEE YING YENG

Mei 2014

Pengerusi : Prof. Nazamid Saari, PhD

Fakulti : Sains dan Teknologi Makanan

Asid γ-Aminobutirat (GABA) merupakan perencat neurotransmiter utama kepada sistem saraf tunjang mamalia dimana ia memainkan peranan penting dalam pengawalan fungsi neurologikal dalam tubuh. Manakala glutamat dekarbosilase (GAD) merupakan enzim yang memainkan peranan penting dalam penghasilan GABA dan ia dikategorikan sebagai enzim intra-sel. Disebabkan oleh peranan fisiologikal GABA terhadap kesihatan, GAD mendapat perhatian daripada pihak industri famaseutikal dan makanan sebagai pemangkin utama dalam proses bio-transformasi GABA.

Dalam kajian ini, *Aspergillus oryzae* NSK yang mempunyai ciri penghasilan GABA yang baik telah diperolehi daripada koji kicap kacang soya. Untuk mendapatkan gambaran yang terperinci terhadap ciri GAD strain tersebut, satu kaedah yang efektif telah dibangunkan bagi tujuan pengekstrakan dan penulenan GAD daripada *A. oryzae*. Tiga kaedah pengekstrakan GAD telah diuji iaitu kaedah mekanikal, kaedah enzimatik dan kaedah pemusnahan permukaan sel melalui bahan kimia. Kaedah mekanikal menggunakan teknik sonikasi merupakan kaedah yang paling efektif dimana sebanyak 1.99 U/mg GAD telah diperolehi. Pengoptimunan yang selanjutnya bagi teknik tersebut berjaya meningkatkan hasil GAD daripada 1.99 U/mg ke 3.50 U/mg dengan peningkatan hasil sebanyak 175%.

GAD yang diperolehi berjaya ditulenkan sebanyak 21 ganda dengan 11.60% GAD tulen diperolehi melalui kombinasi kaedah pemendakan ammonium sulfat diikuti dengan kromatografi cecair yang terdiri daripada kaedah kromatografi songsang gantian ion menggunakan DEAE- Sepharose FF dan dua pusingan kaedah gel filtrasi menggunakan Superdex 200 HR 10/30 column. Kajian elektroforesis terhadap GAD tulen menunjukkan enzim tersebut mempunyai struktur heksamer dengan anggaran berat molekul 240 kDa dan 38 kDa bagi setiap satu subunit. GAD adalah stabil pada

suhu antara 0-40˚C dengan suhu dan pH optima masing-masing adalah 55˚C dan pH 5.5. Nilai K_m dan V_{max} bagi enzim tersebut adalah 10.18 mM dan 5.15 U/ml/min. Aktiviti enzim direncat secara signifikan oleh $FeCl_3$ (62.14%) diikuti mineral sulfat mengikut susunan berikut: $MgSO_4$ (66.32%)> $(NH_4)_2SO_4$ (73.29%)> $NaSO_4$ (80.96%) > $CuSO_4$ (81.64%).

Kestabilan GAD pada julat pH yang luas menawarkan potensi aplikasi yang tinggi dalam industri makanan untuk menghasilkan berbagai jenis makanan tertapai yang tinggi dengan GABA menggunakan kulat berstatus GRAS. Kajian ini turut memberi gambaran yang lebih terperinci terhadap ciri-ciri GAD daripada *A. oryzae NSK* untuk mencapai kapasiti pemangkinan enzim yang maksimum semasa penghasilan makanan-tinggi-GABA dan juga menunjukkan kaedah alternatif penulenan GAD.

ACKNOWLEDGEMENTS

Anyone who has ever written a thesis knows that one doesn't write it alone. I thank my supervisor, Prof. Nazamid Saari, for suggesting many improvements, correcting my manuscript errors, and providing valuable opinions during the course of my Masters Research and thesis writing. I am also thankful to my co-supervisors, Prof. Hasanah and Prof. Raja, whom also have taken their time to check my manuscript and I'm lucky enough to gain further insight in my study from their sharing of knowledge.

I also thank my family for the affection and patient optimism that have kept me going day after day: my father, Lee Kian Ming, and mother, Mary Lumou, brother Milton and sisters Vanessa, Vivian, and Annabelle.

I owe thanks to many people at Food 4 lab 2. My fellow labmats, Chay Shyan Yea, Ariff, Suzie, Faiqa, Khizan, Aizah, Balqis, Najib, Safuan, and etc have been very helpful in stimulating ideas and encouragement for research. I'm also grateful for my special friend, Ooi Lok Yon, for his support in editing the thesis draft.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of **Master of Science.** The members of the Supervisory Committee were as follows:

Nazamid Saari, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Hasanah Mohd Ghazali, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Raja Noor Zaliha Raja Abd Rahman, PhD
Professor
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Member)

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

Declaration By Graduate Student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: _____

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____	Signature: _____
Name of Chairman of Supervisory Committee: _____	Name of Member of Supervisory Committee: _____
Signature: _____	Signature: _____
Name of Member of Supervisory Committee: _____	Name of Member of Supervisory Committee: _____

TABLE OF CONTENT