CHARACTERISATION OF FLAVOUR AND TASTE COMPOUNDS IN *AGARICUS BLAZEI* MURRILL *SENSU* HEINEM., THE CULTIVATED ALMOND MUSHROOM

Tjakko Stijve, Sentier de Clies no 12, CH-1806 St.-Légier, Switzerland.
Maria Angela L. de A. Amazonas, Centro Nacional de Pesquisa de Florestas, Embrapa Florestas, Colombó, Paraná, Brazil.
Véronique Giller, Nestlé Research Center, P.O. Box 44, CH-1000 Lausanne 26, Switzerland.

Abstract

Agaricus blazei Murrill *sensu* Heinem. is a new cultivated medicinal and gourmet mushroom which is currently popular in Brazil, Japan and China. It is also cultivated in the USA, and it has recently drawn the attention of European mushroom growers. Upon investigating the mushroom's pleasant almond flavour, it was observed that benzaldehyde and its precursor benzoic acid were the major components of the volatile fraction. Other benzylic compounds contributing to the flavour were benzyl alcohol, methyl benzoate and 4-hydroxybenzaldehyde. When reconstituting the commercially available dried mushrooms , almond flavour develops, presumably by enzymic conversion of benzoic acid to benzaldehyde and benzyl alcohol. Since benzoic acid is present at concentrations of 1280–3100 mg/kg dry weight, it may contribute to the excellent shelf life of the mushroom. Interestingly, benzoic acid also occurs in several close relatives of *A. blazei*, suggesting that this compound could well be a taxonomic marker. Among the non-volatile taste compounds, mannitol predominated to the extent of 22% on dry weight. Contents of taste-enhancing free glutamic and aspartic acids were comparable to those reported in the White button mushroom (*Agaricus bisporus*). The mycelium of *A. blazei* was found to be poor in nearly all compounds investigated. No almond flavour was observed and its crude protein content was only 13% compared with an average value of 30% in the dried mushrooms. Moreover, it had less than 1% of mannitol and only very low levels of free amino acids. Typical secondary metabolites as urea, free tryptophan and agaritine were even totally absent.

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Introduction

Agaricus blazei Murrill *sensu* Heinem. (Heinemann 1993) is a new cultivated edible mushroom that is already popular in Brazil, Japan and China. It has recently turned up in the USA, where Paul Stamets (2000), the well-known American mushroom grower, has rapidly mastered its culture. The mushroom is an excellent edible, having a pleasant almond taste and a texture that is much better than that of any other agaric, including the White button mushroom, *Agaricus bisporus*, also known as the 'Champignon de Paris'. *Agaricus blazei* Murrill has a variety of common names. In Brazil it is called Cogumelo do Sol (Mushroom of the sun), whereas the Japanese refer to it as Himematsutake. Stamets, capitalising on the popularity of the robust Portobello button mushroom in his country, has proposed the name 'Almond Portobello'.

At present, the mushroom is most widely used for its medicinal virtues. It is said to contain 4 to 6% beta glucans, immuno-potentiating polysaccharides that also inhibit the growth of malignant tumors. Indeed, dried *A. blazei* and its medicinal preparations are widely advertised on the Internet. Still, the mushroom has definitely a future as a gourmet mushroom, because of its excellent nutritional qualities and large gastronomic potential. Commercial cultivation has been established in several countries, including China and Korea, which means that the price will probably soon come down. In a number of European research centres cultivation of *A. blazei* has been initiated with encouraging results. A general article about this mushroom and its domestication was recently published by Stijve & de A. Amazonas (2002).

In this paper the results of an investigation of both the volatile flavour compounds and the non-volatile taste components of *A. blazei* is reported. Although Chang *et al.* (2001) analysed its mycelia for monosodium

glutamate and 5'-nucleotides, we have not found any reports on the composition of its volatile flavour in the available literature.

