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Agaritine content in processed foods containing the cultivated mushroom (*Agaricus bisporus*) on the Nordic and the Czech market

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The level of agaritine was measured in fresh and canned cultivated mushroom (Agaricus bisporus) *as well as in other food products containing* A. bisporus*, by reversed phase high performance liquid chromatography. The two fresh samples were purchased on the open market and contained 212 and 229 mg/kg, respectively. Of the 35 different trademarks of canned mushroom products studied, 25 were based on cut mushrooms and 10 on whole mushrooms. On average, whole mushrooms contained 14.9 ± 6.7 mg agaritine per kg product whereas cut mushrooms contained 18.1 ± 7.8 mg/kg. There was no statistically significant difference between these two values. Agaritine levels in brine were generally slightly lower than the levels detected in canned mushrooms. Thus, the level of agaritine in* A. bisporus *is reduced more than 10 times during the wet canning process, resulting in low levels in canned products. On a portion basis, somewhat higher amounts of agaritine may be found in some other food products (mushroom soup and pasta sauce) containing* A. bisporus.

Keywords: agaritine, *Agaricus bisporus*, cultivated mushroom, shiitake

Introduction

In 1986 American investigators reported tumour formation in bone, forestomach, liver and lungs of mice that were fed raw cultivated mushroom (*Agaricus bisporus*) for 3 days followed by a semi-synthetic diet for 4 days each week of life (Toth and Erickson 1986). The bioactive compounds that are present in the mushroom and were suggested to be responsible for the carcinogenic effects are phenylhydrazine derivatives, of which agaritine (β-N-[γ-L(+)-glutamyl]-4-(hydroxymethyl) phenylhydrazine) is the predominant compound. In addition to agaritine, the mushroom contains the presumed agaritine precursors 4-(carboxy)phenylhydrazine and β-*N*-[γ-L-(+)-glutamyl]-4-carboxyphenylhydrazine, as well as the 4-(hydroxymethyl) benzenediazonium ion, which may be formed by degradation of agaritine. When these phenylhydrazines and related compounds were tested separately as purified chemicals in long-term carcinogenicity studies on mice, both precursors to agaritine as well as the degradation product were identified as animal carcinogens, whereas agaritine itself, unexpectedly, did not give rise to tumours (Toth 1995).

The feeding-schedule with uncooked mushrooms in the original long-term carcinogenicity study of Toth and Erickson (1986) has been criticized by many investigators for creating an unbalanced nutrient intake in the experimental animals, possibly resulting in an oscillation in the animals between periods of *intracellular* deficiency of protective factors and periods with adequate stores of these compounds (Gry and Andersson 1998). It has been speculated whether the unbalanced nutrient intake rather than the suspected carcinogens in the mushrooms resulted in tumour development in the experimental animals.

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Using the same criticized experimental protocol as in the original study, it was recently also shown that life-long feeding of mice with dry-baked mushrooms (approximately 225 °C for 10 min) resulted in increased tumour frequencies, but this time in the forestomach, glandular stomach, duodenum and ovary (Toth *et al.* 1997a). On average the mice consumed around 14 g dry-baked mushroom per day, which corresponds to more than 50% of their body weights. Chemical-analytical studies revealed that mushrooms processed in this way on average retain around 75% of the amount of phenylhydrazines present in raw fresh mushrooms. Parallel experiments, in which mice were fed dry-baked mushrooms according to an experimental schedule creating a much more balanced nutrient intake (only 4.5 g mushroom per day), resulted in non-significant increases in tumour incidence in the lungs, blood vessels, caecum, and colon (Toth *et al.* 1997b). In this study the mice received baked mushrooms 12 h each day for 5 days each week during their life-time. After each mushroom feeding cycle, the mice received a well-balanced semi-synthetic diet for 12 h each day for 5 days, and all day for the remaining 2 days each week. The authors interpreted the absence of a carcinogenic effect as resulting from the lower mushroom consumption in this study compared with the consumption in the studies demonstrating carcinogenic effects when feeding uncooked or baked *A. bisporus* for 3 days a week over the life-time of the mice.

