



**UNIVERSITI PUTRA MALAYSIA**

**EFFECTS OF EXTRACTION, PURIFICATION AND FREEZE  
DRYING CONDITIONS ON ENZYMATIC PROPERTIES OF  
SERINE PROTEASE FROM MANGO (*MANGIFERA INDICA L. CV.  
CHOKANAM*) PEEL**

**MEHRNOUSH AMID**

**FK 2011 30**

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CHOKANAN*) PEEL**



**By**

**MEHRNOUSH AMID**

**Thesis submitted to the school of graduate studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**April 2011**

## DEDICATION

This thesis is dedicated to my loving family: To my dear father, Amir Amid, who has been my inspiration and my strength through all these years, always there for me, never out of reach whenever I needed him, to whom I owe more than I can ever repay. Thank you for your love. To my dear brother, I am grateful for what you are and have always been to me; and to my dear late mother, I have but one wish – which you could be here.



Abstract of thesis presented to the of the Universiti Putra Malaysia fulfilment of the requirement for the degree of Doctor of Philosophy

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By

**MEHRNOUSH AMID**

**April 2011**

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**Faculty: Engineering**

This research studies the extraction, purification, characterization and preservation by freeze drying of serine protease from mango (*Mangifera Indica CV. Chokanan*) peel. Serine protease is one of the most important enzymes widely used in biotechnological and industrial applications. The purification was carried out using expanded bed adsorption (EBA) and aqueous two-phase system (ATPS). In addition, the main factors affecting enzymatic properties of serine protease were optimized using response surface methodology (RSM). It was found that the optimum condition for serine protease extraction was an amount of 50 ml of sodium phosphate buffer at 2.5 °C for 2min. Also, the specific activity of enzyme after extraction was 17.2 U/mg. In continuing experiments the optimal conditions of purification using EBA were proved to be sample load, washing

and elution at flow rate of 100 cm/h and pH 7.5. In addition, heat treatment of unclarified feedstock was found to be an important parameter in decreasing the level of the adsorbed contaminated protein. It was demonstrated that serine protease could be recovered with a purification factor of 11.39 and a yield of 82%, using EBA. Results indicated that EBA is a suitable method that allows recovery of serine protease to be achieved without product loss. Mango peel contains a lot of fiber that needs filtration for recovery with EBA, which is time consuming and costly, so the purification of serine protease was also studied with ATPS polyethylene glycol/ potassium phosphate. The optimum purification factor and yield were obtained when 6000 g/mol of polyethylene glycol, 28.5% (w/w) of tie light length and 5% of NaCl were used. Serine protease purification factor and yield using ATPS were 12.51 and 89%, respectively. Purification with ATPS does not need filtration and thus, this method is more economical, faster and with easy scale up compared to EBA. Yield of serine protease after using freeze drying in the presence of coating agents and activator was increased to 92.2%. The optimum temperature and pH for activity of serine protease were 70°C and 8, respectively. This enzyme was also stable in the presence of inhibitors, surfactants and oxidizing agents. It was additionally found that activity of serine protease was increased in the presence of calcium ions.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KESAN MENGEKSTRAK, PENULENAN SERTA KAEDAH  
PENGERINGANDAN PEMBEKUAN TERHADAP JUMLAH ENZIM DALAM  
SERINE PROTEASE DARI KULIT MANGGA (*MANGIFERA INDICA L. CV.  
CHOKANAN*)**

Oleh

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**April 2011**

**Pengerusi: Profesar Madya Ling Tau Chaun, PhD**

**Fakulti: Kejuruteraan**

Penyelidikan ini mengkaji ekstraksi, penulenan, kecirian dan pengeringan beku protease serin daripada kulit mangga. Protease serin merupakan salah satu enzim yang paling penting digunakan secara meluas dalam aplikasi bioteknologi dan industri. Penulenan dilakukan dengan menggunakan kawasan penyerapan yang luas (EBA) dan sistem air dua fasa (ATPS). Selain itu, faktor utama yang mempengaruhi sifat enzimatik protease serin telah dioptimumkan dengan menggunakan metodologi respon permukaan (RSM) Menurut dapatan kajian, keadaan optimum untuk ekstraksi protease serin adalah sebanyak 50 ml buffer sodium fosfat di paras 2.5°C selama 2 minit. Juga didapati,

