A GC–MS Study of the Volatile Organic Composition of Straw and Oyster Mushrooms During Maturity and its Relation to Antioxidant Activity

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Abstract

Mushrooms are very popular in the market for their nutritional and medicinal use. Mushroom volatiles are not only an important factor in the flavor, but also contain many antioxidant compounds. Antioxidant activity is a very important property for disease prevention. The volatile compositional characteristics of straw mushrooms (*Volvariella volvacea* [Bull. ex Fr.] Sing.) and oyster mushrooms (*Pleurotus ostreatus* [Jacq. ex Fr.] Kummer) during maturity and the mushroom antioxidant activity related to the non-volatiles and volatiles are studied by a chromatographic method in combination with a spectrophotometric method. The volatile compounds of straw and oyster mushrooms are sampled and identified by a combination sampling method, including headspace solid phase microextraction and steam distillation, followed by gas chromatography–mass spectrometry detection. Among all the volatile compounds identified, 1-octen-3-ol and 3-octanone are the two main compounds with the highest amounts in the volatile compositions of straw and oyster mushrooms. During maturity time of the straw mushrooms, the unsaturated 1-octen-3-ol peak area is reduced, whereas the saturated 3-octanone peak area is increased. However, during normal maturity time of oyster mushrooms, the peak areas of 1-octen-3-ol and 3-octanone remain at the same level. 1-Octen-3-ol has a different antioxidant activity from 3-octanone. Combining the results of antioxidant experiments of water extract and main volatile components by the use of a phosphomolybdenum spectrophotometric method, the conclusion is drawn that oyster mushrooms might possess stronger antioxidant activities than straw mushrooms.

Introduction

Mushrooms are very popular in the market for their nutritional and medical use. Mushrooms have especially been widely used as a food or food flavoring material for their unique and subtle flavor. Flavor studies of mushrooms have been of increasing interest (1–9). The substantial base of mushroom volatiles is a series of C8 compounds, especially 1-octen-3-ol (10). The tentative metabolism process of 1-octen-3-ol and 3-octanone has been described (11–13). They could be considered as biologically active compounds, which are not only an important factor in mushroom flavor but also contain crucial bio-information related to antioxidant activity. Mushrooms are recognized as a nutritious food as well as an important source of biologically active compounds (14–18) such as phenolic compounds, terpenes, etc., which usually possess antioxidant activities. Different pathways and enzyme catalyses result in different volatile compositions at different stages of maturity (3). However, there are still few reports focusing on the volatile compositional characteristics of straw and oyster mushrooms related to antioxidant activity.

Straw mushrooms (*Volvariella volvacea* [Bull. ex Fr.] Sing.) and oyster mushrooms (*Pleurotus ostreatus* [Jacq. ex Fr.] Kummer) are widely cultivated in China and frequently used in Chinese cuisine. Many studies of these edible fungi volatiles focus on qualification and quantification analyses but not the potential bio-information. Antioxidant activity related to volatile compositional characteristics of mushrooms, as an important bio-information (11–13), affects the mushroom consumption. Thus, systematical and statistical methods should be established to study mushroom volatile compositional characteristics and the related antioxidant activity, including efficient sampling technique and sensitive detection. The modern chromatographic methodologies could effectively interpret the volatile compositional characteristics. Gas chromatography–mass spectrometry (GC–MS) is a very powerful technique for the separation and detection of volatile organic compounds. Therefore, GC–MS proved suitable for the interpretation of the volatile compositional characteristics of the mushrooms. The early works for the study of antioxidant activity of plant extract were carried out by Chipault and other coworkers (19–21), which affected many post-studies of antioxidant activity of plant extracts, with many potential applications. To date, there are many methods commonly used for measuring total antioxidant activity, such as oxygen radical absorbance capacity (ORAC) assay (22), ferric reducing antioxidant power (FRAP) assay (23), and the phosphomolybdenum spectrophotometric method (24). Compared with FRAP

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and ORAC assays, the phosphomolybdenum spectrophotometric method is simple and applied widely to measure the total antioxidant capacity of plant extract. This method is based on the ability of the antioxidants to reduce Mo (XI) to Mo (IX), which could form the phosphomolybdenum complex.

