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Influence of storage and household processing on the agaritine content of the cultivated *Agaricus* mushroom

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*Agaritine (*N*-(γ-*L*(+)-glutamyl)-4-hydroxymethylphenylhydrazine) was identified and quantified by high-pressure liquid chromatography and used as a marker for the occurrence of phenylhydrazine derivatives in the cultivated* Agaricus bitorquis *and* A. garicus hortensis *mushrooms. Although relatively high levels of agaritine (around 700 mg kg^{-1}) could be found in freshly harvested* A. bitorquis *from early flushes, samples from supermarkets contained less agaritine. The content of 28 samples varied between 165 and 457 mg kg^{-1}, on average being 272 ± 69 mg kg^{-1}. The highest amounts of agaritine were found in the skin of the cap and in the gills, the lowest being in the stem. There was no significant difference in agaritine content of the two mushroom species in our study. Pronounced reduction in agaritine content was observed during storage of mushrooms in the refrigerator or freezer, as well as during drying of the mushrooms. The degree of reduction was dependent on the length and condition of storage and was usually in the region 20–75%. No reduction in agaritine content was observed during freeze-drying. Depending on the cooking procedure, household processing of cultivated* Agaricus *mushrooms reduced the agaritine content to various degrees. Boiling extracted around 50% of the agaritine content into the cooking broth within 5 min and degraded 20–25% of the original agaritine content of the mushrooms. Prolonged boiling, as when preparing a sauce, reduced the content in the solid mushroom further (around 10% left after 2 h). Dry baking of the cultivated mushroom, a process similar to pizza baking, reduced the agaritine content by approximately 25%, whereas frying in oil or butter or deep frying resulted in a more marked reduction (35–70%). Microwave processing of the cultivated mushrooms reduced the agaritine content to one-third of the original level. Thus, the exposure to agaritine was substantially less when consuming processed* Agaricus *mushrooms as compared with consuming the raw mushrooms. However, it is not yet known to what extent agaritine and other phenylhydrazine derivatives occurring in the cultivated mushroom are degraded into other biologically active compounds during the cooking procedure.*

Keywords: agaritine, *Agaricus*, *Agaricus bitorquis*, *Agaricus hortensis*, storage, household processing, cooking

Introduction

The cultivated mushroom *Agaricus bisporus* contains agaritine (*N*-γ-L(+)-glutamyl)-4-hydroxymethylphenylhydrazine), two other phenylhydrazine derivatives (4-(carboxy)phenylhydrazine and β-*N*-γ-L(+)-glutamyl)-4-carboxyphenylhydrazine) presumed to be precursors to agaritine, and the 4-(hydroxymethyl) benzenediazonium ion (Andersson and Gry 2002). The latter compound is believed to be formed when agaritine is metabolized (LaRue 1977). Whereas agaritine is found in high quantities, usually between 200 and 450 mg kg^{-1} fresh weight, the other three compounds mentioned occur at lower levels—one-tenth of the amount of agaritine, or less (Andersson and Gry 2002).

Increasingly more data are accumulating from studies on experimental animals and from *in vitro* studies that the cultivated mushroom *per se* and the phenylhydrazine derivatives in the mushroom have adverse effects on health after chronic oral exposure. Toth and co-workers have demonstrated that life-time feeding of

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Swiss albino mice with raw, dry baked and freeze-dried *A. bisporus*, as well as with three of the four phenylhydrazine derivative constituents of the mushroom, induce tumours in the animals (Toth 2000, Andersson and Gry 2002). Agaritine was the only phenylhydrazine constituent of *A. bisporus* being negative in such studies (Toth *et al.* 1981). However, it was recently observed that agaritine is unstable in oxygenated aqueous solutions (Hajslova *et al.* 2002). Since Toth *et al.* (1981) dissolved agaritine in the drinking water given to the animals, it seems possible that the compound was successively degraded and the animals exposed to lower concentrations of agaritine than expected.

Until it has been established whether consumption of biologically relevant amounts of the cultivated mushroom constitutes an acceptable risk for adverse effects on health or not, it seems advisable to abstain from exaggerated exposures to phenylhydrazines. We have analysed the agaritine content of purchased cultivated mushrooms and determined the distribution of agaritine in the fruit body. To investigate whether storage and household processing of the cultivated mushroom may influence the level of phenylhydrazines in the mushroom, we have also performed a series of experiments using various storage conditions and cooking procedures. The stored or ready to consume products were subsequently analysed for their agaritine content. The agaritine content was used as a marker for all phenylhydrazine derivatives in the cultivated mushroom, assuming all of them to be influenced by the processing in a similar way (Gannett and Toth 1991, Toth *et al.* 1997). Our data show that the agaritine content of the cultivated mushroom is reduced to various degrees by boiling, dry baking, frying and microwave heating of fresh mushrooms, as well as by household drying and storage in the freezer followed by thawing.

