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The biocomposite screen-printed biosensor based on immobilization of tyrosinase onto the carboxyl functionalised carbon nanotube for assaying tyramine in fish products

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Putrescine
Histamine
Tyrosine
Glutathione
Catechol
Phenol
Calcium chloride ($CaCl_2$)
Tyrosinase
Sodium phosphate monobasic (NaH_2PO_4)
Sodium phosphate dibasic (Na_2HPO_4)
Glutaraldehyde

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ABSTRACT

Tyrosinase was immobilized on the carboxyl functionalised single-walled carbon nanotubes modified carbon screen-printed electrode which was employed as an amperometric biosensor for tyramine. The carboxyl functionalised single-walled carbon nanotubes provide a suitable microenvironment for immobilization of enzyme retaining the bioactivity of tyrosinase. A clearly defined reduction current proportional to the tyramine concentration was observed in cyclic voltammetry, which attributed to the reduction of enzymatically produced quinone at the electrode surface. Experimental conditions on the sensing performance of the biosensor were investigated. Under the optimal conditions, the biosensor has a good linearity with the concentration of tyramine in the range of 5–180 μM, with a sensitivity of 0.7414 $A \times M^{-1}$ and a detection limit of 0.62 μM. The biosensor shows high repeatability and long term stability. Selectivity of biosensor towards interfering compounds was studied. Determination of tyramine amounts in food samples showed good recovery in the range of 98–102%. The novel biosensor was successfully applied to determine amounts of tyramine in pickled and smoked fish samples.

1. Introduction

The biogenic amines include the three catecholamines (dopamine, norepinephrine, epinephrine), and the tyrosine metabolites, octopamine and tyramine. Serotonin and histamine are also biogenic amines that play a role in brain function (Gerald, 2010). Biogenic amines are useful chemical indicators to estimate spoilage of foods, particularly fish and fish products, meat, cheese, and fermented foods (Muresan et al., 2008; Carelli et al., 2007).

Biogenic amines are biosynthesized in plant and in animal cells and produced by microbial decarboxylation of amino acids (Huang et al., 2011). As result of microbial decarboxylation activity, tyramine is often produced during the storage and aging of foods (Atta and Abdel-Mageed, 2009; Galgano et al., 2009).

Abbreviations: HPLC, high performance liquid chromatography; CE, capillary electrophoresis; SPE, screen-printed electrode; CV, cyclic voltammetry; SWCNT-COOH, carboxyl functionalised Single-Walled Carbon Nanotubes; Ty, tyrosinase; PBS, phosphate buffer solution; LOD, detection limit; LOQ, limit of quantification; h, Hill coefficient; K_M, the apparent Michaelis–Menten constant; RSD, relative standard deviation.

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It is well known that eating of food containing high amounts of tyramine or other biogenic amines can cause adverse reactions, toxic effects, and diseases, i.e. hypotension, palpitations, nausea, vomiting, headaches and diarrhea (Ferrario et al., 2012; Blob et al., 2007; Himwich and Himwich, 1964). There are two reasons for the determination of tyramine in fish products: its potential toxicity and a possibility of using it as fish product quality indicator.

The most extensively used analytical methods for identification and quantification of tyramine are high performance liquid chromatography (HPLC) combined with different detection techniques (Önal et al., 2013; Spizzirri et al., 2013; Bacaloni et al., 2013; Gianotti et al., 2008), capillary electrophoresis (CE) (Önal et al., 2013; Bacaloni et al., 2013), and quantitative polymerase chain reaction (qPCR) method (Loizzo et al., 2013).

The majority of HPLC analyses apply fluorometric or spectroscopic detection with pre- or post-column derivatization techniques (Atta and Abdel-Mageed, 2009). Additionally, the HPLC analysis of tyramine requires trained personnel in combination with quite expensive instrumentation. In contrast to the classical methods, electrochemical biosensors present simple, rapid and cost-effective solution for the determination of tyramine, especially for in-line, on-line and real-time analysis (Ghasemi-Varnamkhasti et al., 2010).

Detection and quantification of tyramine by means of chemically modified electrodes was described in the literature (Huang et al., 2011). Nevertheless, a promising alternative for tyramine detection and quantification are the biosensors. Biosensing methods are considered better because of their rapidity, high sensitivity and selectivity, inherent miniaturization possibility, and minimal power demands (Vidal et al., 2013; Hernández-Cázares et al., 2011). Tyramine biosensors are usually based on monoamine oxidase or tyramine oxidase. Different techniques were used for immobilization of enzymes on different substrate matrix such as gelatine (Yagodina and Nikolskaya, 1997), collagen membrane (Karube et al., 1980), silanized electrodes by cross-linking with glutaraldehyde (Chemnitius and Bilitewsk, 1996) and chemically modified carbon electrodes (Alonso-Lomillo et al., 2010). Some studies were devoted to biosensors developed employing tyrosinase, able to detect catecholamines from food samples (Apetrei and Apetrei, 2013a; Apetrei and Apetrei, 2013b). Screen-printing technology offers design flexibility, process automatization, good reproducibility in the transducers fabrication and low cost (Li et al., 2012).