Although it is not yet possible to determine whether the consumption of *A. bisporus* constitutes a carcinogenic risk, it is clear that many anthropogenic hydrazines are carcinogenic (Toth 1984). Thus, until it has been shown to be safe to consume *A. bisporus*, it seems advisable to keep the exposure to mushroom hydrazines as low as possible. Whereas dry-baking (approximately 225 °C for 10 min) only marginally reduces the level of phenylhydrazine derivatives in *A. bisporus*, cooking of the mushroom has been reported to reduce the levels substantially (Gannett and Toth 1991, Hajslova *et al.* 1998).

About half of the quantity of *A. bisporus* sold on the Nordic market is sold in the form of canned products. The present investigation reports the level of agaritine in canned cultivated mushroom and in other *A. bisporus* products on the Nordic and the Czech market as measured by reversed phase high performance liquid chromatography (HPLC).

Material and methods

Foods

Fresh and canned *A. bisporus*, as well as a few other products of the cultivated mushroom, were purchased on the open market in Uppsala (Sweden), Copenhagen (Denmark) and Prague (Czech Republic), and sent to the Institute of Chemical Technology, Prague for analysis.

Chemicals

Agaritine (at least 95% pure) was synthesized by Dr Henrik Frandsen at the Danish Veterinary and Food Administration for the Nordic Working Group on Phenylhydrazines in the Cultivated Mushroom, essentially according to the method of Wallcave *et al.* (1979), but with some important modifications (Frandsen 1998). A fresh stock solution of agaritine in water (0.2 mg/ml) was prepared daily from the synthesized standard which was stored in the freezer. The concentration of aqueous working solutions used for calibration ranged from 1 to 100 µg/ml.

Sample preparation

Twenty grams of fresh/canned mushrooms (in the latter case without brine) 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 distilled water. This solution was filtered through a microfilter (ANOTOP® 10; 0.02 µm, Merck) into a vial, and a 20 µl aliquot injected onto the HPLC column.

Five ml of brine from cans were filtered through the microfilter (ANOTOP® 10; 0.02 µm, Merck) into a vial and a 20 µl aliquot injected onto the HPLC column.

Four grams of dried food products containing *A. bisporus* were mixed with 100 ml of a methanol–water mixture (50 : 50, v/v), homogenized for 10 min

in an Ultra Turax (Janke a Kunkel, IKA-Werk), and shaken for 30 min. The crude extract was filtered and the volume of the filtrate adjusted to 200 ml with methanol, 10 ml of the extract was evaporated to dryness and the residue dissolved in 2 ml distilled water. This solution was filtered through a microfilter (ANOTOP® 10; 0.02 μm, Merck) into a vial, and a 20 μl aliquot injected onto the HPLC column.

Identification and quantification

A high performance liquid chromatograph Hewlett Packard HP 1100, equipped with Diode Array Detector, was employed for the analyses of extracts. Separation was made on a 250 × 4.6 mm (5 μm) Supelcosil LC 18 DB column (Supelco), the mobile phase being phosphate buffer (0.05 M NaH_2PO_4, pH 3.3) at a flow rate of 1 ml/min. Analyte peaks were detected at 237 nm.

The detector response was linear in the range 0.05–500 μg/ml. The limit of detection was 3 mg/kg of mushrooms. Relative standard deviation (at a concentration level of 10 mg/kg) was 4%. A chromatogram of a mushroom sample is shown in figure 1.

Results

Two samples of fresh *A. bisporus* were purchased on the open market; one sample in a store in Prague, Czech Republic, the other one in Uppsala, Sweden.

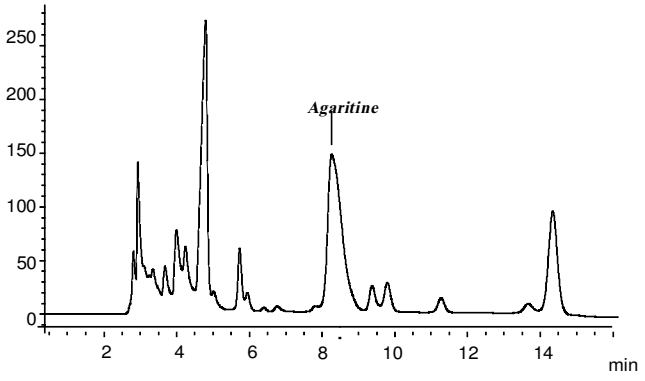

Figure 1. Reversed phase HPLC chromatogram of canned Agaricus bisporus.