Materials and methods

Chemicals and samples

For various reasons, *A. bisporus* is not cultivated in the Czech Republic. Instead, two other species are grown, *A. bitorquis* and *A. hortensis*. Fresh cultivated mushrooms of these species were purchased at the open market in Prague. Studies on household processing used fruiting bodies with a diameter of 4–6 cm. Analytical samples were prepared from at least five fruiting bodies, representing the size distribution of the studied mushroom sample. Water used for preparation of solutions was distilled and further purified using the Milli G RG purification system (Millipore, Germany). Methanol used for extraction was supplied by Penta (Chrudim, Czech Republic), and methanol used in mobile phase was gradient grade purchased from Merck (Darmstadt, Germany). The agaritine standard was synthesized by Dr Henrik Frandsen, Danish Veterinary and Food Administration, according to the method of Wallcave *et al.* (1979), but with some important modifications being introduced (Frandsen 1998). The synthesized agaritine was $> 85\%$ pure as shown at wavelength 200, 237 and 280 nm by high-pressure liquid chromatography (HPLC). The purity factor of the agaritine peak reported by the Hewlett Packard ChemStation software was 999.9 (Wallbron, Germany). The main impurity in the standard (13.6%, coming from the agaritine synthesis) did not change during the experiments reported here (Hajslová *et al.* 2002). The standard was protected from light and stored in the freezer (-18°C).

Sample preparation

Agaritine standard for identification. A stock solution of agaritine in methanol ($0.25\,mg\,ml^{-1}$) 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 under nitrogen and sealed in the freezer. The agaritine standard was found stable during 1 year of storage at -18°C (the amount of agaritine was reduced by $< 1\%$). Before analysis, the agaritine standard was dissolved in 1 ml Milli Q water. The stability of this solution was tested. Less than 5% of the agaritine standard was broken down within 12 h. Fresh agaritine standard solutions were prepared for every analytical occasion, and used within 6 h after preparation.

Fresh mushrooms and mushrooms stored in the refrigerator. Twenty grams of fresh mushrooms were mixed with 100 ml methanol and homogenized for 10 min in an Ultra Turax (Janke a Kunkel, IKA-Werk). The homogenate was shaken for 30 min 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 a 20-µl aliquot injected onto the HPLC column. It is important to handle the methanolic extract within 48 h after it has been prepared and to inject the aqueous solution into the HPLC system as soon as possible (not later than 6 h after the preparation of the solution).

Processed mushrooms and mushrooms stored in the freezer. Ten representative fruiting bodies were sliced and mixed. About 20 g of these slices were processed in various ways: (1) boiled in 250 ml water for 5, 10, 20, 30, 45, 60 or 120 min; (2) dry baked at 200°C for 5, 10, 20, 30 or 45 min; (3) fried on a Teflon pan in sunflower oil at approximately 150°C for 10 min (5 min on each side); (4) frying on a Teflon pan in vegetable oil and in butter at about 100°C for 5 min (some water from the mushroom still remain in the system); (5) deep fried (3–15 min) at 170 or 150°C; (6) dried at 25, 50 or 40/60°C until constant weight; (7) heated in a microwave oven (1000 W, 2450 MHz) for 15, 30, 45 or 60 s; and (8) stored in a freezer for 1 week or 1 month at −18°C. After storage in the freezer, agaritine was extracted from samples with methanol either without thawing, or after thawing for 0.5 min in the microwave oven or for 1 h at room temperature. Processed mushrooms without the medium they were processed in were prepared for analysis of agaritine in the same way as fresh mushrooms.

Dried mushrooms. About 2 g dry mushrooms were mixed with 100 ml of a methanol–water (9:1 v/v) mixture and were then prepared in the same way as fresh mushrooms.

Broth. Broth (5 ml) was filtered through a microfilter into a vial and an aliquot was injected onto the HPLC column.

