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An amperometric biosensor for the rapid assessment of histamine level in tiger prawn (*Penaeus monodon*) spoilage

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Abstract

Histamine could accumulate in seafood when bacteria spoilage commenced and caused histamine poisoning without altering the fish normal appearance and odor. Therefore, a histamine biosensor using immobilized enzyme diamine oxidase (DAO) has been developed for the rapid monitoring of the histamine levels in tiger prawn (*Penaeus monodon*). The histamine biosensor had a response time of <1 min and optimum pH of operation was 7.4 with reproducibility and repeatability ($n = 5$) of 4.87% and 5.26% relative standard deviations (RSD) respectively. Recoveries ranging from 93.11% to 100.58% were obtained for histamine spiked at levels from 5 to 20 ppm. The variation in histamine levels of some tiger prawn samples after a 5-h exposure at temperature of 30 °C ± 2 were studied using the histamine biosensor and the results were comparable to histamine levels determined by an HPLC method. The two methods showed a linear correlation with $R^2 = 0.9612$ ($Y = 0.9164x + 5.58$). The limit of detection was 0.65 ppm of histamine, which is below the indicator level of 50 ppm established by USA FDA. The reusable biosensor is simple and can be used for direct histamine determination without further sample pretreatment, and is suitable for the routine analysis of histamine in tiger prawns to monitor spoilage.

Keywords: Amperometric biosensor; Diamine oxidase; Histamine; Tiger prawn (*Penaeus monodon*)

1. Introduction

Histamine is known as a biogenic amine which is low molecular weight and possesses biological activity (Tombelli & Mascini, 1998). Histamine poisoning is also referred to as 'Scombroid fish poisoning'. The levels of histamine have been suggested as rapid fish spoilage indicators (Male, Bouvrette, Luong, & Gibbs, 1996; Patange, Mukundan, & Kumar, 2005; Tombelli & Mascini, 1998). Histamine poisoning probably occurs frequently in Asia, and was reported in extremely high levels in some salted, and dried fermented products. Other countries outside Asia have also reported cases of histamine poisoning (Lehane & Olley, 2000). The largest outbreak (2656 cases) was recorded in Japan in 1973 (Lehane & Olley, 2000). Histamine exerts its effects by binding to receptors on cellular membranes in the respiratory, cardiovascular, gastrointestinal and haemotological immunological system and the skin in the course of allergic and other actions such as hypotension, flushing, diarrhea, vomiting and headache (Lehane & Olley, 2000). The symptoms may vary between individuals that exposed to the same dose of histamine in contaminated fishery products (Bremer, Fletcher, & Osborne,

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2003). The earliest record of this disease was in 1828. Since then, the worldwide network for harvesting, processing and distributing fish products has made histamine poisoning as a global problem (Lehane & Olley, 2000). USA FDA, an international food safety organization has established 500 ppm as hazardous level for histamine (FDA, 2001). Therefore, this is considered as an indicator of earlier microbial decomposition of seafoods and a guidance level of 50 ppm is considered as the chemical index for fish spoilage. Histamine is generally not uniformly distributed in a decomposed fish (FDA, 2001; Lehane & Olley, 2000). The histamine level of a decomposing fish varied from 50 ppm in one location to exceeding 500 ppm in another location of the tissue. (FDA, 2001; Lehane & Olley, 2000). The fish and fishery products with histamine above that level are prohibited from being sold for human consumption (Gigirey, Craven, & An, 1998). A more detail review in the oral toxicity to humans of histamine in fish muscle suggested that histamine induced poisoning slightly at 80–400 mg/kg (ppm) fish, moderate poisoning at >400 mg/kg and severe at >1000 mg/kg. Based on the assessment of poisoning cases, the guidance levels suggested for histamine content for seafood are for safe consumption: <50 mg/kg; possibly toxic: 50–200 mg/kg; probably toxic: 200–1000 mg/kg and toxic and unsafe for human consumption >1000 mg/kg (Lehane & Olley, 2000).

