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Agaritine content of 53 *Agaricus* species collected from nature

Abstract

Fifty-three different species of the genus *Agaricus* were collected in the Czech Republic during the period 1998-2001 and identified by an experienced mycologist. The samples were analysed for agaritine (N^2-(γ-L-glutamyl)-4-hydroxymethylphenylhydrazine) content, a precursor to a suspected rodent carcinogen. There was a huge variation in agaritine content between species, less variation between samples of a species. Whereas the cultivated mushroom *Agaricus bisporus* commonly contain 200-500 mg agaritine/kg fresh weight, no less than 24 of the 53 species contained agaritine levels above 1000 mg/kg fresh weight. The highest level was found in *A. elvensis* containing up to 10,000 mg/kg fresh weight. Seventeen species contained intermediate levels (125-1000 mg/kg), and twelve species below 125 mg/kg. Some of the species producing low levels of agaritine might be candidates for future strain-development of *Agaricus* mushrooms for cultivation. No correlation could be observed between the agaritine content and size of the mushroom, week of the year when collected, year of collection, or site of collection. Besides occurring in the genus *Agaricus*, some species of the genera *Leucoagaricus* and *Macrolepiota* were also shown to contain agaritine.

Introduction

The commonly eaten cultivated mushroom, *Agaricus bisporus*, belongs to the *Agaricaceae* family. For a long time *A. bisporus* was believed to have been derived from several different wild *Agaricus* species (Toth 2000), and its continued propagation over many centuries to have resulted in several commercial strains which have no exact counterpart in nature. This hypothesis has now been proven incorrect as wild *A. bisporus* populations

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have been identified in various parts of the world (Mozina et al. 1993; Kerrigan 1995). In some countries, strains of other species, such as *A. bitorquis,* are the preferred cultivated varieties (Schulzová et al. 2002).

In 1961 Levenberg isolated a characteristic glutamine-containing compound from the press-juice of *A. bisporus.* He characterised the compound physically and elucidated its chemical structure as N^2-(γ-L-glutamyl)-4-hydroxymethylphenylhydrazine (Figure 1), and gave it the trivial name agaritine. As shown in Table I, the level of agaritine in the cultivated mushroom is fairly high, usually in the range 200 to 500 mg/kg fresh weight (Andersson and Gry 2004), but levels as high as 1700 mg/kg fresh weight have been reported (Liu et al. 1982).

[Fig. 1 inserted about here]

[Table I inserted about here]

Agaritine is the predominant, although not the only phenylhydrazine derivative in *A. bisporus*. The compounds 4-carboxyphenylhydrazine, N^2-(γ-L-glutamyl)-4-carboxy-phenylhydrazine, and 4-hydroxymethylbenzenediazonium ion make up around 10% of the total phenylhydrazines in the mushroom (Levenberg 1962; Chauhan et al. 1984, 1985; Ross et al. 1982).

The potential carcinogenicity of *A. bisporus*, and the possible role of agaritine and the other phenylhydrazine derivatives in inducing this severe adverse effect, has been a matter of controversy for a number of years (Gry and Pilegaard 1991; Gry and Andersson 1998; Toth

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2000; Andersson and Gry 2004). In long-term *in vivo* studies, a diet of fresh or baked *A. bisporus* for 3 days per week over 2 years produced tumours in various tissues of both male and female outbred Swiss mice (Toth and Ericsson 1986; Toth et al. 1997a). An elevated tumour incidence, although not statistically significant, was observed when baked mushrooms were fed to mice according to a more balanced feeding schedule - 12 hours each day for 5 days each week during life-time (Toth et al. 1997b). Similarly, oral administration of 4-carboxyphenylhydrazine or N^2-(γ-L-glutamyl)-4-carboxyphenylhydrazine, or the 4-hydroxymethylbenzenediazonium ion resulted in tumour induction in Swiss mice (Toth 2000). The main phenylhydrazine in the mushroom, agaritine (98% pure), however, did not result in tumour induction when administered in drinking water in a long-term carcinogenicity study (Toth et al. 1981). This observation is unexpected, as agaritine has been shown to bind covalently to mouse DNA (Shephard and Schlatter 1998) and to increase the mutation frequency in forestomach and kidney tissue of lacI transgenic mice *in vivo* (Shephard et al. 1995). As it was recently shown that agaritine is unstable in oxygenated aqueous solution (Hajslova et al. 2002), it cannot be ruled out that the exposure to agaritine was lower than expected in the long-term carcinogenicity experiment.

Until it has been established whether consumption of the cultivated mushroom constitutes a risk for adverse health effects or not, it seems advisable to abstain from exaggerated exposures to phenylhydrazine derivatives (Schulzová et al. 2002). This could be done by growers substituting the strains of the cultivated mushroom producing high levels of agaritine with strains or other *Agaricus* species having comparable organoleptic properties but producing lower levels of agaritine. Sources for such strains may be available from the

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nature. The present study reports on the quantity of agaritine detected in 53 different *Agaricus* species collected in the Czech Republic. The data may also be of interest for mushroom pickers when assessing the edibility of *Agaricus* species to be collected.