Identification and quantification

HPLC was performed using a Hewlett-Packard HP 1100 liquid chromatograph equipped with Diode Array Detector (DAD) and thermostated autosampler. Sample (20 µl) was injected into the chromatographic apparatus. Separation was carried out on column (250 × 4 mm), LiChrospher 100 RP-18 (5 µm), with a precolumn (4 × 4 mm), Li Chrospher 100 RP-18 (5 µm) (all Merck). The mobile phase was methanol–water; the mobile phase gradient 10% methanol held for 5 min, changing to 80% over 15 min, held at 80% methanol for 5 min. The total run time was 25 min; post-time was 5 min. The flow rate was 1 ml min^{-1}; the column temperature 35°C. For identification of agaritine, the retention time, DAD spectra and peak purity software function were used. Quantification of agaritine was made by comparing the peak area with that for a known amount of pure standard. The agaritine was detected at 237 nm. The detector response was linear in the range 0.2 µg ml^{-1} to 2.0 mg ml^{-1}. Under the experimental conditions described for sample preparation, the limit of detection of the method was 0.02 µg ml^{-1} (based on a signal–noize ratio of 3:1), corresponding to 0.2 mg kg^{-1} mushroom.

Method validation

The relative standard deviation (repeatability of measurements) was 4.5% for seven fresh mushroom samples containing around 200 mg agaritine kg^{-1} mushroom, a usual level of agaritine in fresh samples of the cultivated mushroom, *A. bisporus*.

The accuracy of the measurements, expressed as the recovery, was tested in a fresh *Agaricus hortensis* sample with low agaritine content (176 mg kg^{-1} fresh weight) and spiked with agaritine to 376 mg kg^{-1} fresh weight. Five measurements gave an average recovery rate of 91.1%. Neither a commercial analytical standard nor reference materials were available. All mushrooms were analysed in two parallel samples.

Recovery and repeatability were also determined for boiled and potted mushrooms. Pure methanol and mixtures of methanol and water were tested for extraction of agaritine from dried mushrooms. The highest recovery was reached by extracting dried mushrooms with 100 ml 9:1 (v/v) methanol–water mixture.

To test for the homogeneity of samples, 10 different fruiting bodies with a comparable cup diameter were analysed. Using a *t*-test based on a mathematical expectation of 95% and distant results, the relative SD was 6.4%.

Confirmation of the HPLC results was done by using an additional mobile phase, 0.5 M NaH_2PO_4 (pH 3.3 adjusted with H_3PO_4). The retention time of agaritine in the standard analytical system was 3.4 min, whereas it was 6.7 min in the alternative system using 0.5 M NaH_2PO_4 as mobile phase.

Results

Twenty-eight different samples of fresh cultivated mushrooms with a cup diameter of 4–6 cm were purchased from the retail market in Prague, 25 samples being *A. hortensis* (the most commonly marketed mushroom) and the other three *A. bitorquis*. Since no significant difference in agaritine content was observed between the species, only aggregate data are given. The agaritine content of the 28 samples varied between 165 and 457 mg kg^{-1}, the average being 272 ± 69 mg kg^{-1} (median 267 mg kg^{-1}; 10% percentile 201 mg kg^{-1}; 90% percentile 356 mg kg^{-1}). Younger and smaller fruiting bodies contained higher amounts of agaritine than larger fruiting bodies ready to be harvested (4–5 days old with a cup diameter around 4–6 cm), the difference was statistically significant (*t*-test, $\alpha = 95\%$). The agaritine content in mushrooms obtained directly from growers often did not deviate very much from the levels found in mushrooms purchased on the open market (data not shown). However, some samples obtained directly from growers contained significantly more agaritine (700 mg kg^{-1} fresh weight in some samples—cup diameter 4 cm—from early flushes).

Table 1 shows the amount of agaritine detected in various parts of the fruit body of *A. bitorquis* purchased on the open market. The analysed mushrooms had a cap diameter of around 4 cm and an average agaritine content of 249 mg kg^{-1} fresh weight. The highest amount of agaritine was found in the skin of the cap (322 mg kg^{-1}) and in the gills (254 mg kg^{-1}), and the lowest amount was in the stem (194 mg kg^{-1}). The cap with its skin contained 254 mg kg^{-1}. As shown in figure 1, 72% of the agaritine of the whole mushroom was found in the cap (with its skin).