Several methods proposed for histamine detection included gas chromatography, thin layer LC (liquid chromatography), reversed-phase LC and LC with pre-column, post-column or on-column derivatisation technique, and high pressure liquid chromatography (Chemnitius & Bilitewski, 1996; Male et al., 1996; Tombelli & Mascini, 1998). However these methods required complicated and expensive instrumentation and time consuming operation. On the other hand, biosensor technology allows fast, cost effective and specific detection of histamine in seafood spoilage (Male et al., 1996). Biosensors are devices comprising of a selective receptor, normally immobilized in close proximity or integrated to a signal transducer. The transducer converts the biochemical signal into an electronic signal, which can be processed as a readable output (Chaubey & Malhotra, 2002). One of the benefits of a biosensor is its selectiveness to a target analyte. This selectivity is due to the substrate specificity of the biological receptor and freedom from interferences of the reaction products (Chaubey & Malhotra, 2002). Biosensors have now moved from laboratory level to field testing and many were commercialized (Sharma, Sehgal, & Kumar, 2003). Some biosensors have been developed for biogenic amines including a monoamine sensor based on platinum paste screen-printed electrode or collagen membrane (Chemnitius & Bilitewski, 1996; Karube, Satoh, Araki, Suzuki, & Yamada, 1980), a putrescine sensor based on the detection of hydrogen peroxide with micro planar thin-film electrode coupled with a flow injection analysis system (Chemnitius & Bilitewski, 1996) and a hypoxanthine biosensor with nylon membrane attached onto a polarographic electrode (Mulchandani, Luong, & Male, 1989). Many of these biosensors involved the electrochemical oxidation at high positive potential of the hydrogen peroxide produced from the catalytic reaction by the oxidase enzymes. This causes the detection system to be vulnerable to interferences such as ascorbate and urate (Wimmerová & Macholán, 1999). The importance of developing an electrochemical biosensor

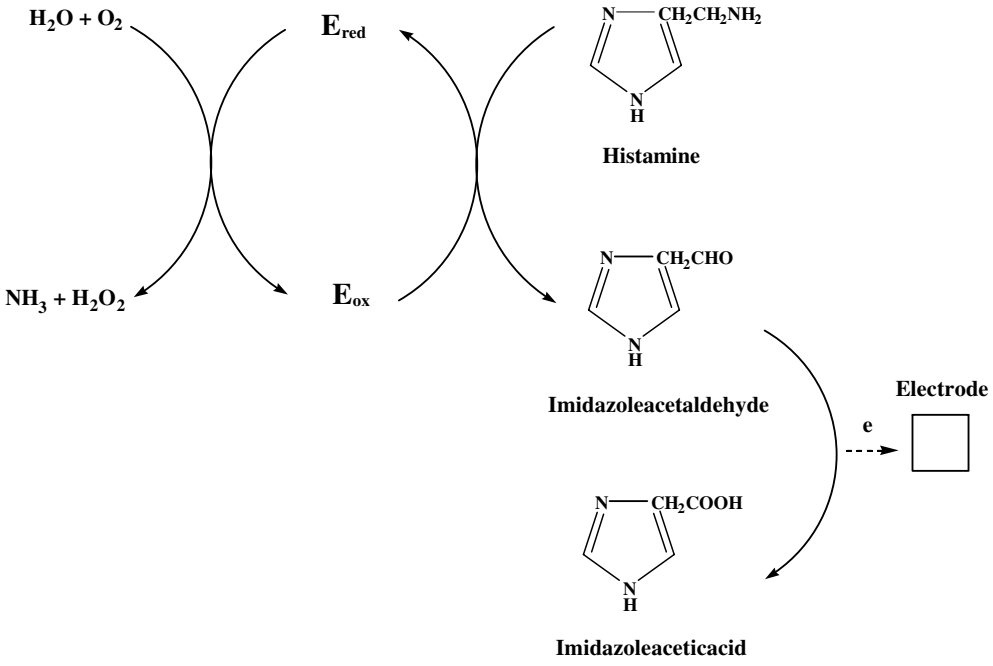

Fig. 1. The mechanism of operation of the histamine biosensor, where the product imidazole acetaldehyde from the enzymic reaction (diamine oxidase, E_{red} and E_{ox}) of histamine was electrochemically oxidized at lower potential of 0.35 V at the screen-printed electrode.

for histamine operating at low potential can be seen when a biosensor for histamine has been developed recently using histamine dehydrogenase immobilized in a redox polymer, which can act as an electron transfer mediator to reduce the working potential (Takagi & Shikata, 2004).

