International Food Research Journal 21(1): 263-268 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Volatile compounds in fresh, cooked fresh, dried and cooked dried *Agaricus bisporus* using ambient temperature vacuum distillation

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Article history

Received: 10 April 2013
Received in revised form: 15 August 2013
Accepted: 16 August 2013

Keywords

Ambient temperature vacuum distillation
HS-SPME
A. bisporus
VC

Abstract

Ambient temperature vacuum distillation in conjunction with headspace- solid phase micro-extraction (HS-SPME) was used to study the volatile compounds (VC) in *Agaricus bisporus*. Different extraction times 2, 3, 4 and 5 h were also tested against VC yield and 4 h was found to be the best extraction time with most of the compounds retrieved. No significant difference was found between the VC extracted in 4 and 5 h. The samples were analysed as fresh, boiled under reflux, dried and dried and then boiled under reflux. The major peaks identified in all treatments were 4-methyl, 3-penten-2one, benzaldehyde, 1-octen-3-ol, 3-octanone, 3-octanol, and 2-octen-1-ol, 1-octanol. Benzyl alcohol and 1-octen-3-one were identified whenever heat was applied while pyrazine, limonene, benzyl isothiocyanate, and 4 phenyl-2-butenal were detected in dried then boiled under reflux samples. Thermal processing affected the relative concentration of VC where a decrease in the relative concentration of the main C8 compounds was observed while other compounds, such as furfural, limonene, benzyl alcohol, 3, 5, 5-trimethyl- cyclohexen-2-one, were formed.

Introduction

Mushrooms have been widely consumed as a food or food ingredients in many cultures and not only as a part of the normal diet, but also as a delicacy because of their highly desirable taste and aroma (Mau *et al*., 1997; Nollet, 2009). Button mushroom, *Agaricus bisporus*, a widely cultivated and commercially available mushroom, possesses a high culinary and commercial value because of its subtle flavour characteristics (Tsai *et al*., 2007). The mushroom volatiles have been extensively studied and aroma compounds responsible for the characteristic flavour of certain mushroom species have been well documented (Wąsowicz, 1974; Maga, 1981; Chen and Wu, 1984; Chen *et al*., 1984; Fischer and Grosch, 1987; Mau *et al*., 1992; 1997; Assaf *et al*., 1997; Lizarraga-Guerra *et al*., 1997; Venkateshwarlu *et al*., 1999).

The characteristic volatile compounds found in button mushrooms (*A. bisporus*) are eight-carbon compounds including 1-octanol, 3-octanol, 3-octanone, 1-octen-3-ol, 2-octen-1-ol, and 1-octen-3-one (Cronin and Ward, 1971; Pyysalo and Suihko, 1976; Fischer and Grosch, 1987). Among them, 1-octen-3-ol is the most important compound associated with fresh mushroom flavour. It is formed from the enzymatic breakdown of linoleic acid (Grosch and Wurzenberger, 1984), which is normally present in high levels in mushroom fruiting bodies (Holtz and Schisler, 1971). During technological processing, the flavour compounds of mushrooms changed due to various chemical reactions (Misharina *et al*., 2009). They noticed that the level of alcohols decreased considerably due to drying process while the concentration of heterocyclic compounds increased.

Different sampling techniques offer individual benefits, but also suffer some limitations such as potential aroma destruction, degradation of VC or artefacts formation. One of the main obstacles encountered by researchers when studying aroma compounds is the choice of a suitable extraction technique to quantitatively and qualitatively represent the original aroma. That is to obtain a representative extract containing all the volatile compounds present originally in the food sample. Several extraction and concentration methods have been used, among them simultaneous distillation extraction (SDE), solvent extraction, static and dynamic headspace, purge and trap. According to Reineccius (2002), the main principles of aroma isolation are volatility, partition, or a combination of both. Several researchers discussed the disadvantages of using SDE mainly the release of volatiles from antifoaming agents and greases used in the joints (Jeon *et al*., 1976). The objective of this study was to investigate the VC changes caused by thermal processing, and ascertain the qualitative and quantitative differences of VC between fresh, cooked, dried and dried and cooked

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mushrooms. The technique of ambient temperature vacuum distillation, and HS-SPME combined with GC-MS was employed.

Materials and methods

Mushrooms

Agaricus bisporus samples were purchased from a local fruit shop in Sydney (Punchbowl, NSW, Australia). Mushrooms were cleaned and weighed.

Sample preparation

a. *Fresh sample (F)*: Fresh *A. bisporu*s sample (50 g) was cleaned, and blended to a puree to fragment hyphae and gills thus facilitating greater release of volatiles. The puree was placed in a 2 L round bottom flask filled with 100 g of distilled water. The flask was put in a water bath set at 25°C and then subjected to ambient temperature vacuum distillation.

b. *Boiled under reflux sample (BR)*: Prepared in the same way as (F), the sample was cooked in hot boiling water for 30 min then left to cool down before subjecting it to the ambient temperature vacuum distillation.

c. *Dried sample (D)*: The mushroom sample 1000 g was cleaned, sliced and then dried in a cabinet drier for 18 h at 40°C and RH % 25. The sample was then cooled down, vacuum packed and stored at -20°C until needed. The dried sample (15 g) was put into the round bottom flask then 180 g of water were added and the sample was subjected to the distillation.

d. *Dried and boiled under reflux sample (DBR)*: Prepared in the same way as (D), cooked then cooled down before subjecting it to vacuum distillation. The extracts collected from a, b, c, and d were then concentrated and analysed via HS-SPME combined with GC-MS.

Ambient temperature vacuum distillation

Initially, ambient temperature vacuum distillation was carried out for 2, 3, 4, and 5 h to test the effect of extraction time on the VC yield. Following this preliminary test, all distillation experiments were performed for 4 h at 30 Pa on the several mushroom samples according to the method published by Ashmore *et al.* (2013).

Concentration using SPME

An SPME holder (Supelco, PA, USA) was used in performing the second stage of the experiment. SPME was performed on the combined cold traps to extract the VC using a fused silica fibre coated with 50/30 µm layer of divinylbenzene/carboxen/polydimethyl-siloxane (Supelco). The fibre was conditioned following the manufacturer's instructions prior to its use and was thermally cleaned between analyses by injecting it to the GC injection port at 250°C for 15 min. The sample vial was placed inside the bath and was allowed to condition for the equilibrium time (10 min) with no fibre exposition. After the equilibrium time, the fibre was introduced into the vial and was exposed to the headspace of the sample during the corresponding extraction time (1.5 h). After extraction, the fibre was inserted into the injection port of the GC for thermal desorption of the analytes.

Identification and quantification of volatiles compounds using GC-MS

The SPME was injected in the splitless mode into the GC-MS and left for 0.5 min allowing the volatiles to desorb. GC-MS analysis was performed using an Agilent 19091S-433 equipped with a DB-5ms column (30 m length * 0.25 mm i.d. * 0.25 µm film thickness). The carrier gas used was helium at a constant flow rate of 1.1 mL/min. The oven temperature was initially held at 30°C for 5 min, and then raised to 250°C at a rate of 3°C/min held at 250°C for 25 min. All the chromatogram peaks were identified by means of the mass spectral library using a PC, then confirmed by comparison with the retention time of the authentic reference (Wiley 275, Hewlett-Packard Company, Palo Alto, CA.). For quantitation, the standard addition method was used where absolute ethanol (10.00 g) and mixture of standards (1-octen-3-ol, 3-octanol, benzaldehyde and nonanal) were prepared at 500 ppm. A volume of 5 µL was added progressively to 1 ml mushroom extract placed in water bath set at 30°C +/-1°C. The calibration curve obtained by plotting GC peak area versus different concentrations of the standards was plotted and the samples were quantified by calculation of each peak area from the calibration line.

Statistical analysis

Experiments were performed in triplicate and results were expressed as the mean value +/- standard deviation (SD) of 3 replicates. Analysis of variance (ANOVA) was used to compare fresh and thermally treated samples and to determine the effect of extraction time on the VC yield.

Results and Discussion

Effect of extraction time on VC yield

The effect of extraction time on the relative concentration of the VC was studied. The longer the extraction time, the higher the content of VC extracted

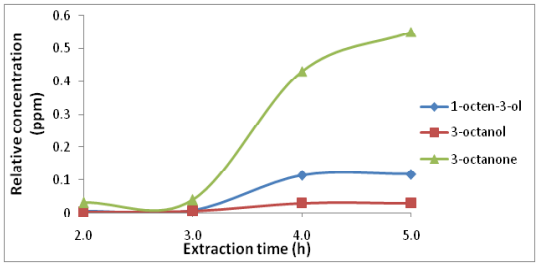

Figure1. Relative concentration of 1-octen-3-ol, 3-octanol and 3-octanone at different extraction times

(Figure 1). The highest relative concentration (RC) of VC was observed after 5 h extraction where 1-octen-3-ol was found to be 0.115 ppm +/-0.002, 3-octanone 0.551 ppm +/-0.021, and 3-octanol was 0.030 +/-0.001. When compared to 4 h extraction, the results showed no significant difference between the 2 extractions times. However, a significant difference was observed between 2, 4, and 5 h and between 3, 4 and 5 h at $p < 0.05$. After 2 h, the RC of 1-octen-3-ol was 0.005 +/-0.000 and it greatly increased after 4 h extraction (0.113 +/-0.021). As for 3-octanol, 2 h extraction resulted in a RC of 0.002 ppm +/-0.000 but then it increased to 0.038 ppm +/-0.008 after 4 h extraction. The two other compounds, 3-octanol and 3-octanone, behaved in the same way as 1-octen-3-ol with no significant difference noticed after 4 h of extraction. No significant difference was observed between 2 and 3 h extractions for 3-octanone (0.031 ppm +/-0.003 versus 0.039 ppm +/-0.003) unlike 1-octen-3-ol whose RC increased almost 3 times when the extraction time was increased. Therefore 4 h was chosen as the suitable extraction time.

Isolation of VC from mushrooms

Ambient temperature vacuum distillation was used to isolate the VC from *A. bisporus* (50 g). The distillate was analysed using HS-SPME and GC-MS. Weurman *et al.* (1970) argued that they failed to obtain residues from food products containing completely odourless non-volatile residues. Their observations were consistent with the results presented in this paper where mushroom aroma was still present in the sample residue.

The fresh "raw" mushroom sample (F) had a strong fresh mushrooms aroma containing six major peaks which were identified as 4-methyl, 3-penten-2-one, 1-octen-3-ol,3-octanone, benzaldehyde, 3-octanol, and 2-octen-1-ol by comparison of their retention times and mass spectra with those of references. The boiled under reflux (BR) mushroom aroma extract had a strong cooked mushroom-like odour. GC-MS analysis showed nine major components which corresponded to 4-methyl, 3-penten-2-one, benzaldehyde, 1-octen-3-one, 1-octen-3-ol, 3-octanone, 3-octanol, 2-ethyl, 1-hexanol, benzyl alcohol, 2-octen-1-ol, 1-octanol (Table 1).

Table 1. Relative concentration (RC) of VC in fresh (F), boiled (BR), dried (D), and dried and boiled (DBR) *A. bisporus*