The mushroom, whether wild-growing or cultivated has a most agreeable almond odour, especially when freshly picked, but it is also noticeable in the dried mushrooms of commerce, when these are soaked in water prior to culinary preparation. Several members of the genus *Agaricus* (e.g. *A. augustus*, *A. subrufescens* and the related secotioid species *Gyrophragmium dunalii*) also possess a more or less pronounced almond aroma. This feature is associated with the presence of benzaldehyde, which has indeed been identified as a major volatile compound in the said mushrooms (Chen & Wu 1984, Rapior *et al.* 2000, Wood *et al*. 1990). Even the ordinary cultivated white mushroom, *A. bisporus* contains benzaldehyde (Cronin & Ward 1971), but in this species the strong 'mushroomy' odour of 1-octen-3-ol predominates (Cronin & Ward 1971, Dijkstra & Wiken 1976, Hanssen 1982, Tressl *et al.* 1982).

Materials and Methods

Mycelia and mushrooms
Dried mycelium and its corresponding mushrooms were obtained from Paul Stamets, Olympia, USA. Several dried cultivars were supplied by commercial growers in the Brazilean States of Paraná and Minas Gerais. A collection of wild-growing *A. blazei* came also from Paraná, made in a heap of mowed and decomposed grass at the National Forest Research Centre, Embrapa Florestas. Other agarics analysed were obtained in Switzerland and in France.

Isolation and gas chromatography analysis of the volatile fraction
Isolation of the flavour compounds was performed by simultaneous extraction and distillation, using pentane-diethyl ether 1:1 v/v as a solvent (Chen & Wu 1984, Römer & Renner 1974) Ten gram test portions were rehydrated with 100 ml water prior to extraction. Gas chromatography and mass spectrometric identification (GC-MS) were carried out under conditions similar to those as described by Rapior *et al.* (2000).

HPLC determination of benzoic acid
An aqueous extract of the test portions was clarified, diluted with methanol, and directly subjected to HPLC using a C-18 bonded silica gel column, and a phosphate buffer-methanol mixture as a mobile phase. Detection was by spectrophotometry at 227 nm (Stijve & Hischenhuber 1984).

Free amino acids
Free amino acids were extracted from the dried mycelium and mushrooms by refluxing several hours with 80% ethanol. After evaporation of the solvent at 40°C under reduced pressure, the remainder was analysed by classic ion exchange chromatography as described in the AOAC manual (Cunniff 1996).

Soluble sugars and sugar alcohols
Simple sugars and sugar alcohols were determined according to the manufacturer's manual of the DX 500 DIONEX system equipped with an ED 40 electrochemical detector. Sugars and sugar alcohols were extracted with water, and separated by ion chromatography on an anion exchange column (CarboPack MA 1). Electrochemical detection of the eluted compounds was by means of a pulsed amperometric detector and quantification by comparison with the peak areas of a series of standards. In addition, a rapid semi-quantitative analysis of the polyols was performed by thin-layer chromatography (Andary *et al*. 1979).

Results and Discussion

Volatile flavour compounds
The volatile compounds identified in *A. blazei* can be divided in several groups (Table I). Benzylic derivatives (benzoic acid, benzaldehyde, benzyl alcohol, methyl benzoate and 4-hydroxybenzaldehyde) predominated, whereas phenylethyl compounds were less important. The C-8 aliphatic volatiles (e.g. 1-octen-3-ol and derivatives) which are well-known as flavour constituents of many other mushrooms (Buchbauer *et al.* 1993), amounted only to 3% of the total surface of the GC signals. Several unidentified compounds were observed, but they seemed quantitatively less important.

Table 1. Volatile compounds (mg/kg dry weight) in *Agaricus blazei*.