Conventional volatile sampling techniques for mushroom volatile compounds include solvent extraction (7,8) and simultaneous distillation extraction (SDE) (10,13,15). These methods require long extraction time, large amounts of solvents, and multiple steps. Moreover, many unstable volatile compounds would be thermally decomposed and degraded during thermal extraction or distillation (25). However, due to the relative simplicity and straightforwardness of SDE and solvent extraction, they are still extensively applied to fragrance-and-aroma characterization procedures, either alone or combined with other sample-preparation procedures (26). The diffusive sampling method is a solvent-saving method for sampling fungus volatiles (27), but the long sampling time makes it unsuitable for the rapid analysis. Solid-phase microextraction (SPME), developed by Pawliszyn and his co-workers (28,29), is a simple and solvent-saving sampling method. SPME has been widely used in environmental (30), biological (31), pharmaceutical (32) and field analyses (33), and fragrance-and-aroma studies (26,34). Headspace is a very important mode of SPME. During HS-SPME, the fiber is exposed in the headspace phase to extract the volatile compounds, but not directly inserted into the matrix. Therefore, HS-SPME has been considered a suitable sampling method for sample preparation in fragrance-and-aroma studies (26,34). In recent years, several applications of the analysis of mushroom volatile compounds by HS-SPME (11,12,14) have been published. The combination of HS-SPME and conventional sampling methods has also been used in the study of plant volatile compositional characteristics (35,36), because it can provide as much helpful volatile information as possible.

The overall goal of this study was to develop a systematical and statistical method for studying the volatile compositional characteristics of straw and oyster mushrooms during maturity and to compare their antioxidant activities. An HS-SPME method in combination with steam distillation (SD) was established to sample volatile compounds from straw and oyster mushrooms at different maturity stages, followed by GC–MS detection. Then the chromatographic method was used to monitor the changing trends of the volatile compositional characteristics of straw and oyster mushrooms during maturity. In order to study the antioxidant activity of straw and oyster mushrooms related to their volatiles and non-volatiles, the phosphomolybdenum spectrophotometric method was developed to measure the antioxidant activities of the water extract of straw and oyster mushrooms and the simulated essential oil made by the standard compounds of 1-octen-3-ol and 3-octanone.

Experimental

Mushroom material

Straw and oyster mushrooms were collected from the Edible Fungi Cultivating Center of Banghu County (Guangzhou, Southern China). After the fruiting body burgeoned, straw and oyster mushrooms grew commercially mature after 4 and 5 days, respectively. During the maturity time of straw mushrooms, the color of the mushroom body gradually turned from white to dark, with an increasing size of the mushroom body. However, during the maturity of oyster mushrooms, the color of the mushroom body gradually turned from grey to white, with an increasing size of the mushroom body. In order to continuously investigate the changing volatile compositional characteristics during mushroom maturity time, the sample collection was conducted on the same mushroom bed once a day in the morning until the mushrooms were grown enough for harvesting. The other immature mushrooms were left growing on the bed until they were mature. All the samples collected were selected for uniformity in size and color. Blemished or diseased samples were discarded. The fresh samples were analyzed within 12 h of collection.

HS-SPME procedure and sample preparation

The experimental conditions of HS-SPME potentially influencing the extraction process included the type of SPME fiber coating and the extraction time. Replicate measurements were performed to optimize HS-SPME conditions in the study. The type of SPME fiber coating preferred in the study was 65 mm polydimethylsiloxane-divinylbenzene (Supelco, Inc., Bellefonte, PA) due to their similar polarities to the non- or mid-polar volatile compounds. An extraction time of 45 min resulted in the best extraction efficiency and was chosen as the optimal extraction time in the work by comparing with other extraction times (15, 30, 45, 60, and 90 min).

Before being ground, the dirt on the surface of each sample was removed. Every 100 g was distributed to one sampling group randomly. Then 2.0 g samples from one group were rubbed with 4 mL deionized water in a commercial glass mortar (Guangdong Huabopi, Guangzhou). After that, 3.0 g sample tissue homogenate from the glass mortar was put into a 15-mL glass vial followed by HS-SPME exposure for 45 min at 25°C. Finally, the volatile compounds were thermally desorbed by inserting the fiber into the GC injector set at 250°C in splitless mode for 5 min. Seven duplicate samplings were performed to study the volatile compositional characteristics of the maturity samples.

SD procedure

SD is a classic sampling technique for the volatiles from complicated samples. The mobile steam carries the volatile compounds out, and then volatile compounds are desorbed and extracted by the organic solvents. In the study, during the SD procedure, 100 g fresh mushrooms were homogenized and then subjected to hydrodistillation for 120 min. Twenty-five milliliters of pentane were used to extract the obtained fraction. Then the organic phase was concentrated to 1 mL by a rotor evaporator (Shenke Instrument, Shanghai, China) at 30°C; 0.5 mL concentrated organic phases were introduced to the GC–MS for subsequent analysis.

GC–MS analysis

Analyses were carried out by use of a Hewlett-Packard (HP) 6890 GC equipped with HP 5973 mass detector system.

Chromatographic separation was performed with an HP-VOC (Agilent Scientific, Palo Alto, CA) capillary column (60 m length × 0.32 mm i.d. × 1.8 µm film thickness). Splitless injection was used. The carrier gas was ultra-pure helium at a flow of 1 mL/min. The injector and transfer line temperature were set at 250 and 280ºC, respectively. The oven temperature program was from 60ºC to 100ºC at ramp rate of 10ºC/min, 100ºC for 5 min, from 100ºC to 110ºC at ramp rate of 1ºC/min, 110ºC for 5 min, and from 110ºC to 240ºC at ramp rate of 10ºC/min. The parameters of the HP 5973 mass detector were: ion mass/charge ratio, 30–550 *m/z*; scan mode. All mass spectra were acquired in the electron impact mode (EI = 70 eV, source temperature, 230ºC)

Preliminary study for the antioxidant activities of straw and oyster mushrooms (24)

The mushroom substantial resource should include the volatile and non-volatile compounds. The corresponding water extracts of straw and oyster mushrooms were obtained for the preliminary study of their antioxidant activities related with the non-volatile compounds. Ten grams ground samples of straw and oyster mushrooms were refluxed with 150 mL deionized water in a water bath of 60ºC for 5 h. Then the water extracts of straw and oyster mushroom were placed in 250-mL volumetric flasks. For spectrophotometric determination, 1.0 mL 3.00 mol/L H_2SO_4, 0.5 mL 0.14 mol/L Na_3PO_4, 0.5 mL 0.20 mol/L $(NH_4)_6Mo_7O_{24}$, and the water extracts of straw and oyster mushrooms (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL) were added to a 10-mL color comparison tube and controlled to the constant volume of 5 mL by the use of deionized water. Then the tube was stuffed and placed in a water bath of 95ºC for 1.5 h. After that, the tube was cooled down by the cold water, and the absorption of the mixed liquid was measured in triplicate at $\lambda = 695$ nm (A_{695}) using a Vis spectrophotometer (VIS-7220G, Beijing Rayleigh Analytical Instrument Corp., China). For the further confirmation of the antioxidant activities of mushroom volatiles, the two main related volatile compounds, namely 1-octen-3-ol and 3-octanone, were mixed as the simulated essential oils according to their normalization amounts in the volatile compositions of straw and oyster mushrooms (the ratio of 1-octen-3-ol to 3-octanone in straw mushroom volatiles: 1:19.6; the ratio of 1-octen-3-ol to 3-octanone in oyster mushroom volatiles: 1.7:1). The different concentrations of the mixture of authentic compounds (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL/L) (Alfa Aesar, Ward Hill, MA) were added to the 10-mL color comparison tube with 1.0 mL 3.00 mol/L H_2SO_4, 0.5 mL 0.14 mol/L Na_3PO_4, and 0.5 mL 0.20 mol/L $(NH_4)_6Mo_7O_{24}$, and analyzed as in the previously mentioned procedures.

Results and Discussion

The volatile compositions of the straw and oyster mushrooms

The volatile compounds were identified according to the standard mass spectra of the National Institute of Standards and Technology MS spectral library. Volatile compounds were considered "tentatively identified" when their mass spectral fit values were at the default value of 85 or above. When available, some volatiles were further confirmed by comparing their retention times with those of the standards. The variability of the retention times between the volatile compounds and corresponding standards was < 0.05 min. The volatile chromatograms of straw and oyster mushrooms obtained by HS-SPME are illustrated in Figures 1 and 2. The reproducibility of volatile compositional characteristics can be evaluated from the standard deviation (n = 7) of the main volatile compounds (> 0.01% of total composition). The corresponding relative standard deviations (RSD) ranged from 1.4% to 12.0%. The fluctuation of the retention time of all the identified peaks was < 0.05 min.

Seventeen volatile compounds of straw and oyster mushrooms thermally desorbed from the SPME fiber coating were tentatively identified according to different degrees of certainty. A series of C8 compounds consisted of the major volatile compounds of straw and oyster mushrooms, which was consistent with the previous reports (6,7,15). The identified volatile compounds could be mainly divided into six groups according to the diverse functional groups, which were alkene, alkane, alcohol, aldehyde, ketone, and other organic compounds. Among these volatile compounds, alcohols, aldehydes, and ketone dominated over the main volatile components. The volatile compositions of straw and oyster mushrooms obtained by SPME were different. Straw mushrooms possessed more aldehydes and ketones but fewer alcohols than oyster mushrooms. The volatile compounds with the highest normalized amounts were benzaldehyde for straw mushrooms and 1-octen-3-ol for oyster mushrooms.

Conventional SD was used to sample the volatile compounds as a complementary method to HS-SPME. Six and eight volatile compounds, respectively, were tentatively identified from straw and oyster mushrooms. Volatile C8 compounds such as 1-octen-3-ol, 3-octanol, and 3-octanone were still made up of the main volatile compositional characteristics, which were also presented in the HS-SPME procedures. Bezeneacetaldehyde, nonanal, diethyl disulfide, and some alkanes were special volatile compounds identified in the SD procedures. The possible reason might be the thermal degradation of some unstable compounds

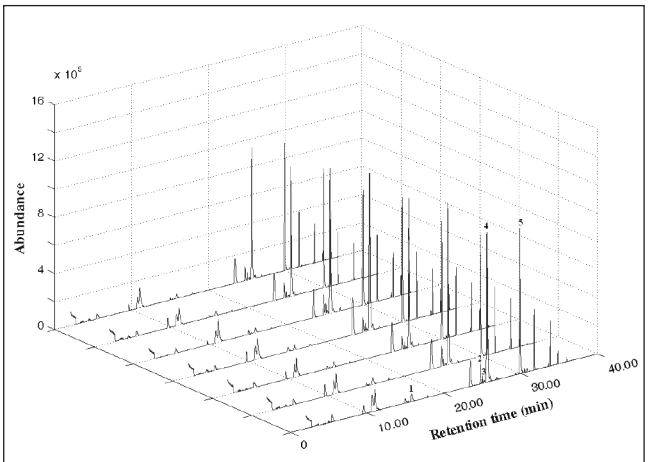

Figure 1. The reproducible volatile chromatograms of the straw mushrooms by HS-SPME. The peak numbers correspond to the typical volatiles continuously studied during the maturity of straw mushrooms. (1-Hexanol, 1; 1-Octen-3-ol, 2; Benzaldehyde, 3; 3-Octanone, 4; Benzeneacetaldehyde, 5).