In this study, we have developed an enzyme based histamine biosensor that can operate at a lower potential. The decrease in the operation potential was achieved by the electrochemical oxidization of the product imidazole acetaldehyde, which was produced from the enzymic reaction of diamine oxidase on histamine (Fig. 1). The biosensor also utilized a photocuring technique for the immobilization of the diamine oxidase enzyme where it was directly entrapped in a photocured membrane and deposited onto a carbon paste screen-printed electrode (SPE). Histamine was then determined using an amperometric method. The biosensor was used to evaluate histamine in tiger prawns and for the monitoring of histamine release during prawn spoilage.

2. Materials and methods

2.1. Reagents

DAO, (0.16 U/mg), histamine dihydrochloride and 2,2-dimethoxyphenylacetophenone (DMPP) and monomer 2-hydroxyethyl metacrylate (HEMA) were purchased from Sigma. Other chemicals were of analytical grade and used as received without further purification. All aqueous solutions were prepared with deionized water.

2.2. Tiger prawn (*Penaeus monodon*) samples

Fresh samples of tiger prawn samples were used in this study. The prawn samples were exposed at 30 °C ± 2 for 5 h before taken for histamine analysis at an interval of 1 h. The prawn's shell, head and tail were removed before 10 g of the tissue were used in histamine analysis either with biosensors or a HPLC.

For biosensor detection, the prawn's body region was blended together with 100 ml of phosphate buffer (pH 7.4, 0.1 M) and the sample was used directly without extraction and pretreatments.

For HPLC method, the samples were extracted as previously described (Mopper & Sciacchitano, 1994). The prawns were blended with methanol and then diluted by 10 times with water. The purification procedure for histamine was then carried out as reported by Vale and Glória (1997). Finally, the extracted samples were derivatized to detect the benzoylated histamine ring at 254 nm (Hauschild, 1993). The benzoyl derivative was stable (Yen & Hsieh, 1991).

2.3. Preparation of histamine biosensor

Photopolymerization technique was applied for DAO entrapment where the monomer 2-hydroxyethyl methacrylate was polymerized into a hydrogel film of photo2-hydroxyethyl methacrylate (photoHEMA) to entrap the enzyme DAO according to Low, Lee, Yamin, and Ahmad (2005). For DAO immobilization, an appropriate volume of HEMA and DAO solution in phosphate buffer (pH 7.4, 0.1 M) was mixed together. After homogenization of the mixture, 5 μl of the resulting solution was drop-coated onto a carbon paste SPE. The electrode was photocured for 300 s in an UV-exposure unit under constant nitrogen gas flow.

2.4. Biosensor characterization

The biosensor evaluation was performed by using an Autolab PGSTAT 12 Potentiostat/Galvanostat. All the experiments were carried out with a three-electrode system consisted carbon paste screen-printed working electrode, platinum rod counter electrode and Ag/AgCl reference electrode. Samples were tested in 5 ml of phosphate buffer (pH 7.4, 0.1 M) at a potential of 0.35 V. The changes of current with different amount of histamine were recorded after 50 s.

2.5. HPLC method for histamine determination

Separation of benzoylated histamine was achieved by isocratic reversed-phase HPLC using a Waters 1500 Series HPLC Pump and a 4.6 mm × 250 mm I.D, a C_{18} column of particle size 5 μm and acetonitrile–deionized water (52:48) as the mobile phase at a flow rate of 1.0 ml/min. Histamine was detected spectrophotometrically at 254 nm with a Water Model 2487 Dual λ Absorbance Detector.

3. Results and discussion

The response range for the histamine biosensor with immobilized DAO was plotted from 0 to 300 ppm histamine (Fig. 2). A linear range from 0 to 60 ppm of histamine

