UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF ENZYME-BASED BIOSENSOR FOR THE DETECTION OF FORMALDEHYDE IN FISH

NUR INDANG BINTI MARZUKI

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By

NUR INDANG BINTI MARZUKI

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

January 2011

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January 2011

Chairperson: Professor Fatimah Abu Bakar, PhD

Faculty : Institute of Bioscience

Usage of formaldehyde as preservative in fish by fisherman in order to maintain its fresh look and avoid microbial spoilage is a big risk to consumer's health. Moreover, its capability to induce carcinogen at certain level arises endeavour to create simple, sensitive and rapid device for formaldehyde determination. Therefore, a novel detection method based on amperometric biosensor coupled with an enzyme, formaldehyde dehydrogenase (FDH) has been developed. To maximize the reaction rate, the enzyme acts as biorecognition was immobilized in Nafion membrane which chemically modified on gold electrode. The enzyme required nicotinamide adenide dinucleotide (NAD^+) as a cofactor which then reduced to NADH at -0.2 volt during enzymatic reaction. Through the electrode this physiochemical changes were converted into electric signal which correlated with formaldehyde concentration. The current measurement was analysed using a computer connected to the transducer.

The optimized formaldehyde biosensor displayed a linear response over the range of 1 to 10 ppm formaldehyde with correlation coefficient (R^2) equals to 0.9865 (RSD <

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3.05%). The limit of detection (LOD) calculated was 0.016 ppm of formaldehyde and the sensitivity was 7.0264 nA ppm^{-1}. The response time of formaldehyde biosensor was found less than 1 min and has an optimum pH of 8. Besides, it also showed reproducibility with no significant different ($p > 0.05$) at 1, 5 and 10 ppm of formaldehyde (n = 10). For selectivity and interferences by ratio study, it was discovered that the biosensor response retained its specificity for formaldehyde and did not respond to equivalent additions of methanol and also ethanol and gave the percentage of formaldehyde recovered ranging from 99.0% to 99.8%.

The developed biosensor has been applied for monitoring formaldehyde level in Indian Mackerel (*Rastrelliger kanagurta*) where the samples stored for ten days at temperature of $4^oC \pm 1$ and were compared to formaldehyde levels determined by the conventional Nash method. As the result, the two methods showed a linear correlation coefficient with $R^2 = 0.9937$ ($y = 0.0542x - 0.0256$) and has no significant different ($p > 0.05$). For determination of formaldehyde in fish tissue, the percentage of formaldehyde recovery was found at the range of 68.3 to 86.25 (RSD $\leq$ 10.81%) after spiked with 1, 5 and 10 ppm of formaldehyde. It also showed stable measurement reading of 90% from the initial value after six months (stored at 4^oC). Another advantage of the constructed biosensor in this work is the assembly of the basic requirements for simplicity, reusability and reagentless system. Thus, it is a promising tool and has a potential application for fast and direct formaldehyde detection in fish.

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

PEMBANGUNAN BIOSENSOR BERDASARKAN ENZIM UNTUK MENGESAN FORMALDEHID PADA IKAN

Oleh

NUR INDANG BINTI MARZUKI

Januari 2011

Pengerusi: Profesor Fatimah Abu Bakar, PhD

Fakulti : Institut Biosains

Penggunaan formaldehid sebagai bahan pengawet oleh nelayan untuk mengekalkan rupa bentuk yang segar dan mengelakkan kerosakan oleh mikrob merupakan risiko yang besar kepada kesihatan pengguna. Lagipun, kebolehannya untuk merangsang pembentukan kanser pada aras yang tertentu membangkitkan usaha untuk mereka cipta alat yang mudah, cepat dan sensitif terhadap formaldehid. Oleh itu, satu kaedah novel pengesanan berdasarkan biopenderia amperometrik berpasangan dengan enzim, formaldehid dehidrogenase (FDH) telah dibangunkan. Untuk memaksimumkan kadar tindak balas, enzim yang bertindak sebagai biopengenal telah dipegunkan di dalam membran Nafion yang diubahsuai secara kimia pada elektrod emas. Enzim ini memerlukan nikotinamide adenine dinukleotide (NAD^+) sebagai kofaktor yang kemudiannya diturunkan menjadi NADH pada -0.2 voltan semasa tindak balas enzim berlaku. Melalui elektrod perubahan fizikokimia ini bertukar kepada signal elektrik yang berkorelasi dengan kepekatan formaldehid. Pengukuran arus dianalisis dengan komputer yang bersambungan dengan transduser. Biopenderia formaldehid yang dalam keadaan optimum mempamerkan julat reaksi linear dari 1 hingga 10 ppm formaldehid dengan koefisien korelasi (R^2) 0.9865 (RSD

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I certify that an Examination Committee has met on **12th May 2011** to conduct the final examination of **Nur Indang Binti Marzuki** on her degree of **Master of Science** thesis entitled "**Development of Enzyme-Based Biosensor for the Detection of Formaldehyde in Fish**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Examination Committee were as follows:

Alfi Khatib, PhD
Faculty Science and Food Technology
Universiti Putra Malaysia
(Chairman)

Mohd Yunus Abd Shukor, PhD
Associate Professor
Faculty Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Md. Zaidul Islam Sarker, PhD
Associate Professor
Faculty Science and Food Technology
Universiti Putra Malaysia
(Internal Examiner)

Salmijah Surif, PhD
Y. Bhg Professor
Faculty Science and Technology
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

NORITAH OMAR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 April 2011

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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:

Fatimah Abu Bakar, PhD
Associate Professor
Faculty Science and Food Technology
Universiti Putra Malaysia
(Chairman)

Abu Bakar Salleh, PhD
Professor Dato'
Faculty Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Nor Azah Yusof, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Lee Yook Heng, PhD
Professor
Faculty Science and Technology
Universiti Kebangsaan Malaysia
(Member)

HASANAH MOHD. GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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DECLARATION

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

NUR INDANG BINTI MARZUKI

Date: 24 January 2011

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