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DEVELOPMENT OF A MICROBIAL BIOASSAY SYSTEM FOR DETECTION OF BORIC ACID USING *Paecilomyces variotii*

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

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

DEVELOPMENT OF A MICROBIAL BIOASSAY SYSTEM FOR DETECTION OF BORIC ACID USING *Paecilomyces variotii*

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April 2011

Chairman: Professor Dato' Abu Bakar Salleh, PhD

Faculty: Institute of Bioscience

Boric acid is a water soluble chemical preservative that has been used as food preservative by some local manufacturers. This chemical is used to preserve food products such as noodle and fish ball in order to inhibit the growth of microorganism, so that the preserved food can stay fresh and longer. However, its usage is prohibited by government of Malaysia as boric acid is considered harmful to human health if consumed in a considerably large quantity. Therefore, the detection method for boric acid is important. To date, no study has been performed to detect boric acid by using microorganism as sensing element. Hence, this study was aimed to develop a simple, fast and environmental friendly bioassay system incorporated with *Paecilomyces variotii* as bioreceptor for detection of boric acid in food. This detection system was based on the measurement of the changes of β-glucosidase produced by the microorganisms in response to the presence of boric acid. The changes of β-

glucosidase concentration were assayed spectrophotometerically and correlated to the concentration of boric acid. In this system, *P. variotii* was grown in cellobiose medium for two days before its mycelia were entrapped in calcium alginate in bead form. In order to optimize the best condition for β-glucosidase production, the important factors such as initial pH, temperature, amount of cell loading, concentration of sodium alginate and calcium chloride were determined. The system was found to show optimum β-glucosidase production when 2% (w/v) sodium alginate and 0.25 Molar calcium chloride were used. Maximum enzyme production was also obtained with initial pH 7 and temperature 45 °C, using 6% (w/v) mycelia after three hours of incubation. By using these optimum operating conditions, a lower detection limit of 0.037% (w/v) was obtained from a linear range of 0% to 0.215% (w/v). The reproducibility of the system was acceptable with an observed relative standard deviation of 4.96% (n=10) and 4.81% (n=10) in the presence of 0.2% (w/v) boric acid and absence of boric acid, respectively. The bioassay system was then applied to determine boric acid in fish ball and the results of recovery ranging from 61% – 86% were recorded for boric acid spiked at different concentrations of boric acid from 0.05% to 0.20% (w/v). The developed microbial bioassay system not only represents a simple, inexpensive and environmental friendly alternative for determination of boric acid, but also offers a new idea and promising approach to detect boric acid.

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

PEMBINAAN SISTEM BIOASAI MIKROB BAGI PENGESANAN ASID BORIK DENGAN MENGGUNAKAN *Paecilomyces variotii*

Oleh

ANG SWI SEE

April 2011

Pengerusi: Profesor Dato' Abu Bakar Salleh, PhD

Fakulti: Institut Biosains

Asid borik merupakan satu bahan kimia pengawet larut air yang digunakan oleh beberapa pengilang tempatan sebagai bahan pengawet makanan. Bahan kimia ini digunakan untuk mengawet produk makanan seperti mi dan bebola ikan untuk merencatkan pertumbuhan mikroorganisma supaya makanan yang diawet dapat mengekalkan kesegaran dan tahan lama. Namum demikian, penggunaannya adalah dilarang oleh kerajaan Malaysia kerana asid borik memudaratkan kesihatan manusia jika dimakan dengan kuantiti yang banyak. Justeru itu, kaedah pengesanan asid borik adalah penting. Sehingga kini, tiada kajian yang dilakukan untuk mengesan asid borik dengan menggunakan mikroorganisma sebagai unsur pengesan. Oleh demikian, kajian ini bertujuan untuk membangunkan satu sistem bioasai yang ringkas, cepat dan mesra alam merangkumi *Paecilomyces variotii* sebagai bioreseptor untuk mengesan asid borik dalam makanan. Sistem pengesanan ini berdasarkan pengukuran

perubahan β-glukosidase yang dihasilkan oleh mikroorganisma apabila bereaksi dalam kewujudan asid borik. Perubahan kepekatan β-glukosidase diuji dengan menggunakan spektrofotometer dan berkadar dengan kepekatan asid borik. Dalam sistem ini, *P. variotii* ditumbuhkan di dalam medium selobios selama dua hari sebelum miselianya diperangkap dalam kalsium alginat dalam bentuk manik. Dalam menilai keadaan yang optimum untuk penghasilan β-glukosidase, faktor-faktor penting yang mempengaruhi penghasilan enzim seperti pH awal, suhu, kuantiti sel yang digunakan, kepekatan natrium alginat dan kalsium klorida ditentukan. Sistem ini didapati menunjukkan optimum penghasilan β-glukosidase apabila 2% (b/i) natrium alginat dan 0.25 Molar kalsium klorida digunakan. Penghasilan β-glukosidase yang maksimum juga diperolehi dengan pH awal 7 dan suhu 45 °C dengan menggunakan 6% (b/i) miselia selepas 3 jam inkubasi. Dengan menggunakan kesemua keadaan operasi yang optimum ini, had pengesanan terendah 0.037% (b/i) diperolehi daripada lingkungan linear 0% sehingga 0.215% (b/i). Kajian perolehan semula bagi sistem ini adalah diterima dengan sisihan piawai relatif (RSD) yang diperhatikan ialah 4.96% (n=10) dan 4.81% (n=10) dalam kewujudan asid borik pada 0.2% (b/i) dan tanpa asid borik masing-masing. Sistem bioasai ini kemudian diaplikasikan untuk menentu asid borik dalam bebola ikan dan keputusan bagi perolehan semula asid borik berada dalam lingkungan 61% – 86% (b/i) telah dicatatkan bagi pelbagai kepekatan asid borik bermula dari 0.05% sehingga 0.20% (b/i) yang ditambahkan. Bioasai mikrob yang diperbangunkan ini bukan sahaja merupakan satu alternatif yang ringkas, murah dan mesra alam untuk menentukan asid

borik, malah menawarkan satu idea baru dan pendekatan yang memberi kebaikan untuk mengesan asid borik.

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Thank you very much.

I certify that an Examination Committee has met on **11 April 2011** to conduct the final examination of Ang Swi See on her Master of Science thesis entitled "Development of A Microbial Bioassay System for Detection of Boric Acid Using *Paecilomyces variotii*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the Master of Science.

Members of the Examination Committee were as follows:

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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:

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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 institutions.

ANG SWI SEE

Date: 11 April 2011

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