Materials and Methods

Samples and chemicals

Different *Agaricus* species were picked at various locations in the Czech Republic (most of them in the central part of Bohemia, close to Prague) during the mushroom growing seasons 1998-2001. All species were determined by one of the authors (Jiri Hlaváček), an experienced mycologist specialized on the taxonomy of the genus *Agaricus*. The *Agaricus* taxonomy remains far from clear, despite the contributions of many authors over the years, especially Pilát (1951), Möller (1952), Essette (1964), Heinemann (1977), Wasser (1980), Cappelli (1984), Knudsen (1992), Bohus (1995), Nauta (2001) and Parra (2003). As a consequence, mycologists advocate different schools of *Agaricus* taxonomy. We have chosen the practical approach of presenting both the taxonomic names as given by the mycologist who determined the mushroom species, J. Hlaváček, who used more narrow concepts identifying a species, and the taxonomic names commonly used in the Western and Northern European countries where broader concepts identifying the various *Agaricus* species are prevailing (Knudsen 1992; Nauta 2001).

Most mushroom samples delivered to the Institute of Chemical Technology (Prague) for chemical analysis of agaritine content were clearly identified and did not require further consultation. In a few cases where the delivered samples were not adequately identified,

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Hlaváček was consulted again. As he unfortunately died during the present study, his colleagues Borovička and Burel took over the confirmation step of a few samples.

Mushrooms were stored in the refrigerator until analysed (Schulzová et al. 2002), which generally was within three days from being collected. We recently showed that the agaritine content of cultivated *Agaricus* purchased on the open market remained unchanged for the first three days in the refrigerator (5°C), after which the agaritine content started to decrease (Schulzová et al. 2002).

Water used for preparation of solutions was distilled and further purified using the Milli Q RG purification system (Millipore, Germany). Methanol used for extraction was supplied by Penta (Chrudim, Czech Republic), and gradient grade methanol for the HPLC mobile phase was purchased from Merck (Darmstadt, Germany). The agaritine standard was synthesised by Henrik Frandsen, Institute of Food and Veterinary Research, according to the method of Wallcave et al. (1979), but with some important modifications being introduced (Frandsen 1998). The synthesised agaritine was more than 85% pure as shown at wavelength 200, 237 and 280 nm by high performance liquid chromatography (HPLC). The purity factor of the agaritine peak reported by the Hewlett Packard ChemStation peak purity software was 999.9. The main impurity (11.9-13.6%) in the standard originated from the agaritine synthesis and was stable during the experiments reported here. The standard was protected from light and stored under oxygen-free nitrogen in the freezer.

Sample preparation

Agaritine standard for identification: a stock solution of agaritine in methanol (0.25 mg/ml)

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was prepared. From this 100 µl were transferred into vials and evaporated to dryness by a stream of oxygen-free nitrogen. The vials were stored sealed in the freezer. Stability tests showed the dry agaritine standard in the freezer to be stable (Schulzová et al. 2002). Before analysis, the agaritine standard was dissolved in 1 ml of Milli Q water and analysed within 6 hours (Schulzová et al. 2003).

Approximately 20 gram of fresh whole mushroom was mixed with 100 ml methanol and homogenised for 10 minutes in an Ultra Turax (Janke a Kunkel, IKA-Werk). The homogenate was shaken for 30 minutes and a crude extract prepared by filtration. The volume of the filtrate was adjusted to 200 ml with methanol, 10 ml of the extract was evaporated to dryness and the residue dissolved in 2 ml Milli Q water. This solution was filtered through a microfilter (25 mm Filter Unit, 5.0 µm PTFE, PP, ThermoQuest) into a vial and an 20 µl aliquot injected (always within 6 hours) onto the HPLC column.

Identification and Quantification

HPLC was performed using a Hewlett-Packard HP 1100 liquid chromatograph (Wallbron, Germany) equipped with diode array detector (DAD) and autosampler. A sample amount of 20 µl was applied to the separating column (250x4 mm), LiChrospher 100 RP-18 (5µm), Merck, with precolumn (4x4 mm), Li Chrospher 100 RP-18 (5 µm), Merck. Optimization studies showed a methanol-water mixture to be suitable as mobile phase for separation; the mobile phase gradient being 10% methanol held for 5 min, changed to 80% over 15 min, and held at 80% methanol for 5 min. Total run time was 25 min, post time was 5 min. The flow rate was 1 ml/min, the column temperature being 35^{o}C. All analyses were run in duplicate. For identification of agaritine the retention time, DAD spectra and peak purity

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software function were used. Agaritine was detected at 237 nm and quantified by comparing the peak area of the analysed sample with the peak area for known amounts of the pure standard. Free amino acids that strongly absorb in the UV were shown not to interfere with the agaritine peak. The correlation coefficient of the UV spectra (normalized absorbance) of agaritine in standard solution and in a real sample was 0.999. The chromatograms and UV spectra of the agaritine standard and an *Agaricus campestris* sample are shown in Figure 2. The detector response was linear in the range 0.2 μg/ml – 2.0 mg/ml, the equation of the linear regression of the calibration curve being y=31.325x + 17.584, with a correlation coefficient of R^2=0.9999.. Under the experimental conditions described for sample preparation, the limit of detection for the method was 0.02 μg/ml (based on a signal/noise of 3:1), corresponding to 0.2 mg/kg fresh weight of mushroom, limit of quantification was 0.6 mg/kg. The repeatability of measurements at agaritine levels commonly found in fresh mushroom samples (200 mg/kg), expressed as the relative standard deviation, was 4.5% for six replicates. Method validation has been presented elsewhere (Schulzová et al. 2002).

[Fig. 2 inserted about here]

The data was analysed by Student's T-test (α=0.05), using the software of Microsoft Excel 2003.

Results

Mushrooms of the genus *Agaricus* were collected in various regions of the Czech Republic during different periods of the growing seasons 1998-2001, and identified by an experienced mycologist. Fifty-three different species were found. Table II shows the

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average agaritine contents of the various *Agaricus* species identified, and, where appropriate, the range in agaritine content, without controlling for mushroom size. When the average agaritine content was calculated, a value of 50% of the detection limit was used for the samples in which no agaritine could be detected. Only a few samples were available for many of the more uncommon *Agaricus* species.