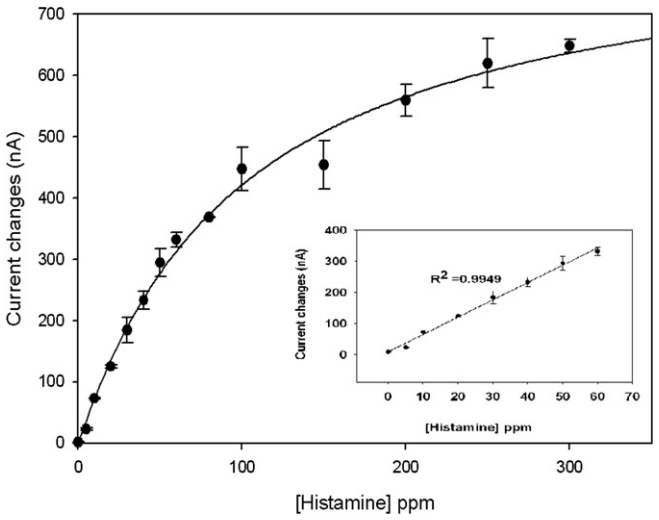

Fig. 2. Response range of histamine biosensor for different histamine concentrations at 0.35 V in phosphate buffer pH 7.4, 0.1 M.

with correlation coefficient of 0.9949 was obtained. This is within the range of spoilage indication of fish and fishery products by the FDA, USA. The linear range obtained was broader compared to that reported by Takagi and Shikata (2004), Carsol and Mascini (1999) and Chemnitius and Bilitewski (1996) using flow injection analysis or Clark oxygen electrode. From Fig. 2, the reproducibility of each data point has a relative standard deviation (RSD) value between 0.22% and 11.19% ($n = 3$) and this indicates that the biosensor showed reproducible responses.

The sensitivity of the histamine biosensor was 5.56 nA ppm^{-1} and showed some selectivity towards putrescine and cadaverine, but the response of diamine oxidase towards putrescine is 17.5 fold lower compared to histamine (Bouvrette, Male, Luong, & Gibbs, 1997; Schwelberger & Bodner, 1997). Because the level of these potential interfering substances in seafood spoilage are normally 10–800 times lower than histamine (Hwang, Chang, Shiua, & Chai, 1997), thus major interference to histamine determination is unlikely. The lifetime of the biosensor was stable up to a month when kept in 5 °C without observable decrease in the response. Using cross-linked extracted pea seedling DAO (25 mg U^{-1}) Chemnitius and Bilitewski (1996) found similar sensitivity for histamine biosensor. The limit of detection of the biosenssor developed in this work was 0.65 ppm, which was calculated based on Fig. 2 as described by Miller and Miller (2000).

The response time of the histamine biosensor is <1 min where stable response could be obtained. However, the response to histamine is pH dependent. Based on Fig. 3, pH 7.4 was the optimum pH because maximum current changes was obtained for a fixed amount of histamine (RSD = 5.53%, $n = 3$). The observed pH effect on the activity of the immobilized DAO was the same as that of the free enzyme assayed by a spectrophotometric procedure. (Ng, Saha, & Choudhuri, 2000). Similar pH also reported for immobilized and native monoamine oxidase used in amines determination (Karube et al., 1980). Besides that, an oxygen sensor for the determination of histamine in fish using fungal DAO proposed by Ohashi et al. (1994) also reported pH 7.4 as the optimum pH. Similar optimum pH was used when amperometric biosensors based on nucleoside phosphorylase and xanthine oxidase were developed for the estimation of fish freshness (Cayuela, Peña, & Pingarrón, 1998; Okuma, Takahashi, Yazawa, & Seimukai, 1992; Yao, 1993).

The histamine biosensor showed reproducibility and repeatability with RSD values of 4.87% ($n = 5$) and 5.26% ($n = 5$) respectively. Its reproducibility and repeatability are improved when compared to a histamine biosensor based on DAO immobilized onto a porous pre-activated nylon membrane that attached to an amperometric electrode operated at 0.70 V (Male et al., 1996). Recoveries of histamine from prawn samples demonstrated by the histamine biosensor ranged from 99.0% to 100% ($n = 3$) (Table 1). This demonstrates the potential of histamine biosensor for the determination of seafood spoilage (Ohashi et al., 1994).

Spoilage of prawn samples was investigated by the histamine biosensor method and compared with conventional HPLC method. Comparable results between both methods (Fig. 4) at 95% confidence level were obtained. For both methods, the histamine levels increased rapidly when the tiger prawn samples were left at room temperature, and this was likely to be caused by bacteria that capable of producing hazardous amounts of histamine in a very short period of time when it is kept at elevated temperatures (Tsai et al., 2005). The good recoveries of the biosensor and satisfactory correlation between the performance of the HPLC and biosensor methods indicated that potential interferences from other biogenic amines is negligible, especially in the prawn samples investigated.