The origin and storage conditions of these fresh mushrooms are not available. Their agaritine contents were 212.4 and 228.5 mg/kg, respectively.

Tables 1 and 2 show the agaritine content of 35 different trade-marks of canned *A. bisporus* on the Swedish, Danish and Czech markets. For 27 of the canned mushroom products the values in the tables are the average based on the analysis of material from three different cans from the same batch of the product. In eight cases where no difference in agaritine content between cans was established in preliminary studies, the data given are the average of two agaritine measurements on materials from the same can. The cans contained between 155 and 400 g of mushroom, including the brine. With a single exception, the mushrooms made up 52–68% of the content in the cans. The exception was product Netherlands XVII which contained 85% whole *A. bisporus*.

Of the 35 different canned mushroom products, 25 were based on cut mushrooms (table 1) and 10 on whole mushrooms (table 2). On average, whole mushrooms contained 14.9 ± 6.7 mg agaritine per kg product whereas cut mushrooms contained 18.1 ± 7.8 mg/kg. There was no statistically significant difference between these two values ($t = 1.21$; $p = 0.24$). It is evident that low and very similar levels of agaritine are found in all the different trade-marks of canned *A. bisporus*. Similar agaritine levels were also usually found in the three separate can samples of the same batch. The level of agaritine was below the detection limit (3 mg/kg) in two of the products. A third product (Netherlands XVII, mentioned above) with whole first quality mushrooms contained 4.4 mg agaritine per kg mushrooms. In the other 32 canned *A. bisporus* products the agaritine levels were between 10.1 and 30.3 mg/kg, and similar low levels of agaritine were also found in the brine of all the 35 canned products (< 3–30.4 mg/l). When the agaritine content of mushrooms and brine was compared in each product, there was a general tendency of a slightly lower amount in the brine than in the mushrooms. Eighteen of the trademarks with canned *A. bisporus* were produced in The Netherlands, six in China, four in France, three in India, two in Spain and one each in the Czech Republic and Sweden. On average products from these countries contained 16.5, 20.5, 10.3, 23.8, 12.8, 15.3 and 28.1 mg agaritine per kg mushrooms, respectively.

In addition to determining the agaritine content in canned *A. bisporus*, we also analysed for this compound in eight miscellaneous food products on the

Table 1. The agaritine contenct (mg/kg) of canned cut A. bisporus *purchased on the Czech, Danish and Swedish markets: average ± standard deviation.*

Country of origin—product code	Weight of product (g)	Agaritine in	
		Mushroom (mg/kg)	Brine (mg/l)
China I	184	16.63 ± 7.16	16.63 ± 3.98
China II	184	20.37 ± 0.72	22.20 ± 2.38
China III	184	23.13 ± 3.86	8.30 ± 1.32
China IV	184	26.2[a]	18.3[a]
China V	400	24.1[a]	12.1[a]
France I	200	25.57 ± 2.48	23.73 ± 1.31
France II	200	12.47 ± 0.38	10.27 ± 0.47
France III	200	< 3	< 3
France IV	200	< 3	< 3
India I	180	21.73 ± 4.65	15.10 ± 5.31
Netherlands I	330	23.33 ± 0.50	11.17 ± 0.59
Netherlands II	200	17.63 ± 2.65	12.70 ± 0.27
Netherlands III	285	11.23 ± 1.53	6.63 ± 0.61
Netherlands IV	330	19.80 ± 0.36	10.97 ± 0.70
Netherlands V	280	20.30 ± 0.20	10.50 ± 0.40
Netherlands VI	280	21.77 ± 1.03	8.20 ± 0.66
Netherlands VII	280	11.33 ± 0.47	6.53 ± 0.35
Netherlands VIII	280	11.05 ± 0.78	7.40 ± 0.14
Netherlands IX	184	30.30 ± 1.28	20.83 ± 0.81
Netherlands X	280	19.67 ± 1.03	9.80 ± 0.56
Netherlands XI	280	11.93 ± 1.27	6.67 ± 2.83
Netherlands XII	280	11.6[a]	7.5[a]
Netherlands XIII	400	28.5[a]	17.5[a]
Spain I	155	12.5	7.1[a]
Sweden I	190	28.13 ± 4.10	30.43 ± 3.76

[a] No variation between cans; two determinations per can.