during the SD procedures. The property of the sample changed greatly during the SD process because of the high temperature. On the other hand, SD was suitable for the sampling of the stable compounds such as bezeneacetaldehyde, nonanal, and diethyl disulfide, which were not sampled by HS-SPME. Although different sampling methods resulted in different sampling efficiencies for some typical volatile compounds according to their normalized amounts, the main volatile compounds were the same compounds both in the HS-SPME and SD procedures, such as 1-octen-3-ol, 3-octanol, 3-octanone, etc. Although HS-SPME could sample more species of volatile compounds than the SD methods, including alkenes, alcohols, aldehydes, and ketones, HS-SPME also lost some water-soluble components of SD. The result tentatively claimed the mutual compensation of HS-SPME and SD. Wilkes et al. summarized the sample preparation for the analysis of flavors and off-flavors in food (37). They considered that few of the methods that were available for analyses of food flavors and off-flavors could be described simultaneously as cheap, easy, and good. Therefore, combining HS-SPME and the conventional SD could obtain more qualitative and semiquantitative information of the volatile compositions of straw and oyster mushrooms during their maturity time than any single method.

The volatile compositional characteristics during mushroom maturity time

In order to obtain the original and representative volatile compositions of mushrooms, it is better to sample and analyze mushroom volatiles immediately as soon as mushrooms are delivered to the lab. The HS-SPME sampling procedure is simple and saves time, which is more suitable for monitoring the volatile compositional characteristics during mushroom maturity time than SD. After the sampling and data-processing method was established, the change of typical volatile compounds of straw and oyster mushrooms during their maturity time, isolated by SPME, were continuously investigated. After the fruiting body burgeoned, it took straw and oyster mushrooms 4 and 5 days to grow mature, respectively. Figures 3 and 4 demonstrate the changing trends of the main volatile compounds' relative amounts in straw and oyster mushrooms during their maturity. The separation of two main volatiles, 1-octen-3-ol and 3-octanone, in the volatile chromatograms of straw and oyster mushrooms obtained during maturity by HS-SPME, are also illustrated in Figures 3 and 4.

For straw mushrooms, the relative amounts of reduced volatiles normally showed reducing trends, whereas oxidizing compounds accumulated during maturity (Figure 3A). 3-Octanone, benzeneacetaldehyde, and benzaldehyde increased gradually during maturity, whereas 1-octen-3-ol reduced and 1-hexanol remained almost at the same level with a few fluctuations. The major compounds found in straw mushrooms were 1-octen-3-ol and 3-octanone, which showed the complementary changing trends during maturity. The peak area of saturated 1-octen-3-ol was significantly reduced, whereas that of 3-octanone was increased. During maturity, the color of the fruit bodies of straw mushrooms turned from white to brown, which might be caused by enzymatic browning. Enzymatic browning is one of the most important color reactions that affect fruits, vegetables, and seafood, and is also a kind of slow oxidation (38,39). During

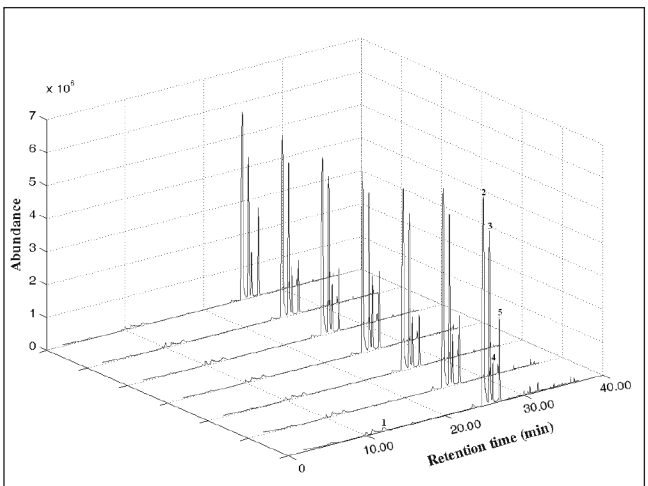

Figure 2. The reproducible volatile chromatograms of the oyster mushrooms by HS-SPME. The peak numbers correspond to the typical volatiles continuously studied during the maturity of oyster mushroom (1,3-Octadiene, 1; 1-Octen-3-ol, 2; 3-Octanone, 3; 3-Octanol, 4; Octanal, 5).

