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Determination of formaldehyde in shiitake mushroom by ionic liquid-based liquid-phase microextraction coupled with liquid chromatography

Jing-fu Liu[a], Jin-feng Peng[a,b], Yu-guang Chi[a], Gui-bin Jiang[a,*]

[a] *Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, PR China*
[b] *Graduate School of Chinese Academy of Sciences, Beijing, PR China*

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Abstract

Using ionic liquid as extraction solvent and 2,4-dinitrophenylhydrazine (DNPH) as derivative agent, formaldehyde in shiitake mushroom was determined by liquid-phase microextraction coupled with high-performance liquid chromatography (HPLC). Shiitake mushroom was leached with water and filtrated, then the formaldehyde in filtrate was derivatized with DNPH and extracted simultaneously into a 10 μl drop of ionic liquid suspended on the tip of the microsyringe, and finally injected into the HPLC system for determination. The proposed procedure has a detection limit of 5 $\mu g\,l^{-1}$ formaldehyde in extraction solution, thus the mushroom sample filtrate could be diluted with a large ratio to eliminate the influence of sample matrix. The method has a relative standard deviation of 3.5% between days for 53.5 $\mu g\,l^{-1}$ formaldehyde standards. High contents of formaldehyde (119–494 $\mu g\,g^{-1}$ wet weight), which is harmful for human beings, were detected in shiitake mushroom. Therefore, strategies must be taken to prevent the accumulation and strictly control the content of formaldehyde in shiitake mushroom.

Keywords: Formaldehyde; Shiitake mushroom; Liquid-phase microextraction; Ionic liquid; HPLC

1. Introduction

Shiitake mushroom (*Lentinus edodes*), usually grows on fallen broadleaf trees, is eaten in Chinese and Japanese meals. Recently, it was much concerned that formaldehyde was detected in shiitake mushroom at 21.3–369.5 $\mu g\,g^{-1}$ level [1]. The source of formaldehyde in shiitake is not very clear at present. One possibility is that formaldehyde is used as disinfectant of air at 5 ml m^{-3} level when the seeds were inoculated into the culture medium. Another possibility is that shiitake mushroom may produce formaldehyde during its growth. Owing to the possible carcinogenic property of formaldehyde [2–4], it is of great importance to develop simple, cheap, sensitive and selective analytical methods to control the contents of formaldehyde in shiitake mushroom.

Many methods including spectrophotometry, fluorimetry, polarography, gas chromatography (GC), and high-performance liquid chromatography (HPLC) have been reported to determine formaldehyde in air, food, water and wood [5]. Most methods were developed based on reaction of formaldehyde with various reagents to form colored derivatives for spectrophotometric detection. In recent years, chromatographic methods including GC [6,7] and HPLC [9–11] have been the most frequently reported one for the determination of formaldehyde. The most commonly used sample preparation procedure for chromatographic determination of formaldehyde is based on its reaction with 2,4-dinitrophenylhydrazine (DNPH) to form the corresponding hydrazone (DNPHo), which is extracted by liquid–liquid

* Corresponding author. Tel.: +86 1062849334; fax: +86 1062923563.
E-mail address: gbjiang@mail.rcees.ac.cn (G.-b. Jiang).

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extraction [12], solid-phase extraction [11] or solid-phase microextraction [7,8]. Although the DNPH derivation method has relatively good selectivity, extraction is commonly used as sample clean-up procedure before chromatographic separation [14]. To our knowledge, the only report on the determination of formaldehyde in shiitake mushroom is the one reported by Lu et al. [1]. In that procedure, formaldehyde in shiitake sample was detected by acetylacetone photometric method after steam distillation, which is very tedious and time consuming.

The purpose of this present study is to develop a simple, sensitive and selective method for determination of formaldehyde in shiitake mushroom. Formaldehyde in shiitake was leached with water, then derivatized with DNPH and simultaneously extracted with drop-based liquid-phase microextraction (LPME), and finally determined by HPLC. 1-Octyl-3-methylimiazolium hexafluorophosphate ($[C_8MIM][PF_6]$) ionic liquid was used as extraction solvent, as it is compatible with HPLC and a large volume drop can be suspended on the tip of a microsyringe needle, which might provide high sensitivity [13,14].