	Cultivar from Paraná, Brazil, Sample 01	**Ditto from Minas Gerais, Brazil Sample 07**	**Wild-growing, collected in Embrapa Florestas, Colombo, Paraná, Brazil**
Intensity of Almond flavour	+++	+	++
Major volatiles* **Benzaldehyde**	2430	428	885
Benzoic acid	2890	2250	1560
Benzyl alcohol	375	254	180
Methyl benzoate	220	107	58
4-hydroxy-benzaldehyde	116	86	99
Other constituents** **2-phenyl ethanal**	110	65	48
2-phenyl ethanol	75	72	65
2-phenyl acetic acid	90	110	42
C-8 compounds*** **1-octen-3-ol**	42	38	45
1-octen-3-one	45	32	15
3-octanol	20	20	n.d.
3-octanone	18	15	12
Aliphatic C5 + C6 ***alcohols****			

* 68–75%, ** 4–6%, *** < 3%, **** < 2% of total volatiles, n.d. = not detected.

As presumed, benzaldehyde is undoubtedly responsible for the almond flavour of the mushroom. Cultivar 01 having the most pronounced odour contained almost six times more of this compound than cultivar 07 in which the flavour was weak indeed. Interestingly, wild-growing *A. blazei* had an intermediate almond odour and benzaldehyde concentration. It must be taken into account, however, that production of flavour compounds by mushrooms depends on the composition of the growth medium/substrate, growth conditions, different stages of growth, and genetic variations of the strains (Jong & Birmingham 1993). We paid special attention to the possible presence of two other compounds possessing a similar odour. However, GC-MS indicated absence of p-tolualdehyde, which gives the fungus *Mycoacia uda* its strong smell of bitter almonds (Sastry *et al.* 1980). In a separate test (Stijve & de Meijer 1999), the collections also proved negative for hydrocyanic acid, which is not only contained in several food plants, but also in a number of cyanogenic mushrooms. Benzyl alcohol and methyl benzoate probably contribute a sweet flowering note. Since the three samples also contained several unidentified volatiles at varying concentrations, their contribution to the overall flavour—although unlikely—cannot be ruled out at this moment. In all three samples benzoic acid was the major volatile compound, but since it is odourless, it does not contribute directly to the mushroom's flavour. Chen & Wu (1983) have rightly pointed out that this compound may well be the precursor of benzaldehyde. They postulated the existence of a reductase system converting benzoic acid into benzyl alcohol and benzaldehyde in both *Agaricus subrufescens* and *A. bisporus.* Evidence for such a system was obtained by blending fresh ordinary white mushrooms with benzoic acid, whereupon the formation of the almond smelling volatiles significantly increased. Oddly, the authors did not report any benzoic acid among the volatile fraction of *A. subrufescens* (Chen & Wu 1984), but this compound was probably not eluted from the Carbowax 20M column the authors used in the GC-MS determinative step.

The enzymes involved in the biosynthesis of benzaldehyde apparently survive for a long time in mushrooms which have been dried at a temperature below 40°C. A one-year-old herbarium collection of wild-growing *A. blazei,* rapidly developed a pleasant almond odour when moistened with water. So did most 12–18 month old samples of *A. blazei* that had been powdered for analysis, and kept in the freezer. This reconstitution experiment failed on a mycelium sample, but this material did not even smell of almonds upon receipt. Since Rapior *et al.* (2000) reported both benzaldehyde and benzoic acid in *Gyrophragmium dunalii*, we examined some adult specimens of this secotioid mushroom that had been kept at -10°C for two years. Upon thawing and concurrent disruption of the tissue cells, the enzymes were apparently reactivated, and a faint but distinct almond odour developed.

It should be pointed out that benzoic acid is also formed by oxidation of benzaldehyde. Since *A. blazei* is dried after harvest on gauze wire in a stream of warm air, it is highly probable that much benzaldehyde is lost in this process, either by volatilisation or by conversion to benzoic acid. This loss of flavour is compensated by the enzymic reaction proceeding upon reconstitution of the mushrooms, provided that the enzymes were not destroyed upon drying.

Benzoic acid

Since literature reports on the occurrence of benzoic acid in mushrooms are scarce indeed, we decided to analyse all available *A. blazei* and some samples of related mushrooms for this particular compound. Since the GC-MS method is too laborious for this purpose, we used the more straightforward HPLC procedure proposed by Stijve & Hischenhuber (1984). For this purpose, a 2,5 g test portion of the powdered dried mushroom was reconstituted in 20 ml water prior to extraction. After clarification, HPLC determination did not pose any problems. All samples contained co-extractives, contributing peaks to the chromatogram, but no interference was observed when subjecting the benzoic acid signal to diode array analysis. The results of this investigation involving 24 samples are listed in table II.

Table 2. Benzoic acid content (mg/kg dry weight) of *A. blazei* and some related mushrooms.

SAMPLE	ORIGIN	BENZOIC ACID CONTENT
A. blazei mycelium	Olympia, WA, USA	42
A. blazei mushrooms, cultivated on cow dung	ditto	1760
Ditto, cultivated on saw dust	ditto	1540
A. blazei mushrooms N = 7	Produced in the Brazilean States Paraná and Minas Gerais	1280–3100
A. bisporus, white, N = 4	Switzerland	58–150
Ditto, brown, N = 4	ditto	90–370
A. bitorquis, wild-growing	ditto	87
A. xanthodermus, ditto	ditto	< 10
A. silvaticus, ditto	ditto	< 20
A. silvicola wild-growing	ditto	1850
A. augustus, ditto	ditto	2540
Gyrophragmium dunalii, wild-growing	Ile d'Oléron, France	1230

Apparently, formation of benzoic acid mainly takes place in the mushrooms, since the mycelium hardly contains any. All *A. blazei* samples, whether cultivated on cow dung, saw dust or bagasse, contain comparable quantities of this metabolite. Cultivated White mushrooms (*A. bisporus*) have low levels of benzoic acid, and the brown variety contains about twice as much as the white. Wild-growing edible *A. bitorquis* has also very little, and the compound was absent from *A. silvaticus* and *A. xanthodermus.* Interestingly, high amounts of benzoic acid were only present in *A. blazei*, *A. silvicola* and *A. augustus*, species belonging to the subsection *Arvenses*, which suggests that the compound could well be a taxonomic marker. That the rare *Gyrophragmium dunalii* contains a comparable quantity of benzoic acid is not surprising. Indeed, this secotioid mushroom and the representatives of the said section *Arvenses* have many morphological and chemical characteristics in common (Guinberteau1999, Rapior *et al.* 2000, Stijve *et al.* 2001).

Since the benzoic acid concentration of the said mushrooms is on the average 2000 mg/kg or 0,2%, it may well act as a preservative, especially in *A. blazei*, which has an excellent shelf life (Stamets 2000). Furthermore, *G. dunalii* can be kept in the refrigerator (at 5–7°C) for as long as two months without apparent degradation (Guinberteau 1999). In addition, during HPLC analysis, these mushrooms produced several more UV-absorbing peaks suggesting the presence of phenolic compounds—one was tentatively identified as p-hydroxy benzoic acid —which probably also have a marked bactericidal action.

It is somewhat puzzling that benzoic acid has not yet been recognised as a significant constituent of these edible mushrooms.). In a study of carboxylic and fatty acids in *Agaricus* mushrooms, Abdullah *et al.* (1994) found only a minor concentration of benzoic acid in cultivated *A. bisporus*, but about 100 times more in an *A. silvicola* extract. Unfortunately, these authors made no attempt to quantify the compound.

Soluble sugars and sugar alcohols

The principal sugars and polyols occurring in edible mushrooms are trehalose, glucose, mannitol and arabitol (Laub & Woller 1984), which may well contribute to the taste. The results of a comparative HPLC analysis of mycelium and mushrooms grown on two substrates are reported in Table 3. Both mycelia and mushrooms have about the same low concentrations of glucose and fructose, but biosynthesis of trehalose and the polyols apparently mainly takes place in the fruit bodies.

Table 3. Soluble sugars and polyols in mycelium and mushrooms of *A. blazei* (percentage on dry weight).