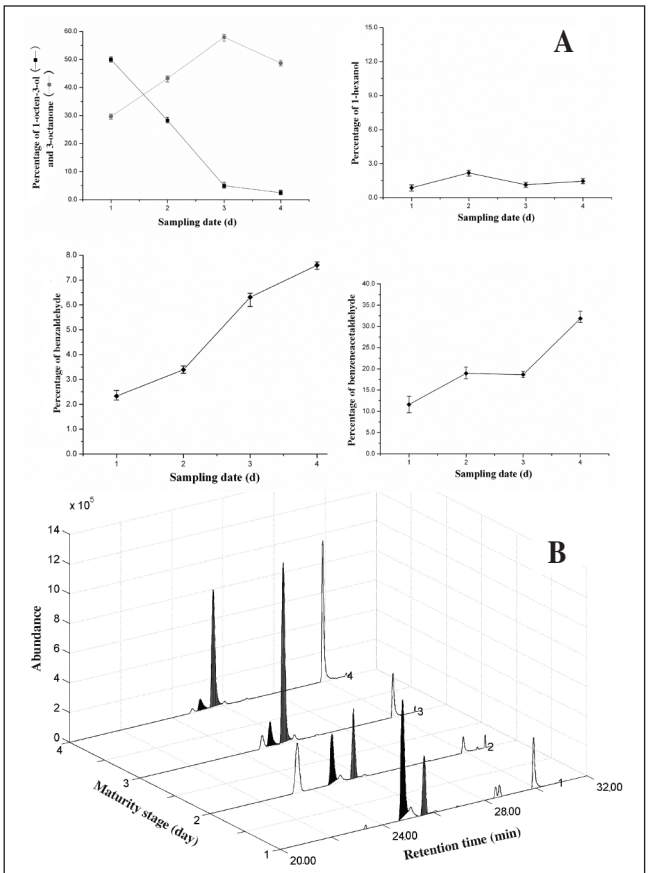

Figure 3. The changing trends of main volatile compounds of straw mushrooms obtained by HS-SPME during maturity. The percentages of typical volatile compounds in the ordinate refer to the corresponding normalized amounts (n = 3) (A). The separation of 1-octen-3-ol and 3-octanone in the volatile chromatograms of straw mushrooms during maturity by HS-SPME. The peak numbers correspond to the main volatile compounds. Black and grey peaks represent 1-octen-3-ol and 3-octanone, respectively. The numbers 1 to 4 refer to the date of mushroom maturity (B).

maturity, bezeneacetaldehyde accumulated, possibly caused by one form of enzymatic browning: Strecker Degradation, which is an interaction of an α-amino acid with a carbonyl compound in aqueous solution or suspension to give carbon dioxide and an aldehyde or ketone containing one less carbon atom. Phenylalanine could decompose to benzeneacetaldehyde via Strecker Degradation and result in the accumulation of benzeneacetaldehyde, which could tentatively suggest that the maturity of straw mushrooms was accompanied by slow oxidation. Moreover, in the previous report, the analyst also found the phenomenon that the brown variety of the cultivated mushroom *Psalliota bispora* va. avellanea produced more 1-octen-3-ol than the white variety (*Psalliota bispora* va. alba). And the amounts of 1-octen-3-ol decreased relatively fast during storage (12). The experimental result in the previous work was consistent with our work, which could also support our idea of slow oxidation during straw mushroom maturity.