Table 2. The agaritine content (mg/kg) of canned whole A. bisporus *purchased on the Czech, Danish and Swedish markets: average ± standard deviation.*

Country of origin—product code	Weight of product (g)	Agaritine in	
		Mushroom (mg/kg)	Brine (mg/l)
China VI	280	12.5[a]	8.2[a]
Czech I	340	15.3[a]	7.3[a]
India II	180	21.20 ± 1.06	11.57 ± 1.75
India III	195	28.57 ± 0.50	14.50 ± 0.40
Netherlands XIV	180	11.83 ± 0.32	6.90 ± 0.00
Netherlands XV	280	12.63 ± 0.50	7.33 ± 1.06
Netherlands XVI	275	10.07 ± 0.58	5.03 ± 0.50
Netherlands XVII	280	4.43 ± 0.31	2.90 ± 0.30
Netherlands XVIII	280	19.47 ± 0.35	9.50 ± 0.10
Spain II	355	13.1[a]	7.2[a]

[a] No variation between cans; two determinations per can.

Nordic market. All these food products were based on a powder, which contained dried/freeze-dried *A. bisporus* and/or a dried mushroom extract, and was meant to be dissolved in water at the time of food preparation. The result of this investigation is shown in table 3. The agaritine content per portion of ready-to-eat product was low, 10 mg agaritine or less, for six of the products. The other two products—a pasta sauce and a mushroom sauce containing a lot of mushrooms—contained somewhat higher amounts

Table 3. Agaritine content of miscellaneous products on the market.

Food product (weight of content in package)	Amount agaritine in dry powder products (mg/kg)	Agaritine content of prepared food (mg/kg)[a]	Amount of agaritine (mg) in one portion ready to eat product
Mushroom sauce (27g)	480.2	3.9	3.7
Mushroom soup I (79g)	152.3	12.0	3.0
Mushroom soup II (20g)	450.5	1.8	9.0
Mushroom soup III (90g)	930.4	62.8	27.9
Pasta sauce I (25g)	211.5	1.6	1.5
Pasta sauce II (61g)	248.6	8.4	5.1
Pasta sauce II (27g)	340.5	2.8	2.6
Pasta sauce IV (50g)	610.5	15.3	15.3

[a] mg/kg fresh weight

of agaritine, 15.3 and 27.9 mg per portion, respectively (table 3).

Discussion

Despite lack of evidence that agaritine is the principal biologically active component in the cultivated mushrooms, analytical work has nevertheless tended to concentrate on monitoring this compound in *A. bisporus* rather than its suggested precursors or decomposition products; presumably because of the relatively large amounts of agaritine present in the mushrooms and the comparative ease with which it can be analysed (Sharman *et al.* 1990). In addition to agaritine, the cultivated mushroom also contains lower quantities of 4-(carboxy)phenylhydrazine, β-N-[γ-L-(+)-glutamyl]-4-(carboxy)phenylhydrazine and the 4-(hydroxymethyl)benzenediazonium ion (Levenberg 1962, Ross *et al.* 1982a, Chauhan *et al.* 1984, 1985). These three nitrogen–nitrogen bond-containing constituents have not been quantified in the present study.

Commercial growing of the cultivated mushroom entails several production steps which may affect agaritine concentration in the harvested mushrooms. Thus, mushroom compost type, order of the flush from which the mushrooms have been harvested, and other culture conditions might influence the agaritine content of fresh mushrooms (Speroni *et al.* 1983). It has been claimed that young fruiting bodies in general contain higher levels of agaritine than old fruiting bodies, the concentration diminishing with age and increase in size of the fruiting bodies (Kelly *et al.* 1962, Chiarlo *et al.* 1979, Fischer *et al.* 1984). On the other hand, different strains/varieties of *A. bisporus* contain comparable levels of agaritine and the season for harvest seems only to have minor influence on the mean agaritine content of the mushrooms (Liu *et al.* 1982, Speroni *et al.* 1983, Fischer *et al.* 1984, Stijve *et al.* 1986).

Also the post-harvest storage and processing of *A. bisporus* may strongly influence the agaritine content of the mushrooms. It is not known whether the other phenylhydrazine derivatives in the mushrooms are influenