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Multiple headspace-solid-phase microextraction: An application to quantification of mushroom volatiles

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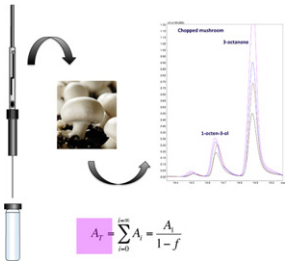
HIGHLIGHTS

- Multiple headspace extraction-solid phase microextraction (MHS-SPME) has been applied to the analysis of *Agaricus bisporus*.
- Mushroom flavor is characterized by the presence of compounds with a 8-carbon atoms skeleton.
- Formation of 8-carbon compounds involves a unique fungal biochemical pathway.
- The MHS-SPME allowed to determine quantitatively 5 target analytes of *A. bisporus* for the first time.

GRAPHICAL ABSTRACT



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ABSTRACT

Multiple headspace-solid phase microextraction (MHS-SPME) followed by gas chromatography/mass spectrometry (GC–MS) and flame ionization detection (GC–FID) was applied to the identification and quantification of volatiles released by the mushroom *Agaricus bisporus*, also known as champignon. MHS-SPME allows to perform quantitative analysis of volatiles from solid matrices, free of matrix interferences. Samples analyzed were fresh mushrooms (chopped and homogenized) and mushroom-containing food dressings. 1-Octen-3-ol, 3-octanol, 3-octanone, 1-octen-3-one and benzaldehyde were common constituents of the samples analyzed. Method performance has been tested through the evaluation of limit of detection (LoD, range 0.033–0.078 ng), limit of quantification (LoQ, range 0.111–0.259 ng) and analyte recovery (92.3–108.5%). The results obtained showed quantitative differences among the samples, which can be attributed to critical factors, such as the degree of cell damage upon sample preparation, that are here discussed. Considerations on the mushrooms biochemistry and on the basic principles of MHS analysis are also presented.

1. Introduction

Solid-phase microextraction is a well established sample preparation technique that has gained an enormous success during the years, dating back to more than 20 years ago. From the pioneer works by Pawliszyn and co-workers published in 1992, the number of publications has grown exponentially up to around 1084 papers, based on the use of SPME, in 2011 [1]. SPME is easy, fast, simple, convenient, and environmentally friendly. However, one of the features of this technique, which turns to be at the same time a drawback, is that SPME performs a non-exhaustive extraction. In SPME, the process of extraction is based on the achievement of equilibria between sample matrix and headspace, and between headspace and fiber coating. A SPME extraction is considered complete when the equilibria are established, although this phase doesn't correspond necessarily to the exhaustion of analytes from

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the sample matrix. This issue makes somehow challenging calibration procedures when SPME is chosen as sample preparation methodology. In order to make quantitation of SPME extracted analytes, a variety of calibration procedures are available to the analyst as suggested by the manufacturer [2]. The decision of which approach is the most convenient (internal standard, external standard or standard addition) depends upon sample matrix (liquid or solid), its complexity and extraction mode (headspace or immersion). The external standard calibration generally succeeds in the construction of calibration graphs with good linear regression coefficients; however, this method doesn't take into account the so called "matrix effect" which causes target analytes to be embedded in a complex matrix, where they establish several uncontrollable interactions with other constituents. The internal standard method is mostly advised for simple matrices, due to the fact that in a complex sample, the standard added can undergo the matrix effect in the same way as the other constituents. In order to avoid the matrix effect, for complex samples the standard addition method can be used, but even in this case, the calibration methodology is in most cases neither feasible nor reliable. The standards added and the native analytes behave differently [3].

An interesting alternative to eliminate the sample matrix effects when analyzing VOCs from solid samples is multiple headspace extraction (MHE). This analytical approach dates back to 1970, when Suzuki et al. introduced a new method for estimating occluded solvents in adhesive tapes [4]. Called "Multiple Phase Equilibration," this calibration procedure was successively employed by McAuliffe in the determination of hydrocarbons dissolved in water [5]. In this last paper, McAuliffe postulated the principles of the MHE theory, reporting equations, calibration graphs and practical implications of the methodology. About 10 years later, Kolb dealt again with the topic through an extensive review focusing on MHS theoretical background and practical calculations over a various range of samples (crude oil, sutures, food, pharmaceuticals) [6]. Quoting Kolb et al., "MHE is in principle a dynamic gas extraction procedure, but carried out stepwise, comparable to a repeated liquid extraction in a separation funnel" [7]. Practically, the same sample is subjected to a number of consecutive extractions, generally corresponding to three or four, at equal time intervals. The total peak area of the target analyte can be drawn from the geometric progression, obtained from the consecutive peak areas of the single extractions:

$$A_T = \sum_{i=1}^{n} A_i = \frac{A_1}{1-f} = \frac{A_1}{1-e^{-q'}}$$

where A_T is the total peak area, A_1 the analyte peak area from the first extraction, f the quotient of the geometric progression, q' a constant which takes into account the distribution coefficient and some instrumental parameters. From this equation, one can easily understand that the two values necessary for the calculation of the total area are A_1 and q'. The latter can be obtained from linear regression analysis of the following equation:

$$\ln \quad A_i = -q' \cdot i - 1 + \ln \quad A_1$$

which corresponds to a $y = mx + b$ type linear equation where the slope $m = -q'$.

Once obtained the A_T value, the real concentration of the target analyte in the original matrix can be gathered from a simultaneous external calibration graph, constructed apart with standard compounds either by direct injection or by MHS-SPME extraction. An extensive discussion on the MHS theoretical principles and practical implications has been presented by Kolb and Ettre in 1991 [8].

In the present study, the theory of multiple headspace extraction has been applied, in combination with SPME, to the quantification of some key compounds released by the mushroom *Agaricus bisporus*. This fungus belongs to the edible mushrooms category; it is better known as "champignon" and can be commonly found on the vegetables counter. Around *A. bisporus* there's a big market, since it is widely used in the food industry (frozen and canned mushrooms, soups, pizza, pasta dressings, aroma extracts, etc.). Literature reports 8-carbon atoms skeleton compounds, such as 3-octanone, 3-octanol, (2E)-octenol, as key compounds of mushroom flavor [9]. Previous studies about *A. bisporus* dealt with packaging and storage vs. flavor quality and biochemical pathway of the mushroom life cycle [10,11]. Once developed the MHS-SPME method, this was applied also to some food formulations containing *A. bisporus*.

2. Experimental

2.1. Samples

Mushrooms belonging to the species *A. bisporus* were purchased in local grocery stores and were immediately analyzed. Upon receipt, samples were divided in two groups and subjected to different preparation procedures: parts were added with distilled water and peanut oil (2:1:1) and homogenized; parts were coarsely chopped and then added with water and oil in the same ratio as above. Also, a commercial cream, used as food dressing and labeled as containing 23% of *A. bisporus*, was purchased in a grocery store and analyzed without any pre-treatment.

About 0.1 g of each sample were put into a 10 mL crimped vial for SPME extraction. A mix of C7–C30 *n*-alkanes (Supelco, Bellefonte, CA, USA) was extracted by SPME and desorbed into the GC–MS system in order to measure the experimental linear retention indices (LRIs).

Stock solutions (1000 ppm) of 1-octen-3-ol, 3-octanone, 3-octanol, benzaldehyde, 1-octen-3-one, benzyl alcohol, phenylacetaldehyde, (2E)-octenol and 1-octanol were prepared in peanut oil and serial dilutions in the range 0.001–20 $\mu g\,g^{-1}$ were extracted by multiple headspace SPME. All the standards were provided by Sigma–Aldrich (St. Louis, MO, USA).

2.2. SPME conditions

SPME extraction was carried out in the headspace mode by means of an AOC-5000 autosampler (Shimadzu, Kyoto, Japan) hyphenated with the GC–MS system. Two different fiber coatings were tested: a 65 μm polydimethylsiloxane/divinylbenzene, 1 cm long; and a 50/30 μm DVB/Car/PDMS, 1 cm long, both provided by Supelco (Bellefonte, CA, USA). After SPME method development, the PDMS/DVB fiber was chosen to extract the volatile components from the mushrooms. Fiber exposure lasted 20 min at 50 °C, under agitation. Analytes were then desorbed for 1 min at 250 °C in the GC injector in splitless mode, equipped with a 0.75 mm ID inlet liner. Multiple headspace extraction was performed through four consecutive extractions, with a 5 min interval between each of them. For external standard calibration, graphs were built-up on 5 points, each corresponding to the total areas (A_T) obtained from the MHS-SPME extraction of standard compounds at different concentrations.

2.3. GC–FID analyses

For gas chromatographic separations, a Shimadzu GC-2010 Plus system was used (Shimadzu, Kyoto, Japan). The split/splitless injector was held at a temperature of 250 °C, and, after sampling time