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Optimisation of ambient temperature vacuum distillation technique for the characterisation of volatile compounds in mushrooms

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Abstract

Experimental equipment has been developed and optimised for laboratory-scale vacuum distillation at ambient temperature of volatile compounds (VC) from mushrooms using button mushrooms (*Agaricus bisporus*) as a model mushroom. The factors which influenced equipment operation and enabled vacuum extraction of VC from mushrooms at ambient temperature included the total operating time, the pressure, and the total amount of the distillate. The results suggest that the amounts extracted are positively correlated with the extraction time: the longer the extraction time, the higher the amount extracted with four hours being sufficient to extract a significant amount of VC. The vacuum distillation can be applied irrespective of the sample size and the condensates can be used in sensory tests as they are free from organic solvent.

Introduction

Mushrooms are widely consumed as a food or food ingredients in many cultures, 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). Edible mushrooms refer to fungal species that are harvested either wild or cultivated. More than 2000 edible species of mushrooms are known, but only 25 are commercially cultivated (Furlani and Godoy, 2008). Edible mushrooms are characterised by short-shelf life (3-4 days at ambient temperature) due to physico-chemical changes after harvest. These changes are due to their high moisture content and high activity of enzymes such as protease or polyphenol oxidase, responsible for decrease in proteins and sugars and for a browning reaction during storage (Manzi, 2004).

Aroma is a characteristic feature of each mushroom species, and can help in distinguishing between them. The aroma VC result from a mixture of organic molecules that exhibit a wide range of chemical structures characterised by different physical and chemical properties (Taylor, 2010). Aroma compounds are present in minute quantities in foods, often at 10^{-9} g level. In order to analyse these compounds, isolation and concentration techniques are needed. Extraction techniques for mushrooms VC in previous studies have mainly been solvent extraction and simultaneous steam distillation extraction. According to Taylor (2010), the aroma profile obtained via solvent extraction represents the relative solubility of various aroma constituents in the organic and aqueous phases. Barcarolo and Casson (1997), showed that the second method causes the formation of secondary flavours as a result of degradation caused by lipid oxidation via heating as well as products resulting from browning reactions, all of them being mixed with the compounds present in fresh mushrooms. Werkhoff *et al.* (1998) argued about the possibility of thermally induced artefacts yielding falsified aroma and concluded that steam distillation produces more representative aromas of the food sample if it is operated under vacuum. Organic compounds with the same molecular formula and belonging to different chemical classes have different odour thresholds. In fact their structure and the structure of the functional group are fundamental in the perception of aroma compounds (Czerny *et al*., 2011).

Different sampling techniques present individual advantages, but also suffer some limitations such as potential aroma destruction, degradation or artefact 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. Traditionally, solvent extraction techniques were employed before GC-MS analyses to isolate VC from mushrooms, but these approaches are

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time-consuming, require large amounts of sample and produce secondary by-products. According to Reineccius (2002), the main principles of aroma isolation are volatility and partition or a combination of both.

Vacuum distillation is not a new extraction method. It has been widely explored in petroleum refining, in the perfume industry, as well as in the concentration and dehydration of foods, herbs, and spices (Greer *et al*., 2008). However, ambient temperature distillation is not so common. The aim of this study is to optimise the ambient temperature vacuum distillation technique, to achieve a mass balance that shows minimal or zero losses, and to obtain an extract with the flavour profile characteristic of recently picked fresh mushrooms.

Materials and methods

Mushrooms

Agaricus bisporus samples were purchased from a local fruit shop in Sydney (Punchbowl, NSW, Australia). Samples of mushrooms (100 g then 50 g) were cleaned, cut and weighed into a 2 L round bottom flask (sample container).

Equipment

Vacuum pump (EDWARDS, Model RV3, Edwards limited. Manor Royal, Crawly, west Sussex RH 109 LW, UK) with pressure gauge, three glass traps (diameter 6 cm) and a water bath (5 L) with thermostat (Townson Mercer, Manchester, UK).

Vacuum extraction

The vacuum distillation apparatus consisted of the sample container immersed in the water bath, three glass traps, an extra trap containing molecular sieve (to prevent VC from entering the pump), the vacuum pump and the pressure gauge (see Fig. 1). The cold traps were kept at temperatures of -12°C, -79°C, and -192°C. These temperatures were achieved by using pre-cooled concentrated NaCl solution, dry ice in ethanol, and liquid nitrogen respectively. Vacuum was applied to the system by opening a release valve built into the pump. The vacuum pump reduced the pressure from atmospheric (105 Pa) to about 20 Pa in approximately 40 min. The air leaks from the system were prevented by using a Teflon tape around all joints.

At first, the pump performance was assessed by successively connecting the round bottom flask and the traps to the pump and monitoring the pressure. Then, water only was introduced into the round bottom flask and the pressure was tested. At this stage, 200 g of water were allowed to boil gently. In order to test the effects of the sample size on the mass balance, 2 sample sizes were used: 100 g and 50 g of mushrooms respectively. The amount of water added to mushrooms had the ratio of 2:1. The temperature of the water bath was set to 25°C. Then, the alignment of the 3 traps and possible leakage at the joints were checked. At some stage of the testing process, more than a 100% mass balance was recorded. The increase in weight of collected liquid was attributed either to a leak in some of the joints or to entry of air containing water vapour during the opening of the system at the end of the experiment. To prevent this phenomenon, silica gel was added to a flask connected to the valve of the round bottom flask (Figure 1).The extraction procedure was tested for 2, 3, 4, and 5 hours and performed in triplicate.

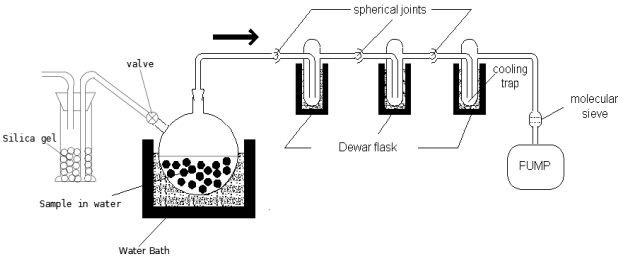

Figure 1. Vacuum extraction apparatus for the volatile compounds in mushrooms

The pressure was reduced from 105 Pa to 19 Pa. The pressure release valve at the vacuum pump was opened gradually at the beginning of the extraction to avoid rapid pressure drop in the sample container resulting in foaming and excessive boiling. Under the effect of vacuum inside the system, the joints were pushed together and the system sealed itself. Without mushrooms, water was evaporated and condensed within two hours. The VC evaporated from the sample were collected in the cold traps. The cold traps were maintained at the required temperature during the whole experiment. When the valve was completely opened the pressure reached a value between 19 and 33 Pa. Table 1 provides details of operating conditions for vacuum distillation.

Table 1. Optimised conditions for ambient vacuum distillation

Sample size	50 g mushrooms and 100 g water
Operating temperature	25°C
Operating pressure	19-33 Pa
Distillation time	4 hours

Results and discussion

The pump performance was tested by connecting successively the system components. The pressure with a single trap was 24 Pa and doubled by the time the

third trap was attached (Table 2). If the release valve was fully open from the start of the experiment when mushrooms were present, the boiling was so intense that some fractions of the sample were pushed into the first trap. Hence, the valve was opened gradually. The effect of the silica gel was also tested to determine whether any intrusion of moisture was affecting the mass balance when turning off the vacuum pump and reverting the system to the atmospheric pressure at the end of the experiment. Table 3 shows that in the absence of silica gel, there was an excess of 1.652 g in the mass balance vs a deficit of 0.003 g in the presence of silica gel.

Table 2. Initial pressure tests

Order of connection to the vacuum pump	Pressure (Pa)
Round bottom flask	22
Trap 1	24
Trap 2	42
Trap 3	48

Table 3. Mass balances performed before and after addition of silica gel

	*Total initial weight (g)	Trap 1 (g)	Trap 2 (g)	Trap 3 (g)	Left over in the flask (g)	Losses (-)/ gains (+) (g)
Before addition	1301.865	224.902	271.453	239.781	567.381	+1.652
After addition	1301.870	229.601	250.964	241.372	579.930	-0.003

*Total initial weight includes flask, valve, neck, mushrooms and water

The contents of all three traps (water and VC) were frozen at the end of the extraction and were left standing overnight at room temperature to allow for condensation to take place. For any extraction time the lowest amount was collected in the third trap (Tables 4 and 5). For 2 and 3 hours, the highest amount was recorded in the first trap but for 4 and 5 hours, the highest amount was recorded in the second one. In terms of aroma, the third trap had the most intense aroma while the first one had the weakest.

Tables 4 and 5 show clearly that the amounts extracted were positively correlated to the extraction time. For 2 hours and 100 g mushroom sample, an average of 78.899+/-2.271 g was extracted vs 108.918+/-12.612 g for 3 hours. The amounts obtained from a 50 g mushroom sample were 24.727+/- 1.942 for 2 h and 47.091+/-8.342 for 3 hours. When the extraction was performed for 4 hours for a 100 g mushroom sample (see Table 4), the volume increased to 190.221 +/-2.932 g but that amount was not significantly different when the extraction time was 5 hours (210.579+/-7.691 g). A similar trend was observed when the sample size was 50 g (Table 5). As shown in Tables 4 and 5, the amounts extracted from the two sample sizes at pressures between 23-47 Pa and at ambient temperature showed similar trends and were characterised by negligible material losses. This proves that this technique can be used irrespective of the sample size.

Table 4. Effects of extractions times on the amounts collected in the three traps, the total amount extracted and losses in a 100 g mushrooms sample (water and VC)

Pressure (Pa)	Extraction time (h)	Trap 1 (g)	Trap 2 (g)	Trap 3 (g)	Total (g)	Amount lost (g)
35	2	30.912	25.852	22.135	78.899	0.009
23	3	45.861	31.594	31.463	108.918	0.007
32	4	58.122	101.325	30.774	190.221	0.002
47	5	86.234	104.535	19.810	210.579	0.005

Table 5. Effects of extractions times on the amounts collected in the three traps, the total amount extracted and losses in a 50 g mushrooms sample (water and VC)

Pressure (Pa)	Extraction time (h)	Trap 1 (g)	Trap 2 (g)	Trap 3 (g)	Total (g)	Amount lost (g)
35	2	13.913	7.951	2.863	24.727	0.005
23	3	21.641	17.124	8.326	47.091	0.005
32	4	28.776	49.482	26.430	104.688	0.003
47	5	42.016	49.812	13.381	105.209	0.002

In this study, ambient temperature vacuum distillation was used and optimised for extraction of VC from *A. bisporus*. During a preliminary extraction experiment without a water bath, the temperature of the round bottom flask and its content dropped due to adiabatic cooling when the temperature of the system is reduced without any heat being exchanged between the system and the environment if the pressure of the system is reduced. Therefore, a water bath set at 25°C was used to maintain a constant temperature. It should be also noted that the vapour pressure related to the temperature and the concentration of a VC depend on the partial pressure of the pure compound and the temperature. Covarrubias-Cervantes *et al.* (2004) showed that when the temperature decreased, volatility also decreased.

As expected, the content of the traps was frozen due to sublimation of condensable gases. These gases then condense on the cold surface appearing as a frost on the trap. This is the reason why the traps were left overnight to allow any condensation to take place and minimising the error given by the balance as the weight is recorded.

Conclusion

The optimised method developed in this study considered several factors affecting the extraction efficiency such as extraction time, boiling effect, and extraction temperature. The method has the

potential to be used as a laboratory technique or at industrial level to obtain unaltered mushroom aroma extracts representative of the original mushroom aroma. Furthermore, the extracts are suitable for sensory analysis as no organic solvent is used in the extraction.

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