An Amperometric Xanthine Oxidase Biosensor for the Determination of Tuna Fish Freshness

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Abstract: An amperometric biosensor for hypoxanthine (Hx) has been constructed and applied to the measurement of fish meat quality. The construction process involves the immobilization of xanthine oxidase in the polymetaphenylenediamine film on the surface of platinum electrode by the electropolymerization technique. Hypoxanthine was measured with the sensor by the oxidation of the enzymatic reaction product, hydrogen peroxide at optimum potentials 0.6 V (vs Ag/AgCl). The use of polymerized film of polymetaphenylenediamine as a matrix for xanthine oxidase immobilization yields enhanced specificity, sensitivity and stability. The linear of 0 mM to 0.8 mM was achieved and the response time was less than 1 minute. Satisfactory results were obtained from the determination of the freshness of tuna tissues stored under different storage time.

Introduction: Estimation of fish freshness is a needful activity in maintaining high product quality in the food industry. When a fish is caught and killed, it loses freshness through its degradation processes. Hypoxanthine is a major metabolite of adenine...
nucleotide degradation that accumulates in fish continuously after death. The level of hypoxanthine is generally used in the food industry as an index for evaluating fish freshness. Various methods (1), including anion-exchange chromatography, thin layer chromatography, precipitation, and capillary electrophoresis have been proposed for hypoxanthine determination. Each requires complicated and time-consuming procedures, and the service of a skilled technician. The establishment of a simple, rapid and accurate method for the determination of fish freshness is required. Electrochemical biosensors are therefore developed as an expedient alternative (2). Most of methods described for the determination of hypoxanthine are based on the enzyme xanthine oxidase (XOD) and amperometric biosensors have been developed (3). XOD has been known for its effectiveness and selectivity in the following enzyme reaction

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\begin{align*}
Hx & \xrightarrow{\text{XOD}} \text{xanthine} + H_2O_2 \\
\text{xanthine} + O_2 & \xrightarrow{\text{XOD}} \text{uric acid} + H_2O_2
\end{align*}
\]

Hydrogen peroxide is produced during the reactions. The concentration of Hx is related to the increase in hydrogen peroxide concentration which can be determined electrochemically. A major problem facing electrochemical biosensors is interferences, any species, other than the analyte present in fish that is oxidizable at the applied potential. Polymer films have the advantage of usually providing protection from interferences. The development of the hypoxanthine sensor based on the immobilization of XOD in polymetaphenylenediamine (poly-1,3-DAB) by electropolymerization technique for the determination of fish freshness is described in this work.

**Methodology:** A platinum electrodes (PtE)(3 mm diameter) was used to develope the hypoxanthine sensor. Prior to sensor preparation, the PtE was polished with 0.3 µm aluminum particles. The PtE was then pretreated in 1 M H₂SO₄ solution with cyclic voltammetry in a potential range between -1.5 V and +1.5 V vs Ag/AgCl reference electrode at a scan rate of 0.1 V/s until a stable cyclic voltammogram resulted. Platinum disc was used as the counter electrode. XOD was immobilized into poly-1,3-DAB film formed by potentiostatic electropolymerization at +0.65 V vs Ag/AgCl for 3 minutes on the pretreated PtE. The enzyme electrode was rinsed with 0.15 M phosphate buffer pH 7.8. Amperometric response of the hypoxanthine sensor to Hx was measured by applied potential at +0.6 V vs Ag/AgCl to the enzyme electrode in 5 ml of 0.15 M phosphate buffer pH 7.8

**Results, Discussion, and Conclusion:** The hypoxanthine sensor constructed by immobilization of XOD into poly-1,3-DAB film by electropolymerization. The optimum concentration of XOD was 0.33 units/ml. The enzyme loading may be influenced by electropolymerization conditions, such as monomer concentration, immobilization times and surface area of electrodes. To test the pH-dependency of amperometric response of the sensor, maximum current output was obtained at pH 7.8. This existence of an optimum pH is due to the amphoteric nature of the amino acid that make up the enzyme, particularly those that are responsible for substrate binding. Protonation can substantially alter the rate of formation and decomposition of the enzyme substrate complex. The Hx sensor gave a linear calibration graph in the range of 0 mM to 0.8 mM with high sensitivity, long-term shelf life(over 30 days). This sensor had response time of less than 1 minute. The determination of Hx content in fish meat
stored under different storage time, it was found that Hx was accumulated with storage time under -8 °C. These results indicate the suitability of the experimental Hx sensor to provide reliable of fish quality.

References:

Keywords: fish freshness, hypoxanthine, xanthine oxidase, electropolymerization, polymetaphenylenediamine, amperometric biosensor.