



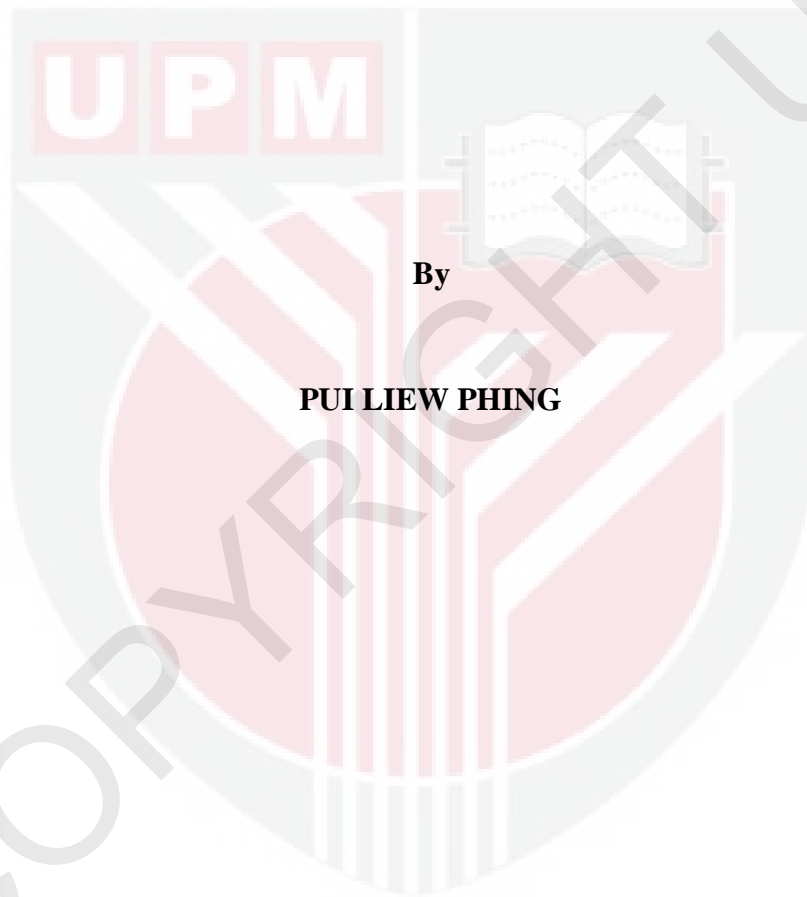
UNIVERSITI PUTRA MALAYSIA

**PURIFICATION OF AND FACTORS AFFECTING 5'-PHOSPHODIESTERASE
FROM GERMINATING ADZUKI (*Vigna angularis* L.) BEAN**

PUI LIEW PHING

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By

PUI LIEW PHING

**Thesis submitted to the School of Graduate Studies, Universiti Putra
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfillment of the requirement for the degree of Master of Science

**PURIFICATION OF AND FACTORS AFFECTING 5'-PHOSPHODIESTERASE
FROM GERMINATING ADZUKI (*Vigna angularis* L.) BEAN**

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Chair: Prof. Hasanah Mohd. Ghazali, PhD

Faculty: Faculty of Food Science and Technology

5'-Phosphodiesterase (5'-PDE) is an enzyme that hydrolyses RNA to form 5'-inosine monophosphate (5'-IMP) and 5'-guanosine monophosphate (5'-GMP) which function as flavour enhancers. The best producer of 5'-PDE was selected from germinated seeds, namely mung bean (*Vigna radiate*), soybean (*Glycine max*), adzuki bean (*Vigna angularis* L.), chick pea (*Cicer arietinum*), black eye pea (*Vigna unguiculata*) and petai (*Parkia speciosa*). In order to ensure there is no contamination during germination, the effects of different surface sterilizing treatments were examined. Sodium hypochlorite at 0.3% (v/v) concentration was able to inhibit mold growth in adzuki bean, soybean and chickpea. Only 0.1% (v/v) sodium hypochlorite was needed to inhibit mold growth in black eye pea and petai, while mung bean required 0.05% (v/v) sodium hypochlorite to inhibit mold growth. 5'-PDE activity was determined using thymidine 5'-monophosphate p-nitrophenyl ester as substrate at pH 7.0 and 55°C. The formation of nucleotide monophosphates, the products of reaction, was determined at 405 nm. As a strong presence of phosphomonoesterase (PME) will reduce the yield of nucleotide

monophosphates as the enzyme hydrolyzes these products into nucleosides and orthophosphate, PME activity was also determined using p-nitrophenyl phosphate as the substrate at 60°C and pH 5.0. Adzuki bean was found to have the highest 5'-PDE activity when germinated for 15 days.

