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RESEARCH PAPER

Determination of Biogenic Amines in Cheese by On-line Solid Phase Extraction Coupled with Capillary High Performance Liquid Chromatography

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Abstract: An on-line solid phase extraction coupled to capillary HPLC method was established for the simultaneous determination of fifteen kinds of biologic amines in cheese. The biogenic amines were concentrated using the solid phase extraction column and transferred by the six-way valve to analytical column for separation and detection. The separation conditions, including gradient elution, pH, flow rate and column temperature on capillary HPLC, composition of on-line SPE mobile phase, pH of the sample solution and switching time of six-way switching valve were optimized. Optimum on-line SPE conditions including 5% of the acetonitrile-water as mobile phase for SPE column, pH 11 of the sample solution and 3 min of valve switching time were chosen in this method. The linear ranges of standard curve for fifteen biogenic amines were 0.25–50.0 mg L^{-1}; LOD was within the range of 0.05–0.25 mg L^{-1} and LOQ was in the range of 0.15–0.80 mg L^{-1}. In the spike and recovery study, four types of cheese were investigated. The recoveries for fifteen biogenic amines on four kinds of cheese ranged were from 79.6% to 118.7% except methylamine, ethylamine, 3-methylbutanamine and 5-hydroxytryptamine; with RSDs from 0.3% to 14.9%, except 3-methylbutanamine and 5-hydroxytryptamine. The method is accurate and reliable for detecting biogenic amines in cheese.

Key Words: On-line solid phase extraction; Capillary high performance liquid chromatography; Biogenic amines; Cheese

1 Introduction

Biogenic amines are a group of basic organic compounds containing nitrogen with low molecular weight, and also indispensable active ingredient in biological living cells[1]. They are widely found in various types of foods, especially protein-rich foods and fermented foods. However, when excessive intake, biogenic amines could damage human's nervous system and cardiovascular system, even cause death. Histamine is the most toxic one in these compounds. Diamine oxidase and histamine methyltransferase enzyme can metabolize histamine in animal body, while putrescine and cadaverine can inhibit the metabolism of these two enzymes, thereby enhancing the toxicity of histamine, besides that, these two amines can react with nitrite nitrosamines, which is potentially carcinogenic[2]. Biogenic amines are produced by decarboxylase that is generated via microorganisms degrade amino acid[3], so the level of biogenic amines is related to microbial contamination and they become the index of food freshness. Cheese is rich of amino acids, if there is spoilage microbial contamination in the long-term fermentation process, it would produce a large number of biogenic amines[4], causing security risk. The development of a simple, rapid and accurate detection of biogenic amines is of importance due to the risks of biogenic amines and potential index of food freshness[5,6].

Currently, biogenic amines can be determined in food by enzyme-linked immunosorbent (ELISA)[7–10], high performance

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liquid chromatography (HPLC)[11–13], liquid chromatography-mass spectrometry(LC-MS)[14,15], gas chromatography-mass spectrometry combined with (GC-MS)[16–18], ion chromatography[19–21], thin layer chromatography (TLC)[22–24] and electrophoresis[25,26] and so on. HPLC coupling with enrichment and purifying method of liquid/liquid extraction (LLE) or off-line solid phase extraction (SPE) is a widely used method for the determination of biogenic amines[27–29]. However, the method needs manual operation and some disadvantages were observed such as poor reproducibility, longer-time spending, inefficiency of sample preparation, and high cost due to the use of disposable SPE column. Zhang *et al*[30] determined 6 biogenic amines by off-line SPE with HPLC in cheese. Sun *et al*[31] established HPLC method to determine five biogenic amines in cheese. In this work a novel method based on on-line solid phase extraction coupled to capillary high performance liquid chromatography was developed for the simultaneous separation and determination of 15 kinds of biogenic amines in cheese under optimal chromatographic conditions. By using an on-line SPE purification technique, this method significantly reduced the matrix effect of samples, simplified sample pretreatment, reduced manual error, as well as greatly improved the efficiency of the analysis. This method was successfully applied to the determination of biogenic amines in cheese.

2 Experimental

2.1 Instruments and reagents

Biogenic amines were analyzed by Agilent 1200 Series capillary liquid chromatograph equipped with two dual pump, autosampler, column oven, six-way valve, DAD detector (Agilent, USA). Zorbax SB- C18 column (35 mm × 0.5 mm, 5 μm) worked as SPE column and Zorbax SB- C18 column (150 mm × 0.5 mm, 5 μm) worked as analytical column were purchased from Agilent Technologies. UC-6200 Ultrasound (USA Ameritech Co.), 5804R centrifuge (Germany Eppendorf Co.), IKA MS3Vortex meter (Germany IKA Co.) and MA-4 ACE broken homogenizer (Japan Nihonseiki Kaisha company) were used for sample preparation.

Methylamine hydrochloride, ethylamine hydrochloride, tryptamine, butylamine, phenylethylamine, 3-methylbutanamine, amylamine, putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, octopamine, 5-hydroxy-tryptamine hydrochloride, tyramine, spermidine, spermine, 1,7-diaminoheptane and dansyl chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, Germany).

2.2 Preparation of standard solution and derived reagents solution

A variety of biogenic amines of 10.00 mg were dissolved in 0.1 M HCl volume to 10 mL to obtain 1000 mg L^{-1} of biogenic amine stock standard solutions. The standard solutions were stored at 4 °C in dark and would be diluted to the standard working solution and the mixed standard working solution with 0.1 M HCl for the further use.

Dansyl chloride solution at concentration level of 5 mg mL^{-1} was freshly prepared by dissolving 250.00 mg of dansyl chloride in 50 mL of acetone.

2.3 Sample pretreatment

5.00 g of cheese was crushed together by broken homogenizer, and 200 μL of 1,7-diaminoheptane (100 mg kg^{-1}) was added as an internal standard. After addition of 10 mL of methanol, the obtained mixture above was suffered from vortex oscillation for 1 min, ultrasound treatment for 30 min, and centrifugation at 10000 *g* for 10 min at 4 °C. The supernatant was collected and the residue was re-extracted. The two supernatants were combined and evaporated to dry under reduced pressure at 40 °C. Finally, it was dissolved with 1 mL of 0.1 M HCl for derivatization.

2.4 Derivatization of BAs

0.2 mL of NaOH (2 M), 0.3 mL of saturated sodium bicarbonate solution and 4 mL of dansyl chloride solution (5 mg L^{-1}) were added into a 5-mL volumetric flask carrying 1 mL of standard solution or sample for extraction. The mixture was allowed to stand at 60°C for 15 min with shaking every 5 min. After derivatization, 200 μL of 25% (m/m) ammonia were immediately added into the mixture to remove unreacted dansyl chloride. After 20 min, it was blown to 5 mL under a gentle flow of nitrogen at 40°C.

2.5 Process of on-line SPE

The analysis process based on Agilent 1200 Series capillary LC with double-binary pump systems was shown in Fig.1. When the initial position of the valve was 12, the sample was purified using on-line SPE. When the valve position was 16 by switching the six-way valve, in this case, SPE column was connected with the analytical column. The target was recoiled to the analytical column for separation and detection by mobile phase of pump 2.

2.6 Conditions for chromatographic separation

Zorbax SB-C_{18} column (35 mm ×0.5mm, 5 μm) worked as SPE column for on-line SPE with 5% acetonitrile-water as isocratic mobile phase at flow rate of 10 μL min^{-1}. Injection volume was set at 1 μL. The switching time of six-way valve is listed in Table1.