Measurement of histamine using user friendly biosensors has many advantages over conventional method such as HPLC (Lange & Wittmann, 2002). Biosensor can be designed for portability and thus allows rapid and *in situ* detection compared with HPLC instrument, which requires

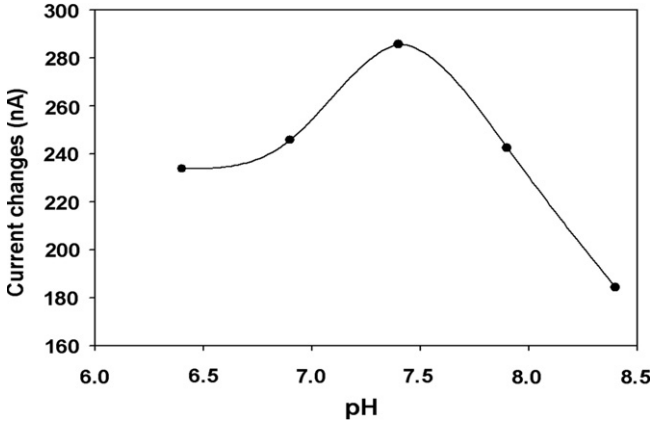

Fig. 3. Effect of pH on the maximum response of the histamine biosensor in 50 ppm of histamine at 0.35 V in phosphate buffer pH 7.4, 0.1 M.

Table 1
The recovery performance of a histamine biosensor for the analysis of histamine in tiger prawn samples (wet weight)

Histamine added (μg)	Histamine found in the sample before spiking (μg)	Expected value (μg)	Found value (μg)	Recovery (%)	RSD (%)
50	436	486	481 ± 3[a]	99.0	0.62
	777	827	826 ± 1	99.9	0.12
	1165	1215	1220 ± 3	100.4	0.25
100	436	536	535 ± 7	99.9	1.31
	777	877	881 ± 2	100.5	0.23
	1165	1265	1272 ± 8	100.5	0.63
200	434	635	637 ± 5	100.3	0.78
	777	977	974 ± 8	99.7	0.82
	1165	1365	1364 ± 4	99.9	0.29

[a] Note: mean ± standard deviation ($n = 3$).

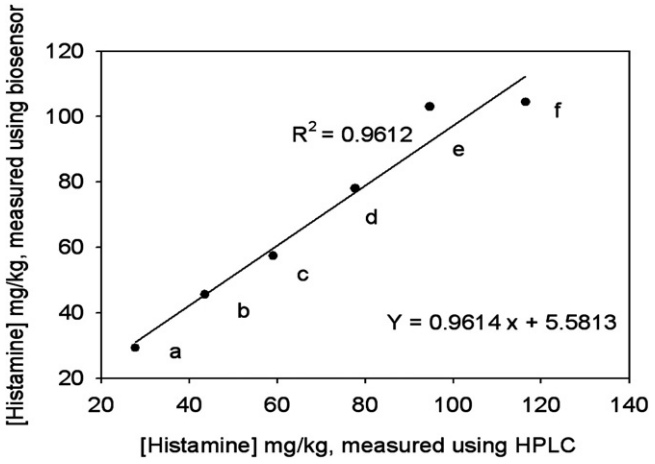

Fig. 4. Correlation of histamine levels determined in prawn samples with both histamine biosensor and HPLC method (Duration at 30 ± 2 °C: a = 0 h, b = 1 h, c = 2 h, d = 3 h, e = 4 h, f = 5 h).

pretreatment of sample such as extraction, neutralization and further clean-up (Lange & Wittmann, 2002).

4. Conclusions

The use of photocurable membrane technology in the fabrication of a histamine biosensor has successfully produced an amperometric histamine biosensor that can be used in the detection of seafood spoilage. The biosensor developed has a response range up to 300 ppm of histamine with the capability of detecting a level of 50 ppm of histamine, an indication level of histamine poisoning. The biosensor exhibited a reproducible and good repeatability characteristic with acceptable RSD values. Histamine level in prawns determined from the biosensor was comparable to conventional HPLC method.

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