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Note

High-performance liquid chromatographic determination of cyclic sulfur compounds of Shiitake mushroom (*Lentinus edodes* Sing.)

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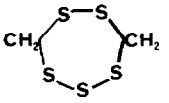
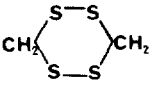
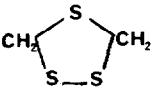
Shiitake (*Lentinus edodes* Sing.) is an edible mushroom highly prized in China and Japan. Sulfur compounds, especially cyclic ones, are flavor impact compounds of Shiitake mushroom. Lenthionine (I) (1,2,3,5,6-pentathiepane, $C_2H_4S_5$) is a cyclic sulfur compound known to possess the characteristic aroma of Shiitake[1–3]. 1,2,4,5-Tetrathiane (II) ($C_2H_4S_4$) and 1,2,4-trithiolane (III) ($C_2H_4S_3$) are two other major cyclic sulfur compounds also reported to be present in the volatiles of Shiitake mushroom[4]. Formation of these sulfur compounds is related to the enzymic activities during drying and/or crushing of fresh mushrooms[4–8].



(I)



(II)



(III)

Determination of sulfur compounds has been invariably carried out by gas chromatography (GC)[9], and a flame photometric detector may be adopted to enhance the sensitivity[10]. However, the decomposition of sulfur compounds during GC separation has been reported frequently[9,11–13].

Until recently, in only a few studies high-performance liquid chromatography (HPLC) was used to separate volatile sulfur compounds in foods[14–16]. Owing to their extraordinarily strong odor, sulfur compounds are known to be important contributors to the flavor quality of many kinds of foods. In the present paper, we report the use of reversed-phase (RP-18) HPLC in analyzing three cyclic sulfur compounds (lenthionine, 1,2,4-trithiolane and 1,2,4,5-tetrathiane) with the characteristic aroma of Shiitake mushrooms and the effect of pH on the formation of these sulfur compounds.

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EXPERIMENTAL

Sample preparation

Fresh Shiitake mushrooms (*Lentinus edodes* Sing.) were obtained daily from a local cultivation house near Hsinchu, Taiwan. The procedure of extraction of the sulfur fraction is shown in Fig. 1. Fresh mushrooms (100 g) were blended with distilled water (500 ml) for 3 min by a Waring blendor, the pH during blending was adjusted by adding 0.1 *M* sodium hydroxide or 0.1 *M* hydrochloric acid solution. Celite (50 g, Wako, Japan) was added to aid the separation of solid and liquid fractions. Both fractions were then extracted thrice by chloroform (E. Merck), combined, and concentrated to minimal volume at reduced pressure. A control experiment was conducted by blending fresh mushrooms directly with chloroform in order to inactivate the enzymic activities.

The sulfur fraction was eluted by *n*-hexane–diethyl ether (9:1) (300 ml, glass-distilled, E. Merck) on a glass column (50 cm × 1.5 cm I.D.) packed with silica gel

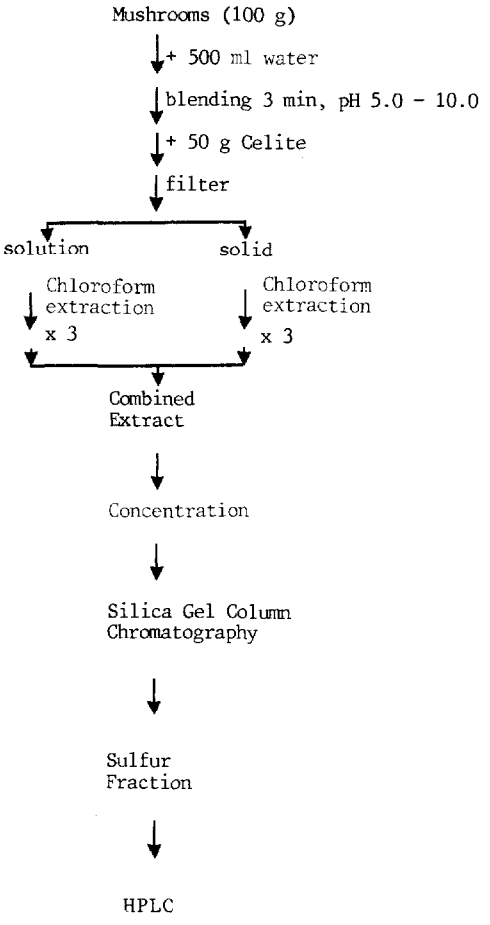

Fig. 1. Scheme of extraction of sulfur compounds from Shiitake mushroom.