Consumers usually purchase the cultivated mushroom as fresh or canned products. When bought fresh and stored for a shorter time, mushrooms are usually kept in the refrigerator. We investigated the influence of storage of *A. hortensis* (obtained directly from the grower) in the refrigerator (5°C) on its content of agaritine. The fresh mushrooms put into the refrigerator contained 393 mg agaritine kg^{-1} fresh weight. A small increase in the agaritine content (around 4%) was registered during the first three days. Thereafter, the agaritine content started to

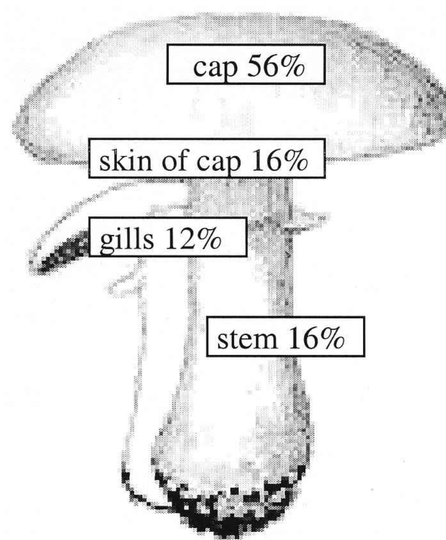

Figure 1. Agaritine content (%) in various parts of the fruit body of Agaricus bitorquis.

Table 1. Agaritine content in various parts of the fruit body of Agaricus bitorquis *purchased on the open market in Prague, Czech Republic. The cup diameter was around 4 cm.*

Part of the fruit body	Weight (%) of the total fruit body	Agaritine content: in specified part (mg kg^{-1})	Agaritine content: of whole mushroom (%)
Cap without skin	59.0	235.6	55.9
Skin of cap	11.4	351.8	16.1
Lamellae and spores	10.5	276.2	11.7
Stem (stipe)	19.1	213.1	16.4
Whole mushroom	100.0	248.8	100.0

decrease, being 75% of the original content after 6 days of storage in the refrigerator, 60% after 10 days and 50% after 14 days.

When bought fresh and stored for longer times, mushrooms are either frozen or dried. The influence of freezing *A. bitorquis* and storing the mushrooms at -18°C for 1 week/1 month on the agaritine content of the mushroom was studied by either extracting agaritine with methanol without thawing or extracting the compound after thawing the mushrooms for 1 h. When purchased, the mushroom sample that was frozen for 1 week contained around 249 mg kg^{-1} agaritine, whereas the sample frozen for 1 month contained around 261 mg kg^{-1}. Calculated on a fresh weight basis before processing, the non-thawed sample contained 147 mg kg^{-1} agaritine after 30 days in the freezer, whereas the thawed sample only contained 56 mg kg^{-1} agaritine. Thus freezing and thawing reduced the agaritine content by 77.3% in our experiment, while only freezing reduced the agaritine content by 41.1%. The reduction was evident but less pronounced after storage in the freezer for 1 week only—25.2% reduction without thawing, 32.9% reduction after 0.5 min thawing in microwave oven and 47.8% reduction after thawing for 1 h at room temperature.

The cultivated mushroom is commonly stored as dried slices. We followed the agaritine content of *A. hortensis* during drying at ambient temperature (25°C), at a constant elevated temperature (50°C) and at programmed elevated temperature (40°C for 2 h followed by 60°C; which is a programme recommended for household drying), respectively. The experiments were carried out with mushrooms cut into slices and continued until the material obtained a constant weight. Drying reduced the mushroom weight by approximately 90%, resulting in a dry weight of *Agaricus* mushrooms between 7 and 10% (w/w) of the fresh mushrooms. As shown in table 3, drying was quicker at higher than at lower temperatures and reduced the agaritine content of the mushrooms to 18–24% (calculated on a fresh weight basis). The agaritine content of the dried product remained at the same level after 1 month of storage at room temperature. Mushrooms were also dried at 70°C, when it took 5 h to reach a constant dry weight. The decrease in agaritine content was 75% at these conditions, but as the sensory/organoleptic quality of the mushrooms was unsatisfactory (the temperature for drying was too high), the data are not shown in table 2.

In contrast to normal drying that resulted in a small decrease in agaritine content of *A. hortensis*, freeze drying (lyophilization) at –51°C for 18 h had no influence on the agaritine content. Dried lyophilized *A. hortensis* (original agaritine content 409.5 mg kg^{-1} fresh weight) contained around 4000 mg kg^{-1} dry weight.

A series of experiments were performed in order to study the influence of various household-processing procedures on the level of agaritine in the cultivated mushroom. Figure 2 shows the effect of boiling 10 sliced fruit bodies (50 g) of cultivated *A. bitorquis* (original agaritine content 360 mg kg^{-1}) in 250 ml tap water for various periods. The mushrooms were boiled in closed jars in steam, thus simulating household preservation and not loosing water during longer boiling times. Similar results to those shown in figure 2 were obtained when the sliced fruit bodies were exchanged for whole fruit bodies (data not shown).