5'-PDE has an optimum pH and temperature of 8.5 and 80°C, respectively. It was stable between pH 7.0 - 8.5. A higher stability was observed at pH 7.5 compared to pH 8.5, and at 60 and 65°C, the enzyme still possessed good catalytic function even after 4 days of incubation. EDTA, Triton X-100 and sodium dodecyl sulfate (SDS) strongly inhibited 5'-PDE activity (inhibition ranged from 75.2-99.4 %), while Tween 80 and Tween 20 were only slightly inhibitory. The enzyme was activated by Ca^{2+} up to 249%, K^+ , Mg^{2+} and Li^+ , when the final concentration of the cations was 10 mM. On the other hand, Na^+ , Zn^{2+} , Ni^+ , Hg^+ , Cu^{2+} , Pb^{2+} , Fe^{2+} , Al^{3+} , Ba^{2+} and Co^{2+} at the same final concentration were inhibitory. 5'-PDE activity increased with an increase in the concentration of Li^+ , Na^+ and Mg^{2+} , until it reaches its maximum at 6 mM followed by a decrease in activity at concentrations beyond 6 mM. The presence of Ca^{2+} in the reaction mixture resulted in a similar trend, but the difference is that maximum activation occurred at 4 mM. K^+ , however, increased the activity of 5'-PDE from 0-10 mM. Al^{3+} decreased the activity of 5'-PDE activity as the concentration of the cation was increased. Thus, it is suggested that 5'-PDE from adzuki bean is a metallo-enzyme.

The yield of 5'-PDE after the four purification steps (acetone precipitation, desalting, anion-exchange chromatography and gel filtration chromatography) was 13.37% with a purification fold of 6.7 and specific enzyme activity of 35.2 $\mu\text{mol p-nitrophenol/min/mg}$

protein. The molecular weight of 5'-PDE using gel filtration chromatography was estimated at 125 kDa. Native PAGE analysis showed one major protein band, while SDS-PAGE estimated the molecular weight of the enzyme to be 124 kDa and indicated that the active/intact enzyme may be composed of two identical polypeptide chains (subunits) with a molecular weight of 62 kDa each. In conclusion, although 5'-PDE from adzuki bean has a high temperature optimum of 80 °C, the enzyme is more stable at 60°C, different cations affected the activity of the enzyme differently and the purified 5'-PDE has an estimated molecular weight of 124-125 kDa.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENULENAN DAN FAKTOR YANG MEMPENGARUHI 5'-
PHOSPHODIESTERASE DARIPADA KACANG ADUKI (*Vigna angularis* L.)
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5'-phosphodiesterase (5'-PDE) merupakan satu enzim yang menghidrolisis RNA membentuk 5'-inosina monofosfat (5'-IMP) dan 5'-guanosina monofosfat (5'-GMP), yang juga berfungsi sebagai penambah perisa. Penghasil 5'-PDE terbaik dipilih daripada kekacang yang dibiarkan bercambah, iaitu kacang hijau (*Vigna radiate*), kacang soya (*Glycine max*), kacang adzuki (*Vigna angularis* L.), kacang kuda (*Cicer arietinum*), kacang mata hitam (*Vigna unguiculata*) and petai (*Parkia speciosa*). Untuk memastikan tidak berlakunya pencemaran semasa percambahan, pelbagai rawatan pengsterilan permukaan digunakan. Natrium Hipoklorit pada kepekatan 0.3% mampu untuk menghalang pertumbuhan kulat dalam kacang adzuki, kacang soya dan kacang kuda. Sebaliknya, hanya 0.1% natrium hipoklorit diperlukan untuk menghalang pertumbuhan kulat dalam kacang mata hitam dan petai manakala kacang hijau memerlukan 0.05% natrium hipoklorit untuk menghalang pertumbuhan kulat. Kacang adzuki mengandungi kandungan 5'-PDE tertinggi dan phosphomonoesterase terendah dalam bentuk

bercambah. Paras 5'-phosphodiesterase dalam kacang adzuki berubah semasa percambahan, di mana 15 jam percambahan menghasilkan kandungan 5'-phosphodiesterase yang paling tinggi. Sebaliknya, paras phosphomoesterase adalah paling tinggi di dalam kacang tersebut pada 9 jam percambahan.

