Talanta 123 (2014) 207–217

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Analytical strategies based on multiple headspace extraction for the quantitative analysis of aroma components in mushrooms

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ARTICLE INFO

Article history:
Received 29 October 2013
Received in revised form
13 January 2014
Accepted 20 January 2014
Available online 6 February 2014

Keywords:
Multiple headspace extraction
Headspace analysis
HS-SPME
Mushroom
Volatile compounds
GC/MS

ABSTRACT

Headspace (HS) and headspace solid phase microextraction (HS-SPME) analysis by gas chromatography–mass spectrometry (GC/MS) have been found to be suitable methods for the analysis of volatile organic compounds. The objectives of this paper are to study the possibilities of multiple headspace extraction (MHE) for the quantitative determination of volatile compounds in mushroom samples and to compare the results obtained using three different sample treatment techniques. For this purpose, HS with two different injection techniques (pressure-loop system and gas-tight syringe autosampling system) and HS-SPME have been studied. Three processes were optimized for the analysis of 20 volatile compounds by experimental design technique based on Central Composite Design (CCD) and Full Factorial Design depending on the used methodology. Once the designs were finished, a trade off among optimum conditions for each compound analyzed was reached.

At optimum conditions, appropriate extraction time and sample amount for the three techniques used were established. Finally, the methods were validated in terms of linearity, detection and quantitation limits and repeatability. The most suitable method was then applied to the quantitative analysis of seven mushroom samples.

A detailed comparison of the analytical performance characteristics of HS and HS-SPME as sample treatment techniques for final GC/MS determination is given. In addition, MHE has been proved to be an adequate technique to avoid matrix effects in complex samples quantitation. Its applicability to the determination of volatile mushroom components, along with its limitations, is discussed in this work.

1. Introduction

Volatile components contributing to mushroom aroma have been widely studied. Analytical methodology for determination of volatile compounds in vegetable matrices is continuously improving due to the important role of these compounds in organoleptic, chemical and nutritional characteristics [1,2]. Due to the fact that aromas present in mushrooms belong to different chemical families (esters, ketones, aldehydes, alcohols, terpens, phenols, and their derivatives), optimization of multicomponent sample preparation procedure is a difficult task. Moreover, there are significant differences in the behavior of the analytes between real samples and standard solutions since distribution constants depend on the composition of each one. In spite of analytical efforts, quantitation of aromas is rather problematic, and in most cases, not fully satisfactory results are obtained [3,4].

Although different sample treatment procedures have been used for the extraction of volatile compounds from mushroom samples [5–10], headspace extraction is now routinely used by scientists in a wide range of disciplines [11]. It is well known that HS is a non-quantitative extraction technique, thus being necessary to calibrate using extracted spiked blank samples. Unfortunately, this is not easy when determining the aroma profile in vegetables as no blank samples can be obtained [12]. A stepped procedure called multiple headspace extraction (MHE), whose theoretical bases were established in the earlier 80's, has been proposed as an alternative to overcome some typical difficulties as the matrix-effects [13–18]. MHE technique is based on the calculation of the area value corresponding to an exhaustive extraction of the analytes from a few steps of consecutive extractions (3 or 4) of the same sample. Thus, the matrix-effect is already eliminated even though obtained area value equivalent to a complete extraction depends only on the amount of analyte and not on the composition of the sample matrix or on the

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http://dx.doi.org/10.1016/j.talanta.2014.01.021
0039-9140

standards matrix. As extensively described by Kolb [19], the total area can be calculated according to Eq. (1):

$$A_T = \Sigma A_i = A_1/(1-e^{-q}) \quad (1)$$

where A_T is the total area, A_i is the peak area of the ith step, A_1 is the area of the first extraction and q is a constant which describes how fast the extraction process proceeds. The value of q can be experimentally obtained by plotting the neperian logarithms of the area values versus the number i of extraction steps, in fact, at equilibrium. A straight regression line is obtained and the slope of this straight line corresponds to q value [19].

$$\ln A_i = -q(i-1) + \ln A_1 \quad (2)$$

From the value of the slope we obtain the quotient Q:

$$Q = e^{-q} \quad (3)$$

Once obtained the A_T value, the real concentration of the target compounds in the original matrix can be gathered from a simultaneous external calibration graph, constructed apart with standard compounds by MHE.

On the other hand, SPME seems to be another attractive alternative for this kind of analysis. Introduced by Pawliszyn [20] SPME is a rapid solvent-free sampling technique that is well suited to the determination of volatile compounds by gas chromatography (GC). Since its introduction, many papers have dealt with the use of SPME for the determination of volatile compounds in the headspace of samples. It is also an excellent tool for comparative studies and semiquantitative determinations [21,22]. Since its development, this technique has become very popular for determining volatile and semi-volatile compounds due to its advantages over conventional extraction methods.

SPME can also be performed in a stepped fashion; this procedure is known as multiple headspace solid phase microextraction (MHS–SPME). The theoretical foundation of this combined technique under equilibrium was reported by Ezquerro et al [23]. MHS–SPME also employs the peak areas of a few consecutive extractions to calculate the amount of analyte of a complete extraction, but this time the analytes are partitioned in a three-phase system (sample matrix, headspace and fiber coating). In this case, the quotient Q is named as β, which has a value between zero and unity ($0<\beta<1$). β can be obtained from linear regression analysis of the logarithmic form of Eq. (2) as it is previously mentioned [23].

In the present study, the potentiality of multiple headspace extraction for the quantitative determination of volatile compounds in complex matrix samples (mushroom) using external solvent calibration has been investigated.

In this work, the multiple extraction method was applied to a particular mushroom species, which is growing up in our territory more and more, *Clathrus archeri*, which is commonly known as the octopus or cuttlefish stinkhorn [24]. *C. archeri* (Phallaceae), is a species native to Africa and Australasia although it is now also naturalized in Europe and North America. The knowledge of volatile compounds concentrations and proportions in this mushroom species will give us valuable information for later use in agroindustrial products. Although 22 volatile compounds had been already identified and qualitatively determined in *C. archeri* samples in previous work [25], to our knowledge, this is the first application of MHE and MHS–SPME to quantitative determination of aroma components of this mushroom. For the quantitative determination of *C. archeri* volatile compounds three different sample treatment techniques have been investigated: HS with two different injection techniques (pressure-loop system and gas-tight syringe autosampling system) and HS-SPME [12,15]. The two HS injection techniques discussed in this work showed differences, therefore a comparison between them has been done.

The extraction processes were optimized and validated in terms of linearity, precision, limits of detection and quantitation and by comparison of the quantitative results obtained by the three techniques. A detailed comparison of the analytical performance characteristics of MHE and MHS–SPME as aroma extraction techniques is given.

2. Experimental

2.1. Chemicals, materials and samples

Individual standard solutions in methanol (HPLC gradient grade, 99.8%, obtained from Prolabo (Leuven, Belgium)) were prepared from volatile compounds studied, 1-butanol (>99%), 1-pentanol (>99%), 6-methyl-5-hepten-2-one (98%), dimethyl trisulfide (98%), acetid acid (100%), 1-octen-3-ol (98%), 1-heptanol (99%), 2-methyl propanoic acid (99%), propanoic acid (99%), butanoic acid (>99%), pentanoic acid (99%), diphenyl ether (99%) and p-cresol (99%), all were supplied by Sigma Aldrich (St. Louis, MO, USA) while limonene/ocimene mixture (90%), isoamyl alcohol (98%), phenol (99%), 2-phenylethanol (100%), indole (99%) and 2-methyl butanoic acid (98%), were obtained by Alfa Aesar (Karlsruhe, Germany). Acetic acid was purchased from Merck (Madrid, Spain). The compounds selection for the study was based on literature [25] and previous work. In it, a qualitative analysis of *C. archeri* was performed and compounds with potential contribution to its aroma were selected. All standard solutions were stored at 4 °C in sealed glass vials completely filled (without headspace) to avoid analyte losses.

Samples of the wild species of *C. archeri* were collected during summer and autumn of 2010, 2011 and 2012 in the forests of Basque Country, Spain. Prior to analysis, two different sample pretreatments were carried out. One part of the mushroom samples were immediately transferred to the laboratory, mincing with a cryogenic grinder (SPEX SamplePrep 6770 Freezer/mill, Metuchen, New Jersey) and analyzed wet with qualitative and quantitative purposes. Other part of the samples was kept in glass bottles, frozen, triturated and freeze-dried at low temperatures (−46/−52 °C) and pressures (0.17/0.22 mbar) in a Cryodos-50 freeze-drier (Telstar, Spain).

For HS-SPME extraction, SPME fibers coated with 85 μm polyacrilate (PA), 100 μm polydimethylsiloxane (PDMS), 75 μm carboxen–polydimethylsiloxane (CAR/PDMS), 65 μm polydimethylsiloxane–divinylbenzene (PDMS/DVB) and 50/30 μm divinylbenzene–carboxen–polydimethylsiloxane (DVB/CAR/PDMS) obtained from Supelco (Bellefonte PA, USA) were used. All of them were thermally conditioned in accordance with the manufacturer's recommendations.

2.2. Optimization of the HS and HS-SPME extraction procedures

HS and HS-SPME extraction parameters can affect the extraction process, and in order to get the highest recovery of the analytes, the optimization of parameters such as extraction temperature, extraction time, fiber type, sample amount, desorption time and stirring speed was performed. Depending on the extraction method used, different kind and number of parameters have to be optimized. If only few factors are involved in the optimization, the most suitable design is a factorial design. Thus, a central composite design (CCD) methodology was used in order to optimize the extraction process in the case of headspace extraction pressure loop system with three variables. The variables and its low, central and high levels were: extraction temperature (Temperature; 60, 75, 90 °C), loop fill time (Loop fill t; 0.015, 0.1, 0.20 min) and vial pressure time (Vial press t; 0.20, 0.35, 0.50 min). In the case of headspace gas-tight syringe