For oyster mushrooms, the peak area of 3-octanol reduced gradually, whereas the peak areas of octanol and 1,3-octadiene remained at almost the same level with a little fluctuation during maturity of oyster mushrooms (Figure 4A). The relative amounts of 1-octen-3-ol and 3-octanone as the two main volatile compounds did not change greatly, although few fluctuations presented during maturity under the normal temperature (Figure 4B). 1-Octen-3-ol and 3-octanone were widespread occurrences in edible fungi, possessing different chemical structures and antioxidant activity. They were two main volatile components in the volatile compositions of straw and oyster mushroom. The significant changing trends of the relative amounts of corresponding main mushroom volatiles during their maturity time may be related with the difference of antioxidant activities of the mushroom volatile compositional characteristics.

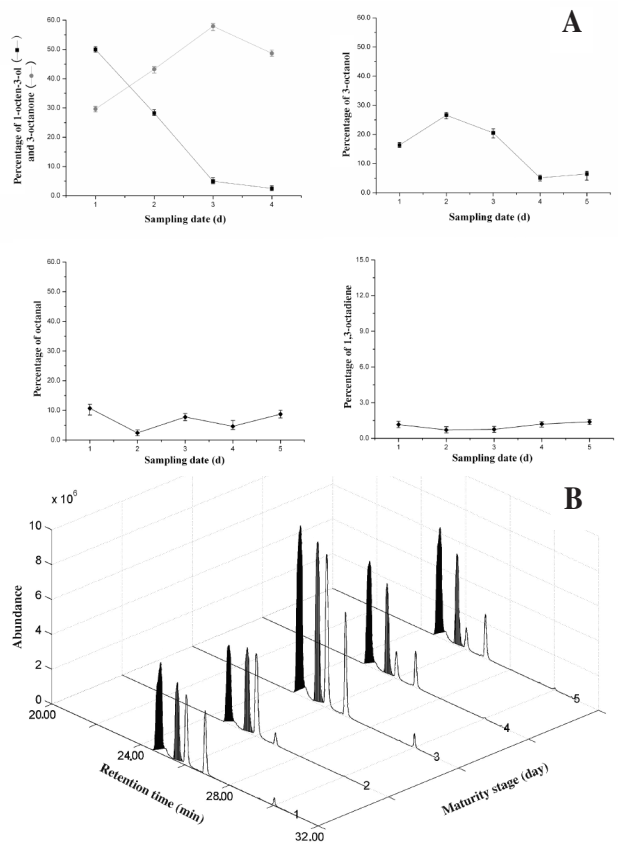

Figure 4. The changing trends of main volatile compounds of oyster mushrooms obtained by HS-SPME during maturity. The percentages of typical volatile compounds in the ordinate refer to the corresponding normalized amounts (n = 3) (A). The separation of 1-octen-3-ol and 3-octanone in the volatile chromatograms of oyster mushrooms during maturity by HSSPME. The peak numbers correspond to the main volatile compounds. Black and grey peaks represent 1-octen-3-ol and 3-octanone, respectively. The numbers 1 to 5 refer to the date of mushroom maturity (B).

The preliminary study of the antioxidant activities of these two mushrooms

In order to study the antioxidant activities of straw and oyster mushrooms, a phosphorusmolybdenum spectrophotometric method was developed for the tentative comparison of the water extract and main volatile components of these two mushrooms. In the previous work, the analyst suggested that the antioxidant activity of the mushrooms could be related to the concentrations of antioxidant compounds in the water extract (14). Thus, the method proposed in our study was based on the reduction of Mo (VI) to Mo (IV) by the water extract or the mixture of 1-octen-3-ol and 3-octanone of straw and oyster mushrooms. At acidic pH, Mo (IV) and Na_3PO_4 could form the green ion-associated complex of phosphorusmolybdenum heteropoly acid, which possesses the maximum absorption in 695 nm (A695) (22,40,41). Thus, A_{695} could tentatively mark the antioxidant activities of the water extract or the mixture of 1-octen-3-ol and 3-octanone of these two mushrooms. Beer's law was obeyed in range of the added mushroom water extract from 0.5 to 3.0 mL (Figure 5). The A_{695} of the water extract of oyster mushroom was higher than that of straw mushrooms, which suggested that the antioxidant activity of the water extract of oyster mushrooms was stronger than that of straw mushrooms. The relative amounts of 1-octen-3-ol and 3-octanone of oyster mushrooms during maturity under normal temperature remained at nearly the same level with a little fluctuation, whereas 1-octen-3-ol showed a reducing