As can be seen in figure 2, boiling resulted in a significant progressive decrease of agaritine in solid mushrooms, whereas the concentration in the aqueous solution increased during the first 15 min. No further increase in the agaritine content of the boiling water was observed thereafter. The most pronounced changes occurred during the first 1 min of boiling. The solid mushrooms had lost 55% of their agaritine after

Table 2. Initial agaritine content on a fresh weight basis before processing, the decrease of agaritine content in dry mushrooms (calculated on fresh weight basis before processing), the time required to reach constant dry matter and the agaritine content in dry product.

Drying temperature (°C)	Agaritine content in fresh mushrooms (mg kg^{-1})	Decrease of agariitine (%)	Constant dry matter after (h)	Agaritine content in dry powder (mg kg^{-1})
25	270.6	18	24	2656
50	210.9	24	7.5	2909
40–60	270.6	19	7	2856

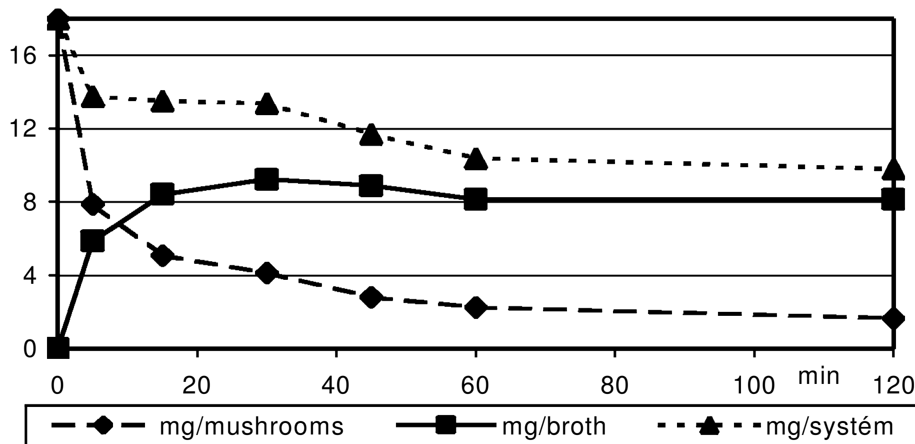

Figure 2. Distribution of agaritine (mg) in mushrooms, broth and whole system.

5 min of boiling and about 90% after 60–120 min of boiling. The total amount of agaritine in the system was also reduced during cooking, being about 75% of the original amount after 5 min and about 60% of the original amount after 60 min of boiling.

In the USA, dry baking of the cultivated mushroom on pizzas is the main food-processing method for the cultivated *Agaricus*. Table 3 shows the influence of baking *A. bitorquis* (340 mg kg^{-1}) on baking paper for 0–45 min at 200°C on its agaritine content. Agaritine was partly degraded by dry baking, the content being reduced by 23% after 10 min and by around 75% after 30 min of baking. Mushrooms are consumable when baked for < 30 min. After 45 min of baking on baking paper, the sensory/organoleptic quality of the mushrooms was unsatisfactory. Baking on baking paper can not be directly compared with baking on pizza since some steam evaporates during pizza baking.

Table 3. Changes in agaritine content during dry baking (at 200°C) of A bitorquis*, originaly having an agaritine content of 340.4 mg* kg^{-1} *fresh weight.*

Time (min)	Weight loss (%)	Decrease in agaritine content (%)
0	0.0	0.0
5	24.1	15.7
10	40.3	22.7
20	76.4	42.6
30	89.9	76.4
45	92.9	89.4

As in the Nordic countries, frying of the cultivated mushrooms in butter or vegetable oils is a much more common cooking procedure than dry baking. We studied the influence of frying sliced *A. hortensis* (298 mg kg^{-1}) in these fats for 5 min on the agaritine content of the mushroom. The temperature of the vegetable oil and butter, respectively, was around 100°C during the entire frying period, the mushroom slices being stirred continuously during the process. After frying for 5 min in vegetable oil, the agaritine content had been reduced to 34.3% of the original agaritine content in fresh mushrooms but only 0.6% of the original agaritine content could be recovered in the oil. Frying sliced mushrooms in butter for 5 min reduced the agaritine content to 69.2% of the agaritine content in fresh mushrooms before processing. Only 2.8% of the original agaritine content was recovered in the butter. In another experiment, whole mushroom caps of *A. bitorquis* was fried at around 150°C in a pan with sunflower oil for 10 min (5 min on each side). The mushrooms originally contained 338 mg kg^{-1} agaritine. Ten minutes of frying reduced the agaritine content by 57%. Although the temperature was higher in this frying experiment with *A. bitorquis* than in the frying experiment with *A. hortensis*, and the frying time was longer, the slightly less pronounced reduction in agaritine content could be due to that whole mushrooms were fried instead of sliced mushrooms.