(50 g, C-200, Wako, Japan). Dipropyl disulfide (2.2 mg, Wako) was added as internal standard.

Chemical syntheses

Lenthionine was synthesized by the method of Morita and Kobayashi[1,2]. Sodium sulfide (E. Merck), sulfur (E. Merck) and formaldehyde (E. Merck) were used as starting materials. 1,2,4-Trithiolane was synthesized according to the method of Gil and MacLeod[17]. 1,2,4,5-Tetrathiane was obtained by eluting the sulfur fraction on a reversed-phase thin-layer plate (20 × 20 cm, RP-18, F-254, E. Merck), with a mobile phase consisting of methanol–water (90:10); the fraction with R_F 0.43–0.50 was isolated.

Analytical HPLC

A Hewlett-Packard (Palo Alto, CA, U.S.A.) 1084B HPLC system was used, which was equipped with two pumps, an adjustable auto-injector, a variable-wavelength UV detector, a built-in integrator and a reversed-phase column (RP-18, 20 cm × 4.6 mm I.D., 5 μm, Hewlett-Packard). A linear gradient from methanol–water (65:35, HPLC grade, E. Merck) to 100% methanol was used. The time of each analysis was 70 min with gradient elution during the first 50 min. The flow-rate of the mobile phase was 1 ml/min. Detection was based on UV absorption at 254 nm.

Gas chromatography–mass spectrometry (GC–MS)

Identification of lenthionine, 1,2,4-trithiolane and 1,2,4,5-tetrathiane was performed on a Hewlett-Packard 5985B GC–MS system. A Hewlett-Packard 5840A gas chromatograph equipped with a fused-silica capillary column (50 m × 0.22 mm I.D., CP-SIL 5 CB, equivalent to OV-1, Chrompack) was connected directly to the mass spectrometer. The operating conditions were as follows: injector temperature, 250°C; temperature program, 50°C–280°C at 4°C/min and held at 280°C for 50 min; carrier gas (helium) velocity, 14 cm/s; temperature of the ion source and all connection parts, 200°C; electron energy, 70 eV; electron multiplier voltage, 2600 V.

RESULTS AND DISCUSSION

Fig. 2 shows the HPLC chromatograms of the sulfur fraction of Shiitake mushroom formed (A) at pH 9.0 and (B) in control samples. More than 30 peaks were detected; however, only four peaks were selected for identification, that is, 1,2,4-trithiolane, 1,2,4,5-tetrathiane, lenthionine and dipropyl disulfide (internal standard). The retention of these sulfur compounds on the reversed-phase column agreed well with their polarity. The significant differences between samples A and B are due to the enzymic activities as reported previously[4].

Fig. 3 shows the effect of pH (5.0–10.0, 1.0 unit per interval) on the formation of sulfur compounds identified in this study. The quantitation was estimated by using dipropyl disulfide as internal standard. Formation of these sulfur compounds is favored around pH 9.0, which is consistent with previous findings[5–7].