[Table II inserted about here.]

A significant difference in agaritine content was observed between species. The amounts detected in individual species varied from non-detectable levels up to several g per kg fresh weight. One sample of *A. elvensis* contained 10 g/kg fresh weight. Some species were characterized by low agaritine content (<100 mg/kg fresh weight), others by intermediate (100-1000 mg/kg fresh weight) or high (>1000 mg/kg fresh weight) levels of agaritine. It should be noted that the allocation of species to groups having these arbitrarily set boarders is hampered by the low number of samples analysed for many species, resulting in less reliable values of average agaritine content.

[Table III inserted about here.]

Nine of the 53 species analysed in our study contained less than 100 mg agaritine/kg fresh weight (Table III), which was the level chosen defining mushrooms with a low agaritine content. Of these nine species with low agaritine content only the relatively rare *A. maskae*, *A. silvaticus* var. *pallidus*, and possibly also *A. caroli* are sought after by mushroom connoisseurs. None of them are common mushrooms.

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Twenty-four of the 53 analysed *Agaricus* species contained high levels of agaritine, more than 1000 mg/kg fresh weight (Table III). In 20 species intermediate levels of agaritine were found, 100-1000 mg/kg fresh weight (Table III), not deviating substantially from the levels reported to occur in the cultivated mushroom, *A. bisporus*.

For the seven species for which more than 10 mushroom samples were analysed (*A. arvensis*, *A. bitorquis*, *A. bisporus* var. *bisporus* (*A. brunnescens*), *A. campestris, A. maleolens*, *A. silvaticus*, and *A. xanthoderma*) it was studied whether a correlation could be found between the agaritine content and size of the mushroom, phenology, year of collection, or site of collection.

No correlation could be found between diameter of the mushroom cap and its agaritine content. A typical example of the poor correlation between cup size and agaritine content is demonstrated by *A. arvensis* ($r = 0.033$; n.s.), Figure 3. These observations contradict the findings of Stijve et al. (1986), that smaller fruit bodies of *A. arvensis* and *A. augustus* contain higher amounts of agaritine than bigger mushrooms of the same species. There was also no correlation in our studies between week of the year when the mushrooms were collected and their agaritine content (data not shown).

[Figure 3 inserted about here.]

Among the 13 *Agaricus* species of which enough mushroom samples had been collected over more than one year, there was in general no statistical difference in the average agaritine content between years (shown by students t-test). The only data that reached

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statistical significance were those of *A. maleolens*. In the years 1998 and 1999 *A. maleolens* contained significantly lower levels of agaritine (51±90 and 25±54 mg/kg fresh weight, respectively), than in year 2000 (165±50 mg/kg fresh weight), t-test, p=0.023. During the first two years the agaritine content was below the detection limit of the analytical method in the majority of the samples, whereas none of the samples collected in year 2000 contained below 100 mg/kg fresh weight. It is our interpretation that this significant difference might have disappeared had larger number of mushroom samples been analysed.

No correlation could be found between agaritine content and geographical region where the mushrooms were collected (data not shown).

In addition to analysing for agaritine in the different *Agaricus* species, we also analysed for agaritine in four species belonging to each of the related genera *Leucoagaricus* and *Macrolepiota.* If agaritine occurs in mushroom species outside the genus *Agaricus*, *Leucoagaricus* and *Macrolepiota* are the genera where agaritine might be found. As shown in Table IV, one of the four *Macrolepiota* species, *M. konradii*, contained low amounts of agaritine, 42 mg/kg fresh weight. Agaritine was also detected in three of the *Leucoagaricus* species – *L. bresadolae*, *L. cretaceus*, and *L. naucinus* - but here the contents were higher, up to 838 mg/kg fresh weight (Table IV). As agaritine has been reported to occur in the cultivated Oyster mushroom *Pleurotus ostreatus* (Burini et al., 1999), we also analysed for agaritine in this mushroom but found none.

[Table IV inserted about here.]

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Discussion

Two research groups, one from the USA and the other from Switzerland, have previously analysed wild-growing *Agaricus* and non-*Agaricus* species for agaritine. Levenberg (1964) examined boiled press-juice extracts from as many as 45 representative genera of wild basidiomycetes collected in the USA. As part of this survey, fruit bodies of 15 different species belonging to the genus *Agaricus* were analysed. Unfortunately, Levenberg used a nomenclature for the mushroom that hardly fit with that used in Europe today. Of the 15 *Agaricus* species, ten were reported to contain agaritine in quantities comparable to those found in *A. bisporus*, whereas five species were devoid of the compound (Table V). The limit of detection for the analytical method (spectrophotometric determination of agaritine by measurement of its rate of enzymatic hydrolysis) used by Levenberg (1964) was not reported. The single sample of fresh *A. bisporus* analysed for its agaritine content was reported to contain 3300 mg agaritine/kg dry weight, which corresponds to approximately 330 mg/kg fresh weight.

[Table V inserted about here.]

Subsequently, Swiss investigators reported HPLC (with a limit of detection in the region 20-25 mg/kg) and thin layer chromatography data on freeze-dried mushrooms, indicating that some wild-growing *Agaricus* species contain substantially higher amounts of agaritine than the cultivated *A. bisporus* (Stijve et al. 1986; Stijve and Pittet 2000). Because a considerable time had elapsed between picking of some mushroom samples and freeze-drying, the latter investigators were unsure that the amounts detected were the

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characteristic levels of freshly harvested wild mushrooms and, therefore, did not give specific information on the quantity of agaritine in the various species.

