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

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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

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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

same sample leaching procedure, LPME and determination procedure were conducted as above described.

3. Results and discussion

3.1. Derivatizing agent concentration

The optimization of derivatizing agent concentration was conducted in 20 ml of HOAc–NaOAc buffer solution (pH 5.0) spiked with formaldehyde and DNPH, and with a derivatization and extraction time of 20 min. Experiments revealed that when 1 $\mu g\,ml^{-1}$ of formaldehyde was reacted, the peak area increased with DNPH concentration in the range of 0.75–6 $mg\,l^{-1}$. Further increasing of DNPH concentration resulted in precipitation of exceeded derivatizing agent due to its limited solubility in water. However, precipitation of exceeded derivatizing agent formed at 6 $mg\,l^{-1}$ of DNPH level if 0.1 $\mu g\,ml^{-1}$ of formaldehyde was derivatized. This is because lower concentration of formaldehyde consumed less derivatizing agent. Further experiments showed that if the DNPH concentration was decreased to 0.6 $mg\,l^{-1}$, no precipitation of exceeded derivatizing agent was observed; even water blank was extracted. Therefore, 0.6 $mg\,l^{-1}$ of DNPH was adopted as derivatizing agent in the following studies.

3.2. Derivatization and extraction time

The influence of sample derivatization and extraction time was studied by using 20 ml of HOAc–NaOAc buffer solution (pH 5.0) spiked with 0.1 $\mu g\,ml^{-1}$ of formaldehyde. Results shown in Fig. 1 indicate that the peak area increased with time over the range of 5–30 min. Further prolonging of derivatization and extraction time, however, resulted in decrease of peak area due to the dissolution of $[C_8MIM][PF_6]$ in sample solution. This result is in agreement with that of previous studies [13,14]. A derivatization and extraction time of 30 min was adopted in the following studies.

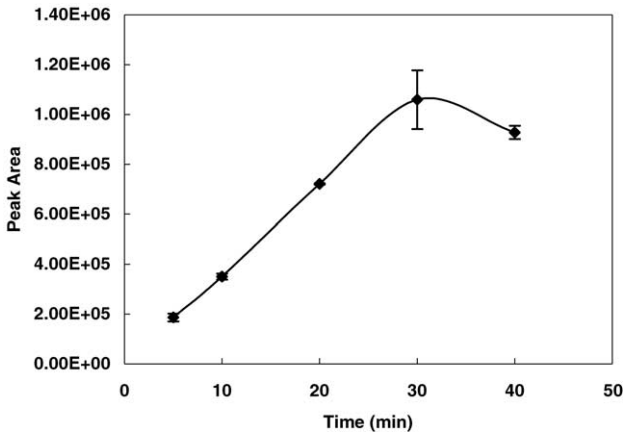

Fig. 1. Influence of derivatization and extraction time on the peak area. In 20 ml of HOAc–NaOAc buffer solution (pH = 5.0) spiked with 0.1 $\mu g\,ml^{-1}$ of formaldehyde was added 0.6 $mg\,l^{-1}$ of DNPH and extracted with 10 μl of $[C_8MIM][PF_6]$.

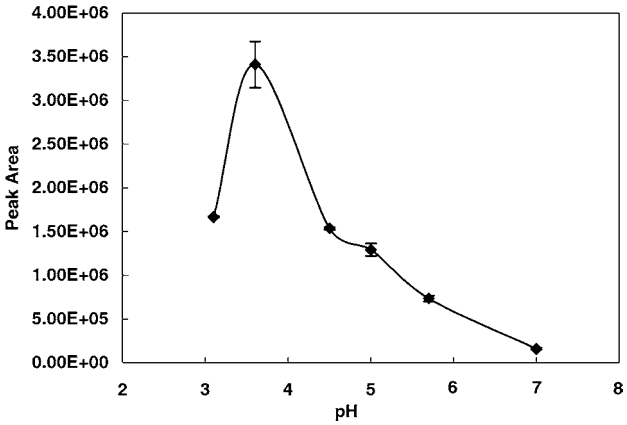

Fig. 2. Effect of pH on derivatization and extraction of formaldehyde. In 20 ml of different buffer solutions, spiked with 0.1 $\mu g\,ml^{-1}$ of formaldehyde and 0.6 $mg\,l^{-1}$ of DNPH, and extracted with 10 μl of $[C_8MIM][PF_6]$ for 30 min.

