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Determination of biogenic amines in oysters by capillary electrophoresis coupled with electrochemiluminescence

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ABSTRACT

Changes in the concentrations of putrescine, histamine, tyramine, phenylethylamine and spermidine were studied by the method of capillary electrophoresis coupled with electrochemiluminescence (CE-ECL) during the storage of oysters at two different temperatures (0 °C and 4 °C). The results showed that, with an increase in the storage time, spermidine and putrescine became dominant. When the oysters were stored at 0 °C, the concentration of spermidine increased from 45.6 mg/kg to 68.5 mg/kg, and that of putrescine increased from 18.6 mg/kg to 28.3 mg/kg. When the storage temperature was controlled at 4 °C, the concentration of spermidine increased from 46.7 mg/kg to 119.7 mg/kg and that of putrescine increased from 19.4 mg/kg to 136.8 mg/kg, respectively. In contrast, the histamine, tyramine and phenylethylamine levels increased slightly throughout the storage period for all of the experimental conditions.

1. Introduction

Biogenic amines (BAs) are alkaline organic compounds that are widespread in meat, alcohol and aquatic products and other foods. Small amounts of BAs are beneficial for the human body. A low level of BAs in food is not considered to be a serious risk. However, if the amount consumed is high enough, or if the normal pathways of amine catabolism are inhibited, various physiological effects, such as hypotension nausea, headaches, rashes, dizziness, cardiac palpitations and emesis, and even death, may occur (Karpas, Tilman, Gdalevsky, & Lorber, 2002; Muñoz-Atienza et al., 2011; Shalaby, 1996). BAs are also considered to be precursors of carcinogens, such as *N*-nitrosamines, and they are an indicator of food quality (Anderson, 2008). BAs are usually generated by microbial decarboxylation of specific free amino acids in fish or in shellfish tissue (Rawles, Flick, & Martin, 1996). BAs are produced at a very low level in fresh fish and their formation is related to bacterial spoilage (Özogul, & Özogul, 2006). Fish muscle is able to support the bacterial formation of a wide variety of amines that come from the decarboxylation of amino acids. Therefore, aquatic products are among the fermented foods most commonly associated with BAs poisoning, and spermidine and putrescine are thought to indicate seafood quality (Önal, 2007). To protect public health, the US Food and Drug Administration has established a guideline level of 50 mg/kg for histamine; as histamine is generally not uniformly distributed, it can be found at a level of 500 mg/kg in other parts of the fish (FDA, 1996). The European Union has established an acceptable level of 100 mg/kg of histamine for fish species of the family Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, and Scombresocidae (Commission Regulation (EC), 2005).

The determination of biogenic amines is not simple because of their structure and because they are usually present at low levels in a matrix such as wine (Vidal-Carou, Lahoz-Portolés, Bover-Cid, & Mariné-Font, 2003); therefore, various analytical methods were used to determine the BAs from aquatic products. Among those were high performance liquid chromatography (HPLC) (Yen, & Hsieh, 1991; Li et al., 2014), capillary electrophoresis (CE) (Park et al., 2010), ion chromatography (IC) (Cinquina et al., 2004; Palermo, Muscarella, Nardiello, Iammarino, & Centonze, 2013), thin-layer chromatography (TLC) (Naguib, Ayesh, & Shalaby, 1995; Romano et al., 2012) and gas chromatography (GC) (Naila, Flint, Fletcher, Bremer, & Meerdink, 2011). HPLC requires extensive sample preparation, including derivatization of the amines to form non-polar volatile compounds (the highly polaramines tend to "stick" to the column and cause "memory effects") (Callejón, Sendra, Ferrer, & Pardo, 2013).

Oysters, which belong to the categories of Mollusca, Lamellibranchia, Anisomyaria and Ostreidae, live in shallow sand and mud. Oysters are famously economic shellfish with a high nutritive

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value, quality protein and variety of amino acids. In addition, the oyster also contains a variety of physiologically active components, which have great potential value in nutrition and medicine (Yang, Lv, Zhang, Zhao, & Ma, 2013). Oysters are a filter-feeding marine organisms, so bacteria can easily invade their bodies. The abundant nutrients in oysters provide a good external environment for the growth and reproduction of microorganisms (Duperthuy et al., 2011). Therefore, the action of microorganisms becomes the main cause of oyster corruption. Amino acids are the necessary precursors for the formation of BAs. There have been few reports on BAs in oysters. In this study, a rapid, simple, sensitive CE-ECL system was used to separate and detect biogenic amines (putrescine, histamine, tyramine, phenylethylamine and spermidine) in fresh oysters. The analysis of the concentrations of BAs in various storage processes in oysters can be act as a detection method that can measure shellfish food freshness. The purpose of this study is to rapidly establish a method for the separation and determination of the BAs content in aquatic foods.