Compound	Rt (min)	RC(F) (ppm)	RC(BR) (ppm)	RC(D) (ppm)	RC(DBR) (ppm)
4-methyl-3-penten-2-one	5.46	0.021+/-0.001	0.023+/-0.002	0.048+/-0.001	0.040+/-0.001
Furfural	7.265	ND	0.059+/-0.001	0.034+/-0.001	0.042+/-0.002
Hexanal	8.631	ND	ND	0.032+/-0.001	0.002+/-0.000
Benzaldehyde	15.952	0.035+/-0.002	0.665+/-0.054	0.544+/-0.063	0.088+/-0.108
1-octen-3-one	16.924	0+/-0	0.021+/-0.004	0.005+/-0.000	0.009+/-0.001
1-octen-3-ol	17.306	0.113+/-0.015	0.071+/-0.010	0.026+/-0.002	0.056+/-0.005
3-octanone	17.653	0.432+/-0.032	0.259+/-0.058	0.114+/-0.017	0.055+/-0.001
3-octanol	18.146	0.038+/-0.008	0.024+/-0.002	0.025+/-0.005	0.020+/-0.003
2-ethyl,1-hexanol	19.631	0.001+/-0.000	0.002+/-0.000	0.068+/-0.002	ND
Limonene	19.623	ND	0.004+/-0.000	ND	0.026+/-0.004
Benzyl alcohol	20.182	ND	0.530+/-0.054	0.655+/-0.046	0.371+/-0.024
Benzyl acetaldehyde	20.443	ND	ND	ND	0.373+/-0.076
2-octen-1-ol	21.927	0.008+/-0.000	0.013+/-0.002	0.021+/-0.004	0.025+/-0.005
n-octanol	22.114	0.006+/-0.001	0.006+/-0.000	0.009+/-0.000	0.006+/-0.001
3,5,5-trimethyl-2-cyclohexen-2-one	24.329	ND	0.021_/-0.000	0.035+/-0.006	0.042+/-0.004
4-phenyl-2-butenal	26.521	ND	ND	ND	0.017+/-0.000
Benzyl isothiocyanate	26.734	ND	ND	ND	0.002+/-0.000

Samples are per 1 mL extract
*ND Not Detected

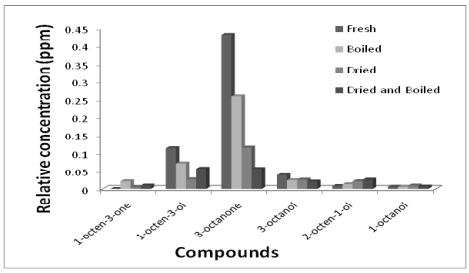

Figure 2. Relative concentrations of main C8 compounds in fresh, boiled, dried and dried and boiled *A. bisporus* mushrooms

The dried sample (D) had a mild aroma but upon rehydration, a stronger cooked aroma developed. The analysis revealed ten major peaks which were identified as 3-hexen-2-one, benzaldehyde, 1-octen-3-one, 1-octen-3-ol, 3-octanone, 3-octanol, benzyl alcohol, 2-octen-1-ol, 1-octanol, 3,5,5-trimethyl-2-cyclohexen-1-one but 4-methyl-3-penten-2-one and 1-hexanol were not detected (Table 1). The dried and boiled under reflux (DBR) sample had a strong cooked aroma where GC-MS analysis showed 17 major peaks which were identified as toluene, furfural, hexanal, benzaldehyde, 1-octen-3-one, 1-octen-3-ol, 3-octanone, 3-octanol, 2-ethyl-5-methyl pyrazine, limonene, benzyl alcohol, benzeneacetylaldehyde, 2-octen-1-ol, 1-octanol, 3,5,5-trimethyl-2-cyclohexen-1-one, 4 phenyl-2-butenal, and benzyl isothiocyanate (Table 1).

The effect of different treatments on the main C8 compounds is shown in Figure 2. The highest amount of 3-octanol was recorded in the fresh sample (F) (0.038+/-0.008) while no significant difference was

found between boiled under reflux (BR) samples (0.024+/-0.002) and dried and boiled under reflux (DBR) samples (0.020 +/- 0.003). However, its relative concentration (RC) decreased when the samples were dried (0.025+/-0.005). As for 1-octen-3-ol, it was found to have its highest RC in F samples (0.113 +/- 0.015). However, the RC was reduced dramatically as heating was involved. When the sample was boiled under reflux, the relative concentration was found to be 0.071+/-0.010 compared to D samples (0.026+/-0.002) and DB (0.056+/-0.005). Benzyl alcohol was detected in all treatments except for F samples with its highest amount recorded in D (0.655+/-0.046) and the lowest in DBR samples (0.371+/- 0.024) but not significantly different when compared to BR samples (0.530+/-0.054) ($P < 0.05$). The concentration of 3-octanone was the highest in F samples (0.432 +/0.032) followed by BR samples (0.259+/-0.058). Its amount was reduced dramatically when the sample was dried (0.114 +/-0.017) and dried then boiled under reflux (0.055+/-0.001). As for 1-octen-3-one, it was absent in F samples while its highest concentration was recorded in BR samples (0.021+/-0.004) followed by DBR samples (0.009+/- 0.001). Its concentration decreased when the sample was dried (0.005+/-0.000). Finally the concentration of benzaldehyde (Table 1) increased significantly when the sample was boiled under reflux (0.665 +/-0.054) and after drying (0.544+/-0.063) compared to F samples (0.035+/- 0.002). When the samples were dried then boiled, the average RC was found to be more than double the amount found in the fresh samples (0.088+/-0.108).