In other parts of Europe, deep-frying is a common way of processing the cultivated mushroom. We studied the influence of deep-frying at 150 and 170°C in vegetable oil on the agaritine content of *A. hortensis* (the original amount was 429 mg kg^{-1}). The

agaritine content was reduced to approximately 50% after 10 min of deep-frying at 150°C or after 5–7 min at 170°C (the optimal cooking time in both cases with respect to organoleptic properties).

Although microwave processing of the cultivated mushroom is less common, the influence of this method of food preparation (1000 W, 2450 MHz) on the agaritine content of *A. bitroquis* was also investigated. The result of this study is shown in table 4. Heating in the microwave own for 1 min reduced the agaritine content of the mushrooms by 65%.

Table 5 summarizes our observations on the influence of storage and household processing on the agaritine content of *Agaricus* mushrooms. All types of storage studied by us as well as all cooking procedures investigated resulted in a reduction of the agaritine level in the two *Agaricus* species investigated, but to what extent (between 20 and 75%) was dependent on the methodology/procedure used. Whether storage and processing has any influence on the level of the other phenylhydrazines occurring in the *Agaricus* mushroom is to a large extent unknown but is now being studied by us. The dynamic of the changes could be described by first-order kinetics. The reaction rate constants for the reduction in agaritine content under the conditions of food preparation described above are shown in table 6.

Table 4. Changes in agaritine content of A. bitorquis *during microwave heating (1000 W, 2459 MHz, 20 g sliced mushrooms). The original content of agaritine was 276.1 mg kg^{-1} fresh weight.*

Time (s)	Weight loss (%)	decrease of agaritine (%)
0	0.0	0.0
15	14.1	40.9
30	25.1	51.4
45	37.5	60.8
60	49.8	64.8

Discussion

Our data indicate that agaritine is present in all parts of the fruit body. The average content in fruit bodies of cultivated mushrooms (*A. hortensis* and *A. bitorquis*) purchased on the open market, and having a cup diameter around 4 cm, was approximately 270 mg kg^{-1}. The amounts found in these two cultivated *Agaricus* species agree fairly well with the overall range of agaritine levels found in fresh cultivated *A. bisporus* — the species commonly cultivated in the other European countries. Such mushrooms obtained

Table 5. Influence of storage and household processing on the agaritine content of Agaricus *mushrooms.*

Process	Conditions (°C)	Time	Amount of agaritine remaining in the mushroom (%)*
Storage:			
Refrigerator	5	6 days	75
Refrigerator	5	14 days	50
Drying	25	24 hours	82
Drying	50	7.5 hours	76
Drying	40–60	7 hours	81
Freezing without thawing	−18	7 days	75
Freezing with thawing	−18	7 days	52
Freezing without thawing	−18	30 days	41
Freezing with thawing	−18	30 days	23
Household processing:			
Cooking	boiling water	5 min	44
Cooking	boiling water	60 min	12
Dry baking	200	10 min	77
Deep frying	150	10 min	50
Deep frying	170	5 min	52
Frying	150	10 min	43
Microwave heating	1000 W, 2450 MHz**	1 min	35

* 100% = agaritine content in fresh mushrooms before processing; ** 20 g sliced mushrooms.

Table 6. Reaction rate constants of the degradation of aragitine in Agaricus *mushrooms stored or processed in various ways.*

Type of storage or household processing	*Agaricus* species studied	Reaction rate constant of degradation of agaritine
Drying 25°C	*A. hortensis*	$1.6 \times 10^{-6} s^{-1}$
Drying 50°C	*A. hortensis*	$8.3 \times 10^{-6} s^{-1}$
Drying 40–60°C	*A. hortensis*	$7.0 \times 10^{-6} s^{-1}$
Cooking	*A. bitorquis*	$9.5 \times 10^{-2} s^{-1}$
Dry baking	*A. bitorquis*	$6.2 \times 10^{-4} s^{-1}$
Deep-frying, 150°C	*A. hortensis*	$1.0 \times 10^{-3} s^{-1}$
Deep-frying, 170°C	*A. hortensis*	$1.2 \times 10^{-3} s^{-1}$
Microwave heating	*A. bitorquis*	$2.2 \times 10^{-2} s^{-1}$