2. Materials and methods

2.1. Reagents and apparatuses

Histamine dihydrochloride, spermidine, putrescine, phenylethylamine, tyramine hydrochloride and $Ru(bpy)_3Cl_2{\cdot}6H_2O$ ($Ru(bpy)_3^{2+}$) were purchased from J&K technology Co., Ltd. An MPI-A-type capillary electrophoresis electrochemiluminescence detection system was obtained from Xi'an Ruimai Analytical Instruments Co., Ltd, including a computer numerical control (CNC) capillary electrophoresis high voltage powerpack, an electrochemical analyzer and a chemiluminescence detector. Disodium hydrogen phosphate dodecahydrate and sodium dihydrogen phosphate were purchased from Shantou Xilong Chemical Industry (Swatow, China). All other chemicals and reagents were used were of analytical grade or better. Ultrapure water prepared by a Millipore Simplicity Ultrapure water device (>18.0 MΩ cm, Millipore, Bedford, USA) was used throughout the experiment.

2.2. Sample preparation

A total of 50 fresh samples, including *Ostrea cucullata*, *Ostrea denselamellosa Lischke*, *Ostrea talienwhanensis Crosse*, etc, are commonly consumed in Xiamen city, in southeastern China. All of the samples were obtained at a local market in October. After being purchased, the samples were iced and transported to the laboratory within 5 h and then were processed according to the following method.

2.3. Measurement of biogenic amines

The samples obtained were mixed. Three copies were divided into equal groups, labelled a (fresh), b (0 °C preservation) and c (4 °C preservation) respectively. An accurately weighed 5.0 g amount of the homogenised oyster was placed into a 25 mL beaker. Next, 10 mL of 6% perchloric acid was added, and the sample was then homogenised for 1 min. Then, the mixture was centrifuged for 10 min at 4000 r/min, and the supernatant was retained. Afterward, the extracting and centrifuging steps were repeated twice. The two supernatants were combined and diluted to 50 mL with 6% perchloric acid. The same method performed to process samples b and c. The resulting solutions were stored at 4 °C in a refrigerator and filtered with a 0.22 μm cellulose acetate membrane before use.

A separation capillary column is of 50 μm id × 50 cm length was used. Prior use, the capillary was flushed sequentially with 0.1 mol/L HCl (10 min), 0.1 mol/L NaOH (10 min) and phosphate buffer (8–12 h). A three-electrode cell with a working electrode, an Ag/AgCl saturated KCl reference and a Pt wire auxiliary electrode were used for the electrochemical measurements (Proestos, & Komaitis, 2008; Bi, Foster, McCormac, & Dempsey, 2007). Every 4 h, the $Ru(bpy)_3^{2+}$ was replaced to reduce the influence of the solvent evaporation and other factors. The maximal voltage of the photomultiplier was set to 800 V, and the capillary was inserted in buffer. The $Ru(bpy)_3^{2+}$ luminescence was detected with cyclic voltammetry (Jiang, Ding, & Sun, 2004; Wang, Feng, & Cai, 2009). After the luminescent signal baseline stabilized, the sample was injected.

2.4. Experimental contents

Optimization conditions: In this work, the conditions that were affected the detection were investigated, including the detection potential, concentration of $Ru(bpy)_3^{2+}$, injection conditions (injection potential and injection time), applied voltage and running buffer pH and concentration.

Accuracy of the method: The recovery test, peak height and migration time relative standard deviation (RSD) were used for verification of the accuracy of the method.

2.5. Storage experiments

The oyster sample was chosen as the sample for the storage experiment. The oyster sample was divided into two portions that were separately stored at 0 °C and 4 °C to study the changes in the BAs levels during the storage period. The levels of BAs were measured at 0, 1, 2, 4, and 8 days. Each sample was investigated in triplicate.

2.6. Statistical analysis

All of the measurements were performed in triplicate. The mean values and the standard deviation were subjected to one-way analysis of variance (ANOVA) using the SPSS Version 17.0 for windows.

3. Results

3.1. Optimization of the detection conditions

3.1.1. Detection potential

The electrochemiluminescence (ECL) intensity of the analytes in a capillary electrophoresis- electrochemiluminescence (CE-ECL) system is significantly affected by the detection potential, which provides a crucial ECL coreaction platform for the analyte and the ruthenium species. Therefore, the relationship between the ECL intensity and the detection potential was studied in the range of 1.0–1.25 V (gradient: 0.05 V). As shown in Fig. 1, the results showed that when the detection potential was lower than 1.00 V, no observable ECL signals appeared in the ECL curves. When the detection potential increased from 1.10 V to 1.15 V, ECL intensity signals for the five types of BAs increased, and then the ECL intensity signal decreased with the increased detection potential ($p < 0.05$). We note that the background signal and the noise increased along with the rise in the detection potential, and so the sensitivity of detection is also be affected. By comparing the signal-to-noise ratio (S/N), 1.15 V was chosen as the optimal potential.

3.1.2. Concentration of $Ru(bpy)_3^{2+}$

The mechanism of the $Ru(bpy)_3^{2+}$ ECL system in solution has been proposed as an oxidative-reductive process (Gao, Xiang, Xu,