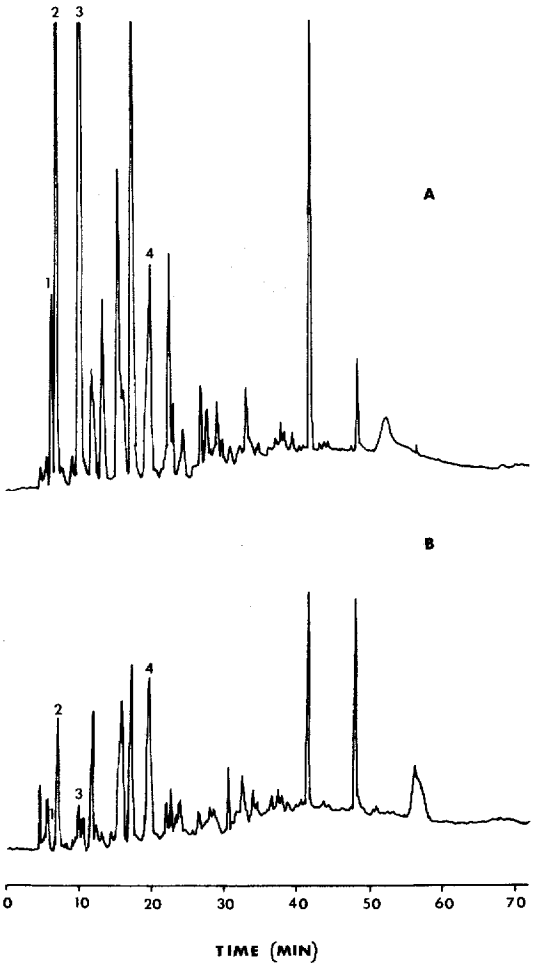

Fig. 2. HPLC separation of sulfur compounds of Shiitake mushroom. (A) Sulfur compounds extracted at pH 9.0; (B) sulfur compounds extracted under control conditions. Peaks: 1 = 1,2,4-trithiolane; 2 = 1,2,4,5-tetrathiane; 3 = lenthionine; 4 = dipropyl disulfide (internal standard).

CONCLUSION

The results indicate that reversed-phase HPLC is a good alternative for analyzing sulfur compounds in foods, especially when the sulfur compounds are thermolabile. The coupling of HPLC with MS is useful in identifying unknown compounds, such as those detected in this study.

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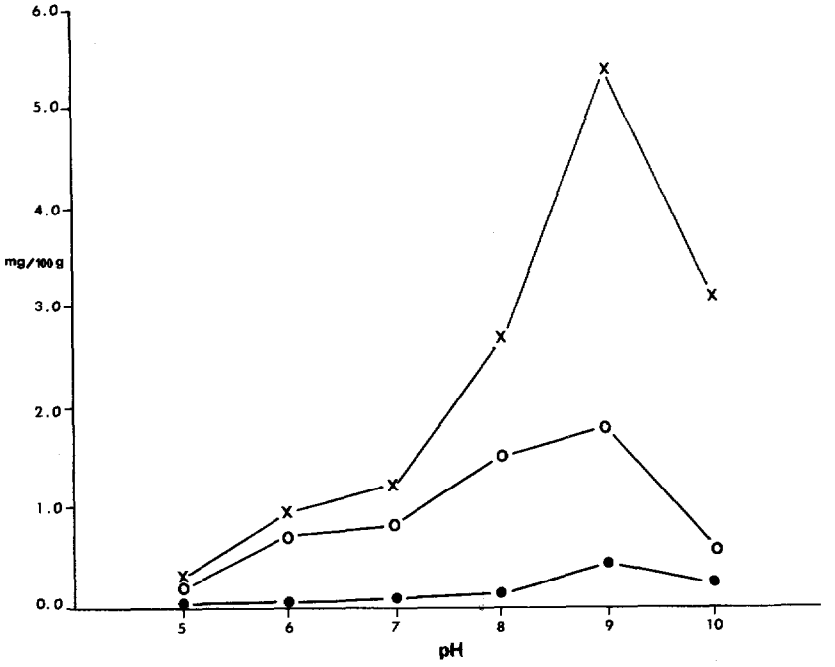

Fig. 3. Effect of pH on the formation of three sulfur compounds detected by HPLC. Values obtained are the average of three determinations, and are expressed in mg per 100 g of mushrooms. ×—×, Lenthionine; ○—○, 1,2,4,5-tetrathiane; ●—●, 1,2,4-trithiolane.

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