3.3. Derivatization and extraction pH

To investigate the effect of sample pH on derivatization and extraction, a series of HOAc–NaOAc buffers in the range of pH 3.6–5.7 was prepared as described in the part of reagents and solutions. Derivatization and extraction at pH 3.1 and 7.0 was performed by using 0.1 $mol\,l^{-1}$ HOAc–NaOAc buffer and water adjusted to the expected pH, respectively. Results shown in Fig. 2 demonstrated that the influence of pH was significant and the highest peak area was obtained at pH 3.6, which was not in agreement with those reported elsewhere [11]. In the following studies, a pH 3.6 HOAc–NaOAc buffer was selected as optimum.

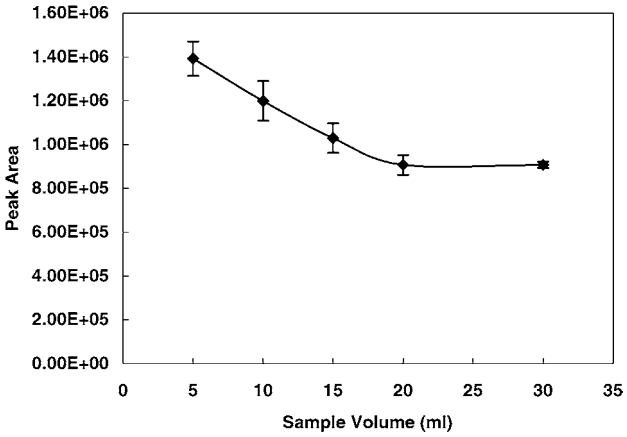

Fig. 3. Influence of sample volume on the peak area. In different volume of HOAc–NaOAc buffer solution (pH = 3.6) spiked with 0.1 $\mu g\,ml^{-1}$ of formaldehyde and 0.6 $mg\,l^{-1}$ of DNPH, and then extracted with 10 μl $[C_8MIM][PF_6]$.

3.4. Sample volume

Sample volume has distinct influence on the method sensitivity. This is because [C_8MIM][PF_6] has relatively large solubility in water, and thus less residual [C_8MIM][PF_6] drop could be injected into the HPLC system for detection with the increase of sample volume [13,14]. In this present study, the peak area was studied in the range of 5–30 ml. Results shown in Fig. 3 indicated that with the increase of sample volume, the peak area decreased significantly from 5 to 20 ml and then decreased very slowly in the range of 20–30 ml. As lower sample volume resulted in inconvenient operation for LPME, a sample volume of 5 ml was adopted in the following studies.

3.5. Analytical performance

Some characteristics of the proposed method were investigated under the above-optimized conditions by using HOAc–NaOAc buffer (pH 3.6) spiked with different concentrations of formaldehyde. The correlation coefficient (R^2) obtained by determining 10 standards covering the linear range of 1.1–856 $\mu g\,l^{-1}$ was 0.9998.

Experiment showed that a small formaldehyde signal was present behind the large reagent peak in the reagent blank chromatogram. This phenomenon was also reported by Oliva-Teles et al. [11]. Many strategies were tried to eliminate this blank signal without success. The pure water was double distilled, and the concentration and volume of DNPH were also varied to give the minimum background peak while providing enough reagents to ensure obtaining linear calibration graph over the studied range. The limit of detection and the precision were determined by measuring five blanks. The detection limit calculated based on three times of the standard deviation divided by the slope of the calibration curve (S/N = 3) was 5 $\mu g\,l^{-1}$, and the precision (R.S.D, n = 5) was 7.2%. The precision (R.S.D, n = 5) between days of the proposed procedure, determined by five successive detections of a HOAc–NaOAc buffer (pH 3.6) spiked with 53.5 $\mu g\,l^{-1}$ formaldehyde, was 3.5%, which demonstrated that the proposed procedure had very good reproducibility.