directly from growers or purchased on the market contain between 80 and 1730 mg agaritine kg^{-1} fresh weight, with typical values of marketed mushrooms between 200 and 450 mg kg^{-1} (Andersson and Gry 2001). Early flushes of *A. hortensis* and *A. bitorquis* sometimes contain higher amounts of agaritine, up to 700 mg kg^{-1}. Studies are ongoing to investigate whether mushrooms of these species collected in nature contain as high levels of agaritine as early flush samples of cultivated mushrooms obtained directly from growers.

Influence of storage on the agaritine content of mushrooms

In agreement with the observation that marketed mushrooms may contain substantially less agaritine than freshly harvested mushrooms, we observed that a substantial fraction of the agaritine is degraded (up to 60%) during post-harvest storage at low temperature (table 5). Since mushrooms sold to consumers on the open market might have been stored at other conditions and/or for longer periods (including the time in the markets), reduction in agaritine content during storage may explain the comparatively lower agaritine content of mushrooms on the market. From a practical standpoint, the use of extended post-harvest storage as a means to reduce agaritine in fresh mushrooms would not be desirable since deterioration in quality would occur (Liu *et al.* 1982).

These observations agree fairly well with data on post-harvest storage of *A. bisporus* reported in the literature. Liu *et al.* (1982) reported that post-harvest storage of the cultivated mushroom for 5 days at both 2 and 12°C reduced the agaritine content by 68%, mainly during the last 2 days of storage. Similarly, two batches of *A. bisporus* purchased from a local store and kept in the refrigerator (4°C) in polyethylene bags lost 2 and 47% during the first week, and after 2 weeks the mushrooms had lost 36 and 76% of their agaritine content, respectively (Ross *et al.* 1982b).

We observed that freezing mushroom samples of cultivated *A. bitorquis* for 1 month followed by thawing, reduced the agaritine content by as much as around 75%, whereas storage of the frozen sample for 1 week only reduced the agaritine by around 48%. Since the degradation of agaritine were much less pronounced in mushroom samples stored frozen for the same period but not thawed before analysis, the more significant degradation in the thawed samples could probably be ascribed to a catalytic breakdown of the toxin by mushroom enzymes. Similar results have been reported by others (Liu *et al.* 1982, Ross *et al.* 1982, Stijve *et al.* 1986, Sharman *et al.* 1990). Liu *et al.* (1982) observed an 80% reduction in agaritine content in frozen and thawed mushrooms, whereas Stijve *et al.* (1986) found an even stronger reduction (98%). The method of freezing, either quickly in liquid nitrogen or slowly at −20°C, did not influence the end result (Sharman *et al.* 1990). It was suggested that the majority of the loss in agaritine occurred during thawing, when disruption of the cellular structures separating the degrading enzymes from the substrate would allow the enzymatic breakdown of agaritine not otherwise possible (Liu *et al.* 1982).

In addition, drying of the cultivated mushroom (in this case *A. hortensis*) reduced its agaritine content, but the temperature influenced the magnitude of the reduction during drying. Drying at lower temperatures, which is the common procedure for mushroom drying, resulted in a smaller reduction in agaritine content then drying at a higher temperature (70°C) (table 5). The latter drying procedure resulted in a much more pronounced reduction in the agaritine level (around 75%) but the sensory/organoleptic quality of the dried mushrooms became poor. In our study, the agaritine content of dried mushrooms ranged from 2650 to 2900 mg kg^{-1} dry weight. These results are comparable with results (1000–2500 mg kg^{-1}) observed by Liu *et al.* (1982) and Ross *et al.* (1982). Fisher *et al.* (1986) observed somewhat higher agaritine levels in dried mushrooms (4600 mg kg^{-1}). In contrast to normal drying, freeze-drying did not result in any significant degradation of agaritine

(calculated on a fresh weight basis before processing). We observed 4000 mg agaritine kg^{-1} dried mushroom, which would correspond to approximately 400 mg kg^{-1} fresh mushroom.