The agaritine contents given for the various *Agaricus* species in the present study should fairly accurately describe the levels in freshly collected mushroom samples, at least when the number of samples studied for a species have been large enough. Several factors may influence the agaritine content of a mushroom. Studies performed with the cultivated *Agaricus bisporus* have revealed that, in addition to hereditary factors, cultural practice employed in production, stage of cropping-cycle (flush), and maturity of mushrooms at harvest may influence the agaritine level in the mushroom (Andersson and Gry 2004). Some of these factors are likely to influence the agaritine content also of wild-growing species. The length of storage after harvest and the conditions during storage are other factors that may influence the agaritine content (Andersson and Gry 2004). This is exemplified by the data given in Table I. The average level of agaritine in *A. bisporus* obtained directly from the growers was 723 ± 276 mg/kg fresh weight, whereas it was 475 ± 221 mg/kg fresh weight in mushrooms purchased on the open market. We have minimized the potential influence of storage on the agaritine content in the present study by analysing samples within three days after being collected.

Already in 1983, Speroni and co-workers suggested that wild-growing *Agaricus* mushrooms contain higher amounts of agaritine than the corresponding cultivated mushrooms. These investigators had noted that a cultivated strain of *A. bisporus* with brown colour phenotype, PSU-351, which was only recently isolated from nature, contained higher agaritine levels than other cultivated *A. bisporus* strains. It was

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hypothesised that this strain had not yet adapted to the less stressing environmental conditions during cultivation and not yet lost or reduced its inherited ability to produce compounds inhibiting growth of certain competing fungi. A similar observation is made in the present study. In the Czech Republic, it is strains of *A. bitorquis* and '*A. hortensis'* that are cultivated. Samples of these cultivated strains have been reported to contain comparable levels of agaritine, varying between 165 and 457 mg/kg fresh weight, with an average content of 272 mg/kg fresh weight (Schulzová et al. 2002). As shown in Table II, *A. bitorquis* and *A. bisporus* collected from nature contain significantly higher amounts of agaritine, 1 473 and 746 mg/kg fresh weight, respectively.

Although too few samples were analysed for some *Agaricus* species to prove their normal agaritine content, enough samples were analysed to conclude on the agaritine content for other species. As shown in Table II and III, no less than 24 of the studied species contained agaritine levels above 1000 mg/kg fresh weight. This amount should be compared with the levels commonly found in *A. bisporus*, 200-500 mg/kg fresh weight. The highest amounts were observed in *A. elvensis* (mean 4445 mg/kg), *A. purpurellus* (4100 mg/kg), *A. augustus* (3995 mg/kg), *A. fissuratus* (3526 mg/kg) and *A. sagatus* (3479 mg/kg). As there is an ongoing discussion whether it is advisable or not to consume fresh cultivated mushroom (Toth 2000; Andersson and Gry 2004), it seems highly questionable to advise consumption of wild-growing *Agaricus* species with even higher agaritine contents than the cultivated species. Some of the species with a high agaritine content, however, have a firm reputation as being good or even excellent culinary delicacies, e.g. *A. augustus*, *A. fissuratus*, *A. essettei* and *A. substramineus*. On the other hand, as indicated in Table II, some of them are

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recognised as bio-accumulating cadmium and other heavy metals from the soil (Řanda and Kučera 2004)

The present study aimed at identifying tasteful, non-toxic *Agaricus* species with a low agaritine content. Such species would be potential sources for strain development for the mushroom cultivation industry, could it be shown they are devoid of other constituents with potentially harmful effects. Only nine of the analysed *Agaricus* species had an agaritine content bellow 100 mg/kg fresh weight. Of these at least five should be classified as non-edible as they may give rise to gastrointestinal symptoms due to formation of phenol. Based on the agaritine data presented in the present report, the most promising species for strain development seem to be *A. caroli*, *A. maskae*, *A. silvaticus* var. *pallidus*, and possibly *A. squamulifer*. Additional studies are required to establish whether these species fulfil the criteria necessary to be good substitutes for *A. bisporus* as cultivated mushrooms.

We have not addressed the question whether the presence of agaritine within the genus *Agaricus* may have chemotaxonomic significance. However, we have noted that the various sections of the genus *Agaricus* seem to be characterized by different abilities to produce agaritine (Table IV). Stijve et al. (1986) have made a similar observation.

The question whether agaritine may be found in mushrooms outside the genus *Agaricus* has been addressed also by other investigators. Levenberg (1964) was unable to detect agaritine in any of the 30 non-*Agaricus* species analysed (detection limit for the analytical technique not mentioned), and Stijve and co-workers in any of 39 wild-growing and 30

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cultivated non-*Agaricus* species (Stijve et al. 1986; Stijve and Pittett 2000). Two reports on popular cultivated mushrooms are in conflict with these data. Hashida et al. (1990) found very low levels of agaritine in shiitake (*Lentinus edodes*), 0.82 mg/kg fresh weight, and Burini et al. (1999) very high levels (average level 680 mg/kg fresh weight; range 478-905 mg/kg) in the Oyster mushroom (*Pleurotus ostreatus*). With such low quantities of agaritine in fresh shiitake, it came as no surprise that Andersson et al. (1999) were unable to detect agaritine in a Swedish sample of canned shiitake, and that Stijve et al. (1986) were unable to detect agaritine in fresh shiitake using an analytical technique having a limit of detection around 20-25 mg/kg fresh weight. Further studies are needed to determine whether fresh shiitake mushrooms contain low levels of agaritine. The report that agaritine occurs in the Oyster mushroom, is, however, most likely an artefact due to the use of an unspecific analytical method. The only other explanation, that the cultivated strains of *Pleurotus ostreatus* may vary substantially in their ability to produce agaritine seems less likely. Neither Swiss investigators (Stijve et al. 1986; Stijve and Pittett 2000), nor we in the present study could identify agaritine in this species, although the limit of detection was as low as 0.2 mg/kg fresh weight in the present study. Furthermore, there is no indication that agaritine production can be suppressed to negligible levels by the method of cultivation.