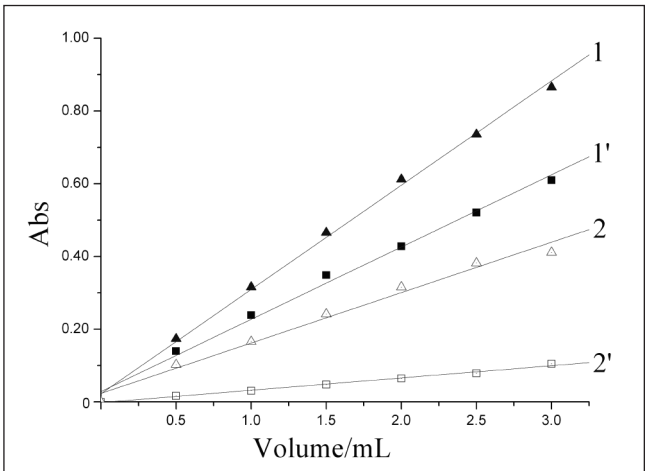

Figure 5. The antioxidant activities of the water extract of oyster (Line 1) and straw mushrooms (Line 1'), and the antioxidant activities of the mixture of 1-octen-3-ol and 3-octanone, according to the volatile compositions of oyster (Line 2) and straw mushrooms (Line 2').

trend with the accumulation of 3-octanone during straw mushroom maturity. 1-Octen-3-ol and 3-octanone were two main volatile components and could be considered related with the antioxidant activities of mushroom volatile compounds. The further conformation of the antioxidant activities of mushroom volatile compounds related with 1-octen-3-ol and 3-octanone was conducted. The A_{695} of the mixture of 1-octen-3-ol and 3-octanone according to the normalization amounts of oyster mushroom volatile composition was higher than that of straw mushrooms in the linear range of 0.5–3.0 mL/L. The tentative result suggested that the antioxidant activity of volatile compounds of oyster mushrooms might be stronger than that of straw mushrooms. The antioxidant activities of mushroom water extract and volatile compositions demonstrated the same comparison results. The mushroom substance base includes the volatile compounds and the non-volatile compounds in the water extract. Combining the antioxidant activities of mushroom water extract and volatile compositions, the conclusion could be drawn that oyster mushrooms possessed the higher antioxidant activity than straw mushrooms.

Conclusions

A preliminary method based on a combination sampling method (HS-SPME and SD) followed by GC–MS detection was developed to study the volatile compositional characteristics of straw and oyster mushrooms. Seventeen volatile compounds, respectively, of straw mushrooms and oyster mushrooms were sampled and identified, which included alkene, alkane, alcohol, aldehyde, ketone, and other organic compounds. C8 volatiles consisted of the major volatile compositions of straw and oyster mushrooms. The continuous investigation of volatile compositional characteristics during the maturity of straw and oyster mushrooms was performed to distill potential bio-information. 1-Octen-3-ol and 3-octanone were two main volatile compounds. During the maturity time of straw mushrooms, the peak area of unsaturated 1-octen-3-ol was reduced, whereas the peak area of saturated 3-octanone was increased. However, for oyster mushroom the relative amounts of 1-octen-3-ol and 3-octanone remained at nearly the same levels during maturity. The antioxidant activities of straw and oyster mushrooms were preliminarily studied by the combination of the antioxidant experimental results of their volatile compounds and non-volatile water extract using the phosphorusmolybdenum spectrophotometric method. The antioxidant activities of both the mixture of 1-octen-3-ol and 3-octanone and the water extract of oyster mushroom were higher than those of straw mushrooms. The tentative results suggested that oyster mushrooms might possess stronger antioxidant activity than straw mushrooms.

Acknowledgments

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