Very low levels of agaritine have been reported in dried Italian *A. bisporus*, whereas extremely high amounts have been detected in dried sliced *Agaricus* mushrooms on the market in the UK, <5 and 6520 mg kg^{-1} dry weight, respectively (Sharman *et al.* 1990). Unfortunately, it is not known how these two samples have been produced. Since freeze-drying (lyophilization) has no or a very minor influence on the agaritine content in *A. bisporus* (Liu *et al.* 1982) and *A. hortensis* (present paper), it seems likely that the sample from UK had been freeze-dried. In accordance with this suggestion and our findings, Swiss investigators found between 2110 and 7800 mg agaritine kg^{-1} dry weight in lyophilized samples of the cultivated mushroom (Fischer *et al.* 1984, Stijve *et al.* 1986).

Influence of household processing on the agaritine content of mushrooms

Although some people have the habit of consuming the fresh cultivated mushroom raw in salads, the mushrooms are usually consumed processed: boiled, dry-baked or fried. To make it possible to estimate the dietary intake of agaritine and give advise on suitable cooking procedures, we investigated in a series of studies the influence of household processing on the agaritine content of the mushroom to be consumed. As summarized in table 5, household cooking procedures reduce the agaritine content of the cultivated mushroom, commonly to levels around 40–80% of the original content.

The cooking time for mushrooms depends on whether they will be consumed as such (usually a short cooking time), or whether they are used to give flavour to a dish or a sauce (often very long cooking times). In our study, shorter boiling times (5–15 min) reduced the agaritine content of the mushrooms by around 55–70%, whereas longer boiling times (1–2 h) reduced it with as much as around 90%. Some, but not all, of the agaritine was found in the boiling broth. Since the level of agaritine in the broth was constant with boiling times between 15 min and 2 h, the total degradation of agaritine in the system increase with boiling time. Other investigators have made similar observations (Liu *et al.* 1982, Hashida *et al.* 1990). Fischer *et al.* (1984), on the other hand, observed no agaritine degradation in cultivated *A. bisporus* blanched for 5–7 min.

In Europe, the cultivated mushroom is preferably fried. Depending on the frying procedure (type of fat, frying time and temperature and use of whole or cut mushrooms), the reduction in agaritine content of the fried mushrooms was between 30 and 65% (table 5). Very little agaritine could be recovered from the frying fat. In addition, deep-frying reduced the agaritine content (by approximately 50%).

In the USA, on the other hand, the cultivated mushroom is most often consumed as dry-baked at a high temperature (200–225°C, 5–10 min) on pizzas. This type of preparation only reduced the agaritine content of *A. bitorquis* by around 25%. Gannett and Toth (1991) analysed for agaritine and other phenylhydrazine derivatives in extracts from fresh or baked *A. bisporus* (10 min at 225°C). In their study, baking reduced the level of agaritine by 27%, 4-(carboxy)-phenylhydrazine by 10% and β-*N*-[γ-l-(+)-glutamyl]-4-carboxyphenylhydrazine by 23% when compared with the unprocessed fresh mushroom. In a parallel experiment, the fresh or baked homogenate of *A. bisporus* was analysed for the 4-(hydroxymethyl)benzenediazonium ion. Baking reduced the content by 41%. These observations have been confirmed by Toth *et al.* (1997).

In addition, microwave processing of the cultivated mushroom resulted in the degradation of agaritine. A heating time of 1 min in the microwave oven resulted in a 65% reduction in the agaritine content of *A. bitorquis*.

Taken together, our data and those of other investigators show that the consumer of household-processed cultivated mushroom is also exposed to substantial quantities of agaritine. Clearly, the lowest exposure to agaritine occurs when canned products of the cultivated mushroom (*A. bisporus*) are consumed (Andersson *et al.* 1999). Canned *A. bisporus* usually contains $<10\%$ of the agaritine content detectable in fresh mushrooms on the open market (Liu *et al.* 1982, Stijve *et al.* 1986, Hajslova *et al.* 1998, Andersson *et al.* 1999).

Unfortunately, very little data are available on the consumption of the cultivated *Agaricus* mushroom, making exposure assessments to the phenylhydrazines in the mushroom difficult. The estimated annual per capita intake in the Nordic countries varies between

0.6 and 2.4 kg, of which between 33 and 70% are fresh mushrooms, the rest being preserved mushrooms, mainly sold in cans (Andersson and Gry 2002). Since the per capita intake is an estimation of the average intake and does not take into consideration the fact that the mushrooms usually are consumed only by a part of the population, the per capita consumption is not very useful in expressing the consumption among people eating this mushroom. Danish data, based on a limited consumer study, indicate that only around 50% of the population consume the cultivated *Agaricus* mushroom and that 5% of the population consumes five times the median intake and 0.1% thirty times the median intake (Andersson and Gry 2001).

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