5'-PDE mempunyai pH optima dan suhu optima 8.5 dan 80 °C, masing-masing. Ia adalah stabil pada julat pH 7.0 – 8.5. Kestabilan yang lebih tinggi diperhatikan pada pH 7.5 berbanding dengan pH 8.5, dan pada 60 dan 65 ° C, aktiviti enzim masih memiliki fungsi sebagai pemangkin yang baik walaupun selepas 4 hari pengeraman. EDTA, Triton X-100 dan dodecyl natrium sulfat (SDS) menghalang aktiviti 5'-PDE dengan kuat (perencatan adalah antara 75.2-99.4%), manakala Tween 80 dan Tween 20 hanya menghalang aktiviti dengan sedikit. Ekstrak enzim kasar itu dirangsang oleh Ca^{2+} kepada 249%, K^+ , Mg^{2+} dan Li^+ , apabila kepekatan akhir kation adalah 10 mM. Namun, Na^+ , Zn^{2+} , Ni^+ , Hg^+ , Cu^{2+} , Pb^{2+} , Fe^{2+} , Al^{3+} , Ba^{2+} dan Co^{2+} pula merencatkan pada kepekatan yang sama. Aktiviti 5'-PDE meningkat dengan peningkatan kepekatan ion logam Li^+ , Na^+ dan Mg^{2+} , sehingga ia mencapai maksimum pada 6 mM diikuti dengan penurunan dalam aktiviti pada kepekatan melebihi 6 mM. Kehadiran Ca^{2+} dalam campuran tindak balas menyebabkan trend yang sama, tetapi perbezaannya adalah pengaktifan maksimum berlaku pada 4 mM. K^+ pula menunjukkan peningkatan aktiviti 5'-PDE dari 0-10 mM. Al^{3+} mengurangkan aktiviti 5'-PDE dengan peningkatan kepekatan kationnya. Oleh itu, adalah dicadangkan bahawa 5'-PDE dari kacang adzuki merupakan enzim metalo.

Hasil 5'-PDE selepas empat langkah penulenan (pemendakan aseton sejuk 1:1.25, kromatografi penyahgaram, kromatografi penukar anion dan kromatografi penurasan gel)

adalah 13.37% dengan lipatan penulenan sebanyak 6.7 dan aktiviti enzim spesifik sebanyak 35.2 μmol p-nitrophenol/min/mg protein. Berat molekul 5'-PDE asli yang dianggar menggunakan kromatografi penurasan gel adalah 125 kDa. Analisis PAGE natif menunjukkan satu jalur protein utama manakala SDS-PAGE menunjukkan bahawa anggaran berat molekul adalah 124 kDa, yang mungkin terdiri daripada dua rantai polipeptida yang serupa (subunits) dengan berat molekul sebanyak 62 kDa setiap satu. Kesimpulannya, 5'-PDE dengan suhu optimum dan kestabilan suhu yang tinggi telah diperolehi dari kacang adzuki, dan 5'-PDE yang telah dituliskan mempunyai berat molekul 124 - 125 kDa.

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I certify that a Thesis Examination Committee has met on 14 February 2012 to conduct the final examination of Pui Liew Phing on her degree entitled “Purification of 5’-phosphodiesterase from germinating adzuki (*Vigna angularis* L.) beans and factors affecting its activity ” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1988. The committee recommends that the students be awarded the Master of Science. Members of the Thesis Examination Committee were as follows:

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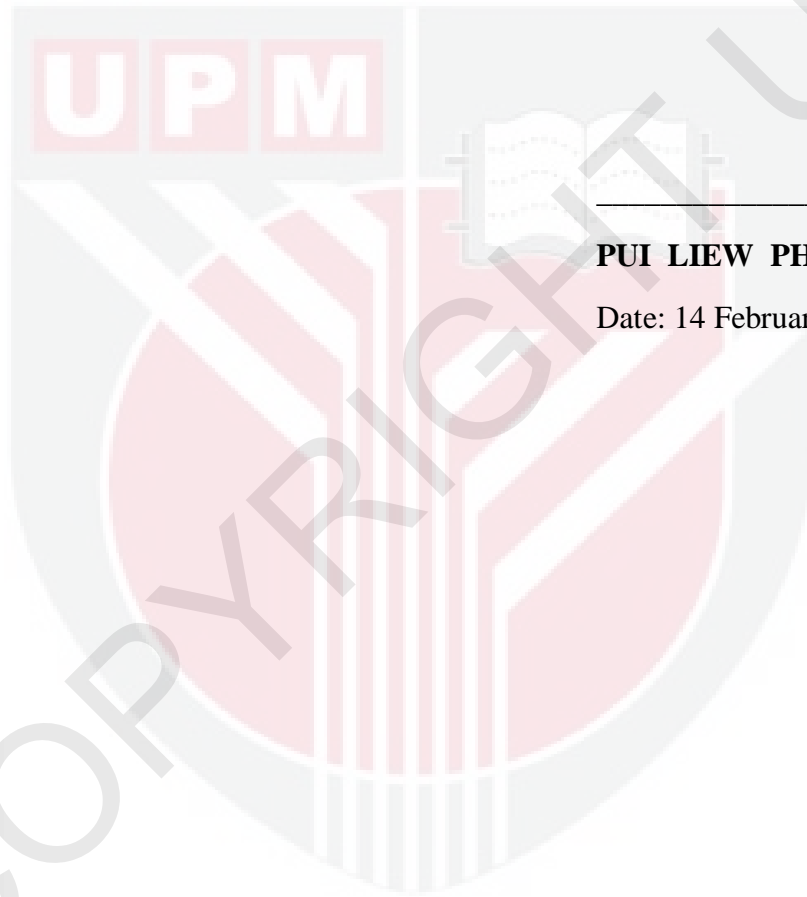
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DECLARATION

I declare that the thesis is my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



PUI LIEW PHING

Date: 14 February 2012

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