Table 1
Formaldehyde content in shiitake mushroom (mean ± S.D., n = 3) and the spiked recoveries (mean ± S.D., n = 3)

Sample	Water content (%)	Formaldehyde content ($\mu g\,g^{-1}$ wet weight)		Recovery (%)
		Added	Found	
1	12.8	0	355 ± 15	
		415	687 ± 17	80 ± 4
2	11.1	0	494 ± 24	
		415	917 ± 50	102 ± 12
3	11.7	0	332 ± 8	
		415	697 ± 25	88 ± 6
4	12.4	0	119 ± 6	
		415	501 ± 12	92 ± 3

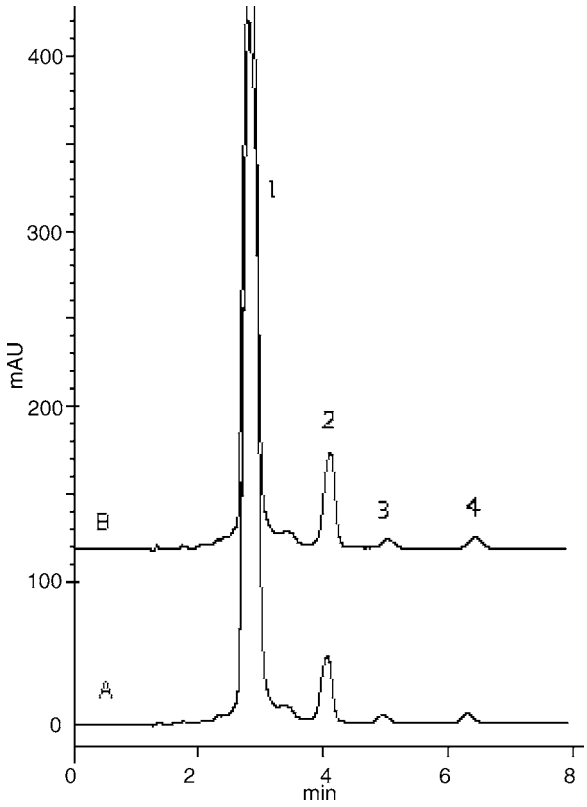

Fig. 4. Typical chromatograms of water blank and shiitake mushroom. (A) shiitake mushroom; (B) shiitake mushroom sample spiked with formaldehyde standard. Peaks identified as: (1) DNPH; (2) formaldehyde-DNPHo; (3) and (4) unknown substance.

3.6. Sample analysis

The proposed method was applied to determine formaldehyde in dried shiitake mushroom with determined water contents as shown in Table 1. Results shown in Table 1 indicate that the contents of formaldehyde in shiitake mushroom were in the range of 119–494 $\mu g\,g^{-1}$ wet weight, which is in agreement with that reported in reference [1]. The recoveries were in the range of 80–102%. These satisfactory recoveries obtained with such a simple sample pretreatment procedure should owe to the high sensitivity of the proposed method, i.e. the sample filtrate was determined with very large diluting ratio and the influence of sample matrix could be eliminated efficiently. Fig. 4 showed the typical chromatograms of a shiitake mushroom sample obtained without and with spiking 415 $\mu g\,g^{-1}$ formaldehyde standard before leaching with water. Experiments showed that the derivative agent peak obtained after spiking standard was significantly lower than that before spiking. This is because more derivative agent was consumed when formaldehyde standard was spiked into the sample.

4. Conclusions

A simple sample preparation procedure, based on LPME by using ionic liquid as extraction solvent and DNPH as derivative agent, coupled with HPLC was proposed for the determination of formaldehyde in shiitake mushroom. As large drop volume (10 μl) of ionic liquid could be suspended on the tip of the microsyringe for extraction, this procedure was very sensitive with a detection limit as low as to 5 $\mu g\,l^{-1}$ in the extraction solution. This high sensitivity benefits the simple sample pretreatment for determination of formaldehyde in shiitake mushroom as the influence of sample matrix could be eliminated with a large ratio dilution of sample filtrate, while the existed method need a tedious steam distillation procedure. The method precision was satisfactory with relative standard derivatives of 7.2% in day for blank solution, and 3.5% between days for 53.5 $\mu g\,l^{-1}$ formaldehyde standards. The formaldehyde contents in shiitake mushroom (119–494 $\mu g\,g^{-1}$ wet weight) are high enough to affect the health of consumers thus, it is necessary to investigate the source of formaldehyde and develop appropriate strategies to prevent the accumulation and strictly control the content of formaldehyde in shiitake mushroom.

Acknowledgements

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