	Mycelium		**Mushrooms grown on saw dust**		**Mushrooms grown on bagasse**	
	1.	**2.**	**1.**	**2.**	**1.**	**2.**
Arabitol	0,01	0,01	0,13	0,13	0,18	0,18
Trehalose	0,01	0,01	0,83	0,85	1,72	1,76
Mannitol	0,75	0,74	17,83	17,78	22,63	22,61
Glucose	0,62	0,61	0,66	0,70	0,23	0,23
Fructose	0,07	0,06	0,06	0,06	0,08	0,10

These results are different from those reported by Chang *et al.* (2001) who found in the mycelium 3,14% arabitol, 4,53% glucose and 2,39% trehalose. The values for the sugars are higher than those we measured both in the mycelium and in the mushrooms. The high level of arabitol and the absence of mannitol reported by the Chinese scientists can only be explained by assuming an analytical error. Indeed, arabitol is a polyol that is typical for some subsections among the Boletales (Andary *et al.* 1979), but it is only a minor constituent of dark-spored gilled fungi like *Agaricus* species, which invariably contain much mannitol. This also applies to cultivated *A. bisporus* that has an average content of 20% mannitol on dry matter (Laub & Woller 1984).

TLC screening of other *A. blazei* samples revealed that arabitol content was often below detection, whereas mannitol concentrations were in the range of 15–22%. The sum of the sugar concentrations fluctuated between 0,5–3% on dry weight.

Free amino acids

The total free amino acid content of the mushrooms was about 10 times higher as that of the mycelium (Table 4). The presence of seven essential amino acids was noted. The sum of glutamic acid, glutamine, aspartic acid and alanine amounted to more than 70% of the total. Low levels of ornithine and γ-amino butyric acid were also observed in the mushroom extracts, but no quantitation was attempted. Interestingly, the free amino acid pattern of *A. blazei* mushrooms resembles that of the cultivated *A. bisporus* as reported earlier (Eisenhut *et al*. 1995, Kurkela *et al*.1980).

Table 4. Free amino acid content (percentage on dry weight) of *Agaricus blazei* cultivars.

	MYCELIUM (N = 2)	**MUSHROOMS (N = 4)**
Aspartic acid	0,05–0,12	0,62–0,97
Threonine*	0,04–0,05	0,08–0,12
Serine	~ 0,01	0,10–0,18
Glutamic acid	0,02–0,10	1,28–1,75
Glutamine	n.d.	0,65–0,90
Proline	n.d.	0,05–0,12
Glycine	n.d.	0,03–0,08
Alanine	0,10–0,12	0,48–0,75
Valine*	0,08–0,15	0,10–0,16
Isoleucine*	~ 0,02	0,05–0,08
Leucine*	0,04–0,05	0,08–0,11
Tyrosine	n.d.	0,12–0,21
Histidine	0,02–0,03	0,05–0,07
Lysine*	0,04–0,06	0,11–0,13
Phenylalanine*	~ 0,01	0,10–0,14
Arginine	0,03–0,05	0,15–0,32
Tryptophan*	n.d.	0,05–0,12
TOTAL	**0,45–0,77**	**4,10–6,21**

n.d. = not detected, * essential amino acids.

As Yamaguchi (1979) has rightly pointed out, the free amino acids aspartic and glutamic acids are mainly responsible for bringing out the umami taste in mushrooms. These monosodium glutamate-like (MSG-like) amino acids are present at appreciable concentrations and contribute therefore to the palatable taste of *A. blazei*.

The results for most of the free amino acids in the mycelium agree rather well with those reported by Chang *et al*. (2001), although these authors could not detect any glutamic acid. Summarising it can be said that the mycelium was found poor in taste-active compounds (flavour components, mannitol, and MSG-like amino acids). In addition, the mycelium's crude protein content was only 13% compared with an average value of 30% measured in the dried mushrooms. Moreover, noting absence of free tryptophan in the mycelium and its relative high content in the mushrooms, we subjected a methanolic extract to TLC analysis for other typical secondary metabolites (Stijve et *al*. 1986). Not surprisingly, urea and agaritine, which amounted both to 0,5–1% in the mushrooms, were found to be conspicuously absent from the mycelium.

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