Since the discovery of carbon nanotubes, significant efforts were made to develop applications of this novel materials in sensing applications (Iijima, 1991). Carbon nanotubes provide a large surface area for higher enzyme loading and a biocompatible environment that helps enzymes to preserve their biocatalytic properties. Furthermore, such materials promote electron transfer reactions involving enzymes (Rivas et al., 2009).

Enzymes are macromolecules with redox centres deeply embedded within their structures. Therefore, it is very difficult for the enzymes to exchange electrons directly with the electrode surface (Luong et al., 2004). However, carbon nanotubes can form a network and project outward from the electrode, acting like bundled ultramicroelectrodes that permit access to the active sites of the enzymes, facilitating direct electron transfer (Anthony et al., 2002).

The most frequent biogenic amines in fish and seafood associated with spoilage are histamine, tyramine, putrescine and cadaverine (Visciano et al., 2012). They are formed by bacteria naturally present in decomposed fish that decarboxylate the corresponding free amino acids (Önal et al., 2013). The reason for the monitoring of tyramine in fish and fish products is double: as indices of spoilage and to prevent potential toxicity on human health (Apetrei et al., 2013; Yüksel Gença et al., 2013).

The present work is aimed to develop an improved amperometric tyramine biosensor by immobilizing of tyrosinase on carboxyl functionalised carbon nanotubes thick film of screen-printed electrodes for detection of tyramine level in different fish products. To achieve this aim, tyrosinase was immobilized onto carboxyl functionalised carbon nanotubes thick film of screen-printed electrodes by casting method followed by cross-linking with glutaraldehyde. The electrochemical performances of the biosensor were investigated and optimized by cyclic voltammetry and amperometry. The biosensor characteristics including enzymatic kinetics, influence of scan rate, calibration curve and limit of detection in the detection of tyramine were investigated. Finally, the biosensor was employed to quantify tyramine amount in pickled and smoked fish samples.

2. Material and methods

2.1. Chemical and solutions

All reagents were of high purity and used without further purification. The reagents used, tyramine, putrescine, histamine, tyrosine, glutathione, catechol, phenol and $CaCl_2$ were purchased from Sigma–Aldrich. The enzyme, tyrosinase (EC 1.14.18.1, from mushroom) was purchased from Sigma. A 5 mg $\times$ mL^{-1} solution of tyrosinase (Ty) in buffer phosphate solution (0.01 M, pH = 7) was used for the enzyme immobilization. The phosphate buffer solution (PBS) was prepared from sodium phosphate monobasic (NaH_2PO_4) and sodium phosphate dibasic (Na_2HPO_4) salts from Aldrich. Water purified with a Milli-Q system (Millipore Milli-Q, Badford, MA, USA) was used for preparation of all aqueous solutions.

2.2. Apparatus

Voltammetric and amperometric measurements were performed on a Biologic Science Instruments SP 150 potentiostat/galvanostat using the EC-Lab Express software. An Elmasonic S10H ultrasonic bath was used for dissolving and homogenization of solutions. For pH measurements an Inolab pH 7310 was used. For stirring of solutions in amperometric measurements a magnetic stirrer (Velp, Italy) was used. For separation of liquid extract containing tyramine from fish samples a centrifuge (Cencom II, JP Selecta-Spain) was used.

2.3. Electrodes and electrochemical cell

Screen-printed carbon electrodes (4 mm diameter, S = 12.56 mm^2) purchased from Dropsens (110SWCNT) were used for biosensor construction. These disposable screen-printed carbon electrodes modified with carboxyl functionalised Single-Walled Carbon Nanotubes (SWCNT–COOH) are designed for the development of (bio)sensors with an enhanced electrochemical active area and enhanced electronic transfer properties (www.dropsens.com). A three-electrode configuration was used. The reference and the counter electrode integrated in the SPE were used (counter electrode – carbon, reference electrode – silver). Cyclic voltammograms were registered from −0.5 to +0.5 V (the scan started at 0 V) at a scan rate of 0.05 V $\times$ s^{-1} (except otherwise indicated).

2.4. Fabrication of biosensor (Ty–SWCNT–COOH/SPE)

5.0 mg $\times$ mL^{-1} of tyrosinase solution was prepared with 0.01 M phosphate buffer solution at pH 7.0. 50 μL of 0.01 M phosphate buffer (pH 7.0) containing 5 mg $\times$ mL^{-1} of Ty was drop coated onto 12.56 mm^2 area of SWCNT–COOH thick film, and dried at 4 °C for