Solid-phase micro-extraction (SPME)

An important aspect in analysis of volatiles using SPME is the correct choice of fibre. In other words, taking into consideration that different compounds have different polarities, the chemical nature of the fibre will reduce the discrimination towards very nonpolar and polar volatile compounds (Pelusio *et al.*, 1995). Thus, the grey fibre (medium polarity) to characterise aroma samples from their volatile composition was chosen for the analysis of the mushroom species, as previous studies have shown that it gives the most complete screening of the volatile profile (Díaz *et al*., 2002; Pinho *et al*., 2008).

The effect of thermal processing on the volatile compounds was also investigated and is tabulated below. These compounds are assumed to be generated in three ways: (i) release of volatiles already existing in mushrooms, (ii) degradation of amino acids, sugars and nucleotides and (iii) Maillard reaction between amino acids and reducing sugars (Li *et al*., 2011). Picardi and Issenberg (1973) documented the changes in the volatile composition during heating, Cho *et al.* (2006) reported mostly saturated and unsaturated alcohols and ketones with eight carbon compounds. Mau *et al*. (1992) reported that the concentration of 1-octen-3-ol throughout the entire crop cycle varied between 19.3 and 37.2 ppm. They also showed that its concentration decreases during postharvest storage at 12°C as the lyases' activity dropped and that the greatest amount detected is pH dependent. On the other hand, Mau *et al.* (1993) argued that the content of 1-octen-3-ol changes during the crop cycle, peaking at the third flush. Taking into consideration that the samples tested here were of an unknown strain, unknown flush cycle and with unadjusted pH, 1-octen-3-ol was not found to be the most abundant volatile compound but rather a major compound contributing to the aroma of the fresh and thermally processed *A. bisporus.*

The compound 1-octen-3-one could not be detected in fresh mushrooms but when the sample was thermally processed (boiled under reflux or dried), the compound was detected. This shows that this compound is generated via heat and that this technique gives a true representation of the aroma of the fresh produce. This is in agreement with Picardi and Issenberg (1973) where 1-octen-3-one was only detected when the mushrooms were cooked. According to them, the appearance of 1-octen-3-one can be explained by 2 possible scenarios. Either the 1-octen-3-one is an important "character impact" compound in the flavour of cooked mushrooms and in this case, this compound plays a distinctive role in the differentiation between fresh and cooked mushrooms, or 1-octen-3-one exist in both fresh and cooked samples, but its level is well below the detection limits of the GC-MS used and in this case, a large difference between both concentrations is observed. Li *et al.* (2011) showed that during cooking, the chemical composition of the volatile changes due to partial loss of existing compounds and formation of new ones as a result of various chemical reactions. Furthermore, Werkhoff *et al*. (1998) argued about the possibility of thermally induced artefacts yielding falsified aroma and concluded that the distillation technique produces more representative aromas of the food sample if it operates under vacuum.

Conclusion

Ambient temperature vacuum distillation was used to determine the effect of 2, 3, 4, and 5 h extraction times on the VC. The longer the extraction

time, the higher the content of VC extracted. A significant difference was observed between 2, 4, and 5 h and between 3, 4 and 5 h at $p < 0.05$ and thus 4 h was chosen to be the most suitable and efficient extraction time. The effect of heat treatments was also studied and it was found that 1-octen-3-one was only detected when the sample was heated. As for the other C8 compounds, heat application whether boiling under reflux, drying or drying then boiling, led to the decrease of the relative concentration of VC. Other compounds, such as furfural, limonene, benzyl alcohol, 3, 5, 5-trimethyl- cyclohexen-2-one, were formed when heat was applied.

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