2. Experimental

2.1. Reagents and solutions

Formaldehyde standard (1070 mg l^{-1}) was obtained from the National Research Center for Reference Material (Beijing, China). Working solutions were prepared daily by appropriate dilution of the stock solutions with water. Analytical-grade DNPH was obtained from Beijing Chemicals Corporation (Beijing, China) and was dissolved in acetonitrile to prepare a 30 mg l^{-1} solution. Reagents for the synthesis of $[C_8MIM][PF_6]$, including 1-methylimidazole (99%), 1-chloroctane (99%) and hexafluorophosphoric acid (60 wt.% solution in water) were obtained from Acros Organics. HPLC-grade acetonitrile was purchased from Scharlace Chemie SA (Barcelona, Spain). All the other chemicals were analytical-grade reagents (Beijing Chemicals Corporation, Beijing, China). To decrease blank, de-ionized water was double distilled with a silicon glass distilling system and was used throughout.

The pH 3.6, 4.0, 4.5, 5.0 and 5.7 acetic acid–sodium acetate (HOAc–NaOAc) buffer solutions were prepared by dissolving 0.8, 2.0, 3.2, 5.0, 10 g of $NaOAc{\cdot}3H_2O$, and 13.4, 23.4, 6.8, 3.4, 1.3 ml of 6 mol l^{-1} of HOAc in five 50 ml flasks, respectively, and diluted to volume with water.

The synthesis and physicochemical properties of 1-octyl-3-methylimiazolium hexafluorophosphate ($[C_8MIM][PF_6]$) ionic liquid were described in our previous study [13].

2.2. Extraction procedure

The LPME procedure was similar to that described in our previous study [13,14]. Briefly, 10 μl of $[C_8MIM][PF_6]$ was withdrawn into a 50 μl microsyringe (Agilent), and the microsyringe was clamped into place such that the needle of the syringe was immersed into the 5 ml sample solution held in a vial, then a 10 μl of $[C_8MIM][PF_6]$ drop was exposed to the sample by depressing the plunger and the magnetic stirrer was turned on. After stirring for the prescribed time, the $[C_8MIM][PF_6]$ drop was retracted into the microsyringe and then injected into the HPLC system for determination. To suspend a 10 μl $[C_8MIM][PF_6]$ drop, the tip of the microsyringe needle was sheathed with a 3-mm long polytetrafluoroethylene (PTFE) tube with (0.6 mm i.d. and 1.8 mm o.d.).

2.3. HPLC determination

The LC-VP (Shimadzu, Japan) liquid chromatographic instrument consists of an SCL-10Avp system controller, two LC-10ATvp pumps, and an SPD-M10Avp diode array detector (DAD) set at 352 nm. Data acquisition and process were accomplished with a Class-VP Workstation (Shimadzu, Japan). The analytical column was a 250 mm × 4.6 mm i.d. C_{18} column (Inertsil ODS-P, GL Sciences Inc., Japan, 5 μm particles). The mobile phase was a mixture of acetonitrile and water (70 + 30 (v + v)) delivered at a flow rate of 1.2 ml min^{-1}.

2.4. Sample preparation and determination

Dried shiitake mushroom samples were purchased from different shops of local market and were produced from different places of China. The obtained samples were kept in capped bottles to prevent contamination from air. Before determination, samples were cut into small pieces of about 10 mm × 2 mm × 3 mm size and mixed. The water content of shiitake mushroom was determined according to China national standard method (GB 12531–90, determination of moisture in edible fungi). Briefly samples were dried by heating at 135 ± 2 °C for 2 h, and the water content was calculated based on the sample weight before heating (W_1) and after heating (W_2): $(W_1-W_2)/W_2$.

For determining the formaldehyde content, 3.00 g of shiitake sample and 100 ml of water were added to a 250 ml flask and capped; then the mixture in flask was ultrasonicated for 30 min and kept at room temperature for 18 h; finally the mixture was filtrated with 0.45 μm micropore membrane. For extraction of the formaldehyde, 10 μl of the filtrate was added into a mixture of 0.1 ml of 30 mg l^{-1} DNPH solution and 4.9 ml of HOAc–NaOAc buffer (pH 3.6) held in a 10-ml vial and then the liquid-phase microextraction was conducted as described above.

In order to determine the spiked recovery at 415 μg g^{-1} (wet weight) spiked level, 1 ml of 1245 μg ml^{-1} formaldehyde standard solution was added into a mixture of 3.00 g shiitake sample and 99 ml of water, and then conduct the