aktiviti khusus enzim setelah ekstraksi adalah 17.2 U/mg. Dalam percubaan seterusnya keadaan yang optimum daripada penulenan menggunakan EBA telah terbukti mengandungi timbangan sampel, cucian dan elusi yang mengalir dengan kelajuan 100 cm/h dan pH 7.5. Selain itu, bahan makanan ternakan tanpa klasifikasi yang dipanaskan merupakan parameter penting dalam mengurangi kadar protein tercemar yang terserap. Hal ini menunjukkan bahawa protease serin boleh dipulihkan dengan faktor penulenan 11.39 dan menghasilkan 82%, dengan menggunakan EBA. Keputusan kajian menunjukkan bahawa EBA adalah kaedah yang sesuai bagi membolehkan pemulihan protease serin tercapai tanpa kehilangan produk. Kulit Mangga mengandungi banyak serat yang perlu filtrasi untuk pemulihan dengan EBA, yang memakan masa dan mahal, dan oleh itu penulenan protease serin juga di kaji bersama dengan polietilena glikol ATP / fosfat potasium. Faktor penulenan dan hasil optimum diperolehi ketika 6000 g / mol glikol polietilena, 28.5% (W /W) bercahaya rendah dan 5% NaCl digunakan. Faktor penulenan protease serin dan penghasilan menggunakan ATPS adalah 12.51 dan 89% untuk pertama dan kedua berikutnya. Penulenan dengan ATP tidak memerlukan penapisan dan oleh yang demikian, kaedah ini lebih ekonomik, lebih cepat dan dengan kesampaian skala mudah berbanding dengan EBA. Penghasilan protease serin setelah menggunakan pengeringan beku bersama dengan agen pelapisan dan penggerak akan meningkat menjadi 92.2%. Suhu optimum dan pH untuk aktiviti protease serin adalah 70 °C dan 8 untuk pertama dan kedua berikutnya. Enzim ini juga stabil jika ada agen inhibitor, surfaktan dan pengoksidaan. Seterusnya didapati aktiviti protease serin meningkat dengan adanya ion kalsium.

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To the many others who have come into my life in my years at UPM, some of whom have grown to be more than course-mates and acquaintances, I thank you for your friendship.



I certify that Thesis Examination Committee has met on the 8 April 2011 to conduct the final examination of Merhnoush Amid on her Doctor of Philosophy thesis entitled “Effect of extraction, purification and freeze drying conditions on the enzymatic properties of serine protease from mango (*Mangifera Indicav Cv. Chokanan*) peel” in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and University Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree.

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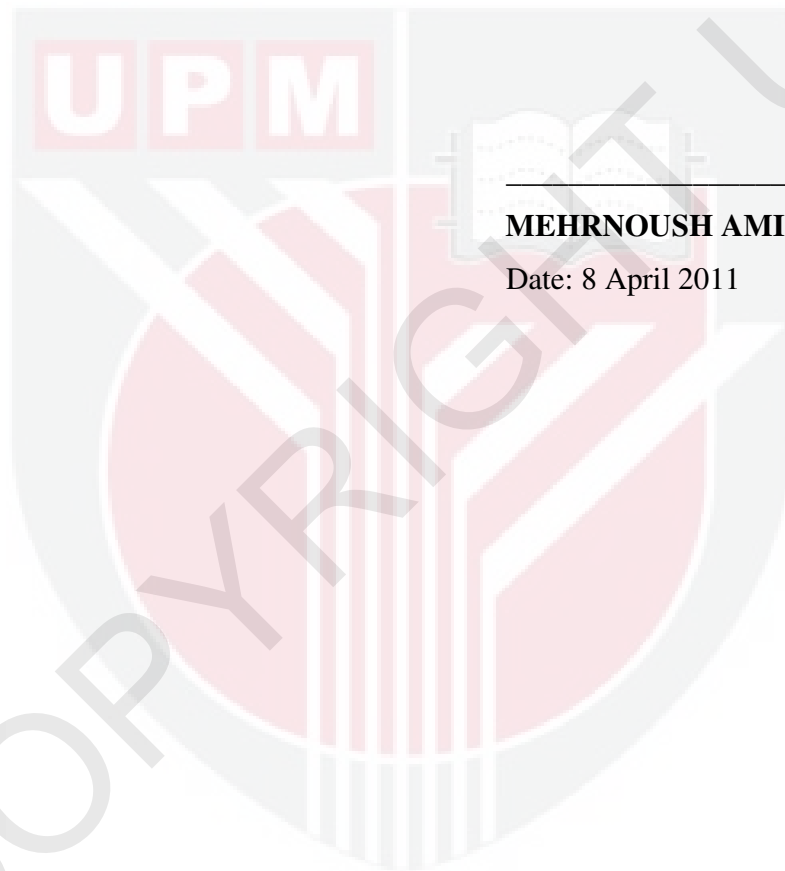
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## DECLARATION

I declare that the thesis is my original work except for 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.



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