However, the data in the present study clearly show that agaritine is present in some species of the genera *Leucoagaricus* and *Macrolepiota*, which both are saprotrophic mushrooms closely related to the genus *Agaricus* (Singer 1986). The compound was detected in *Leucoagaricus bresadolae*, *L. cretaceus*, *L. naucinus*, and *Macrolepiota konradii* (detection limit 0.1 mg/kg), but not in one other species of *Leucoagaricus* and

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three other species of *Macrolepiota* (Table IV). Stijve et al. (1986) found no agaritine in *Leucoagaricus pudicus*, *Macrolepiota procera* and *Macrolepiota rachodes*.

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Agaritine content of 53 *Agaricus* species collected from nature

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Table I. Agaritine content (mg/kg fresh weight) of methanolic extracts of fresh *Agaricus bisporus* as determined by HPLC. If samples were not freeze-dried (which does not influence the agaritine level) shortly after being obtained, they were analysed within 24 hours.

Reference	**Number of samples**	**Diameter of cap (cm)**	**Content**	
			Average	**Range**
Samples directly from growers				
Kelly et al., 1962*	1	not given	~ 400	-
Daniels et al., 1961*	1	not given	~ 220	-
Levenberg, 1964*	1	not given	~ 3300***	-
Liu et al., 1982	14 **	3.0-4.5	880	330-1730
Speroni and Beelman, 1982	1	not given	2 190***	-
Speroni et al., 1983	8 **	2.5-5.0	-	1700-5100***
Fischer et al., 1984	2 **	5.0-6.0	304±6.0; 438±2.5	- -
Sharman et al., 1990	2 **	not given	~ 180	80-250
Andersson et al., 1994	1	not given	340	-
Samples from the market				
Ross et al., 1982b	2	not given	440; 720	170-1170
Fischer et al., 1984	11	2.0-5.5	368±45	94-629
Hashida et al., 1990	1	not given	228	-
Stijve et al., 1986	5**	not given		160-650
Burini et al., et al., 1999	5	not given	820	630-980
Andersson et al., 1999	2	not given	212; 229	-

* gravimetric method; ** different strains: *** mg/kg dry weight (approximately 10% dry matter)

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Table II. Agaritine content (average±S.D.) and usefulness as food (A = popular as food; B = seldom used as food; C = not suitable as food) of different *Agaricus* species collected from the nature in the Czech Republic.

Culi-nary useful-ness	*Agaricus* species (preferred nomenclature according to Hlaváček (1983, 1984a, 1984b, 1984c)	*Agaricus* species - preferred nomenclature according to Knudsen (1992) and Nauta (2001)	Section accord-ing to Nauta (2001)*	Number of samples	Mean content (mg/kg fresh weight)	Range (% with undetectable levels) (mg/kg fresh weight)
B	*A. aestivalis* (F.H. Møller) Pilát	same	Ag	2	824±200	682 - 966
C	*A. altipes* (F.H. Møller) Pilát	same	Ag	1	713	-
A#	*A. arvensis* Schaeff.:Fr.	same	Ar	15	987±334	475 - 1 551
A#	*A. augustus* Fr.	same	Ar	6	3 995±1 885	2 264 - 7 553
A#	*A. perrarus* Schulzer	*A. augustus* Fr.	Ar	2	1 003±512	841- 1 164
B	*A. biberi* Hlaváček (1)	same	?	1	524	-
A	*A. bisporus* (J.E. Lange) Imbach var. *bisporus*	same	B	11	997±490	550 - 2 066
A	*A. bisporus* (J.E. Lange) Imbach var. *avellanea* (J.E. Lange) Singer	same	B	4	639±38	599 - 672
A	*A. bisporus* (J.E. Lange) Imbach var. *albidus* (J.E. Lange) Singer	same	B	3	746±173	613 - 990
A	*A. bitorquis* (Quél.) Sacc.	same	B	11	1 473±781	216 - 3 006
B	*A. bresadolianus* Bohus	same	Sp	1	111	-
B	*A. caespitipes* Hlaváček (2)	*A. bohusii* Bon		2	1 855±24	1 838 - 1 872
A	*A. campestris* L.:Fr.	same	Ag	22	487±475	n.d. (14%) - 2 230
B	*A. cappellianus* Hlaváček (3)	*A. vaporarius* (Pers.) Capelli		11	1 652±826	233 - 3 456
A	*A. caroli* Pilát (4)	*A. benesii* (Pilát) Pilát	Sa	1	n.d.	-
B	*A. devoniensis* Orton	same	B	1	2 089	-
B	*A. elvensis* Berk. & Broome (5, 6)	*A. bohusii* Bon	B	8	4 445±3 074	2 080 - 9 986

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A#	*A. esseteii* Bon (7)	*A. sylvicola* (Vittad.) Peck	Ar	7	2 605±702	1 744 - 3 958
A#	*A. excellens* (F.H. Møller) F.H. Møller (8)	*A. urinascens* (F.H. Møller & Jul. Schäff.) Singer var. *excellens* (F.H. Møller) Nauta	Ar	3	1 539±70	1 481 - 1 617
A#	*A. fissuratus* (F.H. Møller) F.H. Møller (8)	*A. arvensis* Schaeff.	Ar	4	3 526±858	2 430 - 4 553
B	*A. fuscofibrillosus* (F.H. Møller) Pilát	same	Sa	3	597±521 52	152 -1 170
?	*A. heimii* Bon		?	5	1 521±574	1 024 - 2 317
B	*A. impudicus* (Rea) Pilát	same	Sa	2	253±358	n.d. (50%) - 506
A	*A. kotlabae* Hlaváček (9)	same	?	7	1 451±510	963 - 2 341
A	*A. langei* (F.H. Møller) F.H. Møller	same	Sa	1	978	-
A#	*A. leucotrichus* (F.H. Møller) F.H. Møller	*A. arvensis* Schaeff.	Ar	5	1 156±912	383 - 2 534
B	*A. lutosus* (F.H. Møller) F.H. Møller	same	M	1	2 269	-
A#	*A. macrocarpus* (F.H. Møller) F.H. Møller	same	Ar	1	1 501	-
A#	*A. macrosporus* (F.H. Møller & Jul. Schäff.) Pilát (4)	*A. urinascens* (F.H. Møller & Jul. Schäff.) Singer var. *urinascens*	Ar	1	1 049	-
B	*A. maleolens* F.H. Møller (8)	*A. bernardii* (Quél.) Sacc.		19	52±76	n.d. (64%) - 207
B	*A. maleolens* F.H. Møller var. *bernardioides* Hlaváček (A)	*A. bernardii* (Quel.) Sacc.	B	7	186±246	n.d. (29%) - 693
A	*A. maskae* Pilát (10)	*A. litoralis* (Wakef. & A. Pearson) Pilát	Sp	2	63±89	n.d. (50%) - 127
A#	*A. nivescens* (F.H. Møller) F.H. Møller (8)	*A. osecanus* Pilát	Ar	3	584±281	355 - 898
C	*A. pearsonianus* Hlaváček (9)	*A. xanthodermus* Genevier	?	6	103±63	n.d. (17%) - 185
C	*A. phaeolepidotus* (F.H. Møller) F.H. Møller	same	X	2	285±207	139 - 431
C	*A. pilatianus* Bohus	same	X	4	40±46	n.d. (50%) - 82

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C	*A. pilatianus* Bohus var. *iodoformicus* Hlaváček (1)	same	X	1	89	-
B	*A. porphyrizon* P.D. Orton	same	M	1	2 283	-
B	*A. porphyrocephallus* F.H. Møller	same	Ag	1	354	-
C	*A. praeclaresquamosus* A.E. Freeman (11)	*A. moelleri* Wasser	X	4	8±16	n.d. (75%) - 31
C	*A. praeclaresquamosus* Freeman var. *terricolor* (Møll.) Bon & Cappelli (12)	*A. moelleri* Wasser	?	3	386±276	72 - 589
B	*A. purpurellus* (F.H. Møller) F.H. Møller	same	M	1	4 100	-
?	*A. romagnesii* Wasser (13)	*A. bresadolianus* Bohus	Sp	1	60	-
B	*A. sagatus* Fr.		M	1	3 479	-
B	*A. semotus* Fr.	same	B	3	2 755±396	2 299 - 3 021
A	*A. silvaticus* (Schaeff.) Fr.	same	Sa	14	210±305	n.d. (57%) - 920
A	*A. silvaticus* (Schaeff.) Fr. var. *pallidus* (F.H. Møller) F.H. Møller	same	Sa	1	n.d.	-
B	*A. slovenicus* Hlaváček nom. inval.		B	4	1 955±841	1 072 - 3 327
A	*A. squamulifer* (F.H. Møller) Pilát (4)	*A. benesii* (Pilát) Pilát	Sa	2	110±156	n.d. (50%) - 220
A#	*A. substramineus* Courtec. (14)	*A. urinascens* (F.H. Møller & Jul. Schäff.) Singer	Ar	3	2 570±66	2 502 - 2 634
A#	*A. sylvicola* (Vittad.) Peck	same	Ar	6	1 592±446	733 - 1 940
A	*A. tenuivolvatus* (F.H. Møller) F.H. Møller		?	4	1 400±1 361	231 - 3 309
C	*A. xanthodermus* Genev.	same	X	12	12±31	n.d. (67%) - 108

(1) Hlaváček, 2001; (2) Hlaváček, 1999; (3) Hlaváček, 1987; (4) Pilat, 1951; (5) Berkeley and Broome, 1865; (6) Hlaváček, 2000; (7) Bon, 1983; (8) Møller, 1952; (9) Hlaváček, 2002; (10) Pilat, 1954; (11) Freeman, 1979; (12) Bon and Cappelli, 1983; (13) Wasser, 1977; (14) Courtecuisse, 1985. *Ag=Agaricus; Ar=Arvenses; B=Bitorques; M=Minores; Sa=Sanguinolenti; Sp=Spissicaules; X=Xxanthodermi. #Advice not to consume this species due to high heavy metal content may exist.

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Table III. *Agaricus* species with low, intermediate or high levels of agaritine. Section, according to Nauta (2001) is given within brackets (Ag= Agaricus; Ar=Arvenses; B=Bitorques; M=Minores; Sa=Sanguinolenti; Sp=Spissicaules; X=Xanthodermi).

Agaritine content		
low (<100 mg/kg f.w.)	**intermediate (100-1000 mg/kg f.w.)**	**high (>1 000 mg/kg f.w.)**
A. bresadolianus (Sp)	A. aestivalis (Ag)	A. augustus (Ar)
A. caroli (Sa)	A. altipes (Ag)	A. augustus var. perrarus (Ar)
A. maleolens (B)	A. arvensis (Ar)	A. bitorquis (B)
A. maskae (Sp)	A. biberi	A. caespitipes (B)
A. pearsonianus	A. bisporus var. avellanea (B)	A. devoniensis (B)
A. pilatianus (X)	A. bisporus var. bisporus (B)	A. elvensis (B)
A. pilatianus var. iodoformicus (X)	A. bisporus var. hortensis (B)	A. essettei (Ar)
A. praeclaresquamosus (X)	A. campestris (Ag)	A. excellens (Ar)
A. romagnesii (Sp)	A. fuscofibrillosus (Sa)	A. fissuratus (Ar)
A. squamulifer (Sa)	A. impudicus (Sa)	A. heimii
A. silvaticus var. pallidus (Sa)	A. langei (Sa)	A. kotlabae
A. xanthoderma (X)	A. maleolens var. bernardoides (B)	A. leucotrichus (Ar)
	A. nivescens (Ar)	A. lutosus (M)
	A. phaeolepidotus (X)	A. macrocarpus (Ar)
	A. porphyrocephalus (Ag)	A. macrosporus (Ar)
	A. preclaresquamosus var. terricolor	A. porphyrizon (M)
	A. silvaticus (Sa)	A. purpurellus (M)
		A. sagatus (M)
		A. semotus (B)
		A. slovenicus (B)
		A. substramineus (Ar)
		A. sylvicola (Ar)
		A. tenuivolvatus
		A. vaporarius (B)

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Table IV. Average agaritine content (mg/kg fresh weight) in some mushroom species of the genera *Leucoagaricus* and *Macrolepiota* collected in the Czech Republic.

Species as determined by Hlavácek	**Species as interpreted by Knudsen (1992) and Noordeloos et al. (2001)**	**Content (mg/kg fresh weight) (n=sample size)**
Leucoagaricus bresadolae (Schultz.) Bon	*Leucoagaricus americanus* (Peck) Vellinga	368 (n = 1)
L. cinerascens Migl. & Coccia	*L. leucothites* (Vitt.) Wasser *var. carneifolius* (Gillet) Vellinga	n.d. (n = 3)
L. cretaceus (Bull.:Fr.) Moser	*L. cretaceus* (Bull.:Fr.) Moser	838 (n = 2)
L. naucinus (Fr.) Sing.	*L. leucothites* (Vitt.) Wasser	68 (n = 2)
Macrolepiota rachodes var. *bohemica* (Wichansky) Bellù & Lanzoni	same	n.d. (n = 2)
M. konradii (Huijsman ex P.D. Orton) Marchand	*M. mastoidea* (Fr.) Sing.	42 (n = 1)
M. permixta (Barla) Pacioni	same	n.d. (n = 2)
M. rachodes (Vitt.) Sing.	same	n.d. (n = 2)

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Table V. Agaritine levels reported by other investigators in various *Agaricus* species collected from nature.

***Agaricus* species**	**Agaritine content**			
	Levenberg (1964) mg/kg d. w.	**Stijve and Pittet (2000) mg/kg d.w.**	**Stijve et al. (1986) mg/kg dry weight**	**Present study mg/kg fresh weight**
A. abruptibulbus		500 - 5 000		
A. argentatus	+			
A. arvensis		15000-25000	6 500 (200 - 18 500)	987 (475 - 1 551)
A. augustus		15000-25000	8 000 (1 000 - 22 000)	3 995 (2 264 - 7 553)
A. benesi	-			
A. bernardii		doubtful		
A. blazei		500 - 5 000		
A. bisporus		5000 - 15 000		997 (550 - 2 066)
A. bitorquis		15000-25000	7 100 (500 - 20 000)	1 473 (216 - 3 006)
A. campestris	+	5000 - 15 000	2 600 (200 - 10 000)	487 (n.d.-2 230)
A. comtulus	+	500 - 5 000		
A. crocodilinus	+			
A. cupreobrunneus		n.d.		
A. edulis	+			
A. excellens			4 600 (300 - 9 000)	1 539 (1 481 - 1617)
A. fuscofibrillosus		n.d.		597 (152 - 1 170)
A. geesterani		n.d.		
A. haemorrhoidarius		n.d.	n.d.	
A. hondensis		n.d.		
A. hortensis	+	5000 - 15 000		746 (613 - 990)
A. langei		n.d.	n.d.	978
A. lilaceps		500 - 5 000		
A. macrosporus		15000-25000	8 600 (700 - 25 000)	1 049
A. meleagris			n.d.	
A. micromegathus	+			
A. nivescens		500 - 5 000		584 (355 - 898)
A. niveolutescens		500 - 5 000	4 000	
A. patersonii	+			
A. perrarus	+	15000-25000	12 500	1 003 (841 - 1 164)
A. phaeleopidotus		n.d.		285 (139 - 431)
A. placomyces		n.d.		
A. porphyrocephalus		500 - 5 000		354
A. semotus		500 - 5 000		2 755 (2 299 - 3 021)
A. sterlingii	-			
A. subperonatus		5000 - 15 000	2 500	2 788 (2 121 - 3 456)
A. subrutilescens	-			
A. silvaticus	-	n.d.	n.d.	210 (n.d. - 920)
A. sylvicola	-	5000 - 15 000	5 200 (500 - 12 000)	1 592 (733 - 1 940)
A. vaporarius		500 - 5 000	1 000	1 382 (914 - 1851)
A. xanthoderma	+	n.d.	n.d.	12 (n.d. - 108)

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Agaritine content of 53 *Agaricus* species collected from nature

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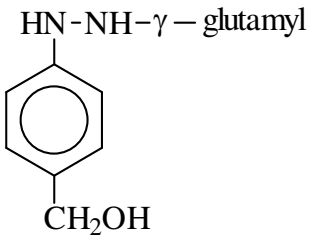

Fig. 1 The molecular structure of agaritine (N^2-[γ-L-glutamyl]-4-hydroxymethylphenylhydrazine).

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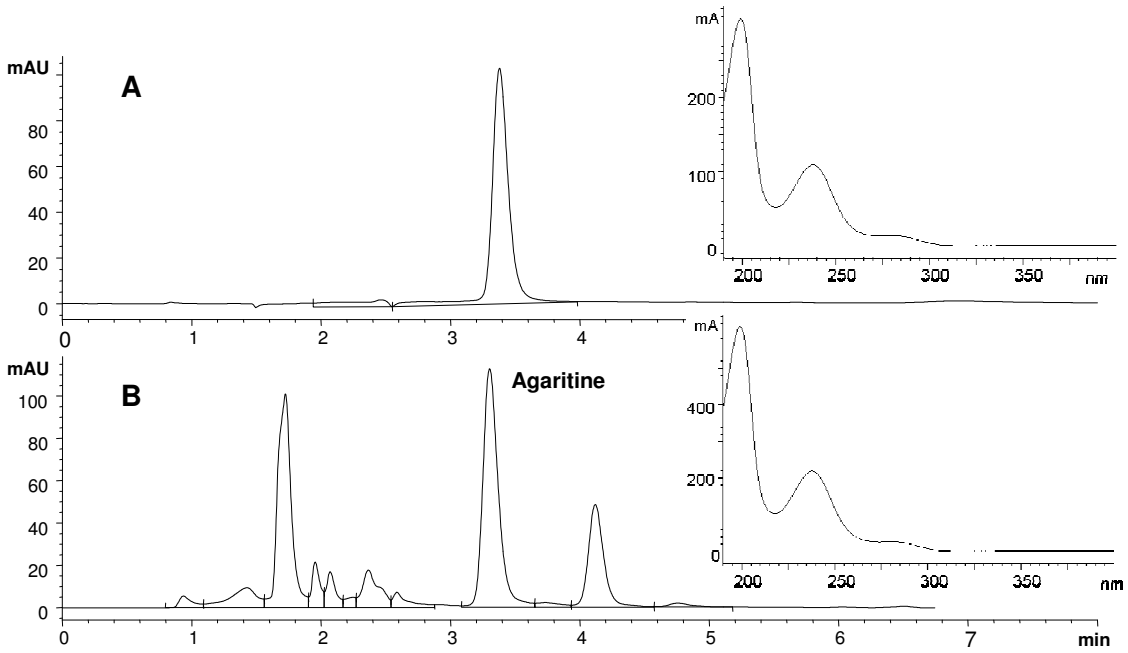

Figure 2. HPLC/UV (237 nm) chromatogram and UV spectrum of (A) agaritine standard - 25 µg/ml, and (B) sample of *Agaricus campestris* with 270 mg agaritine per kg fresh weight.

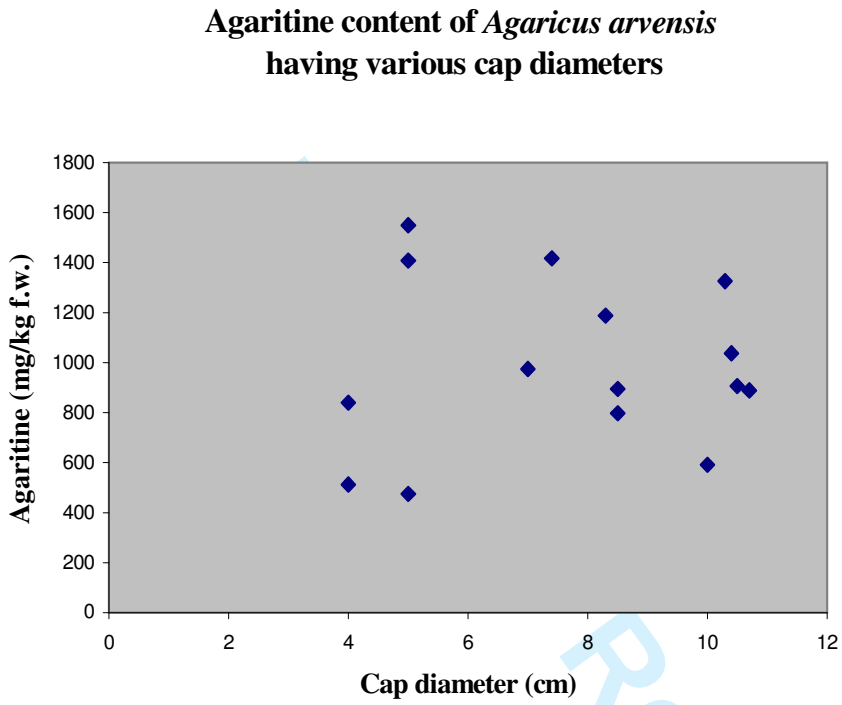

Fig. 3. Absence of a correlation between agaritine content (mg/kg fresh weight) and cap diameter of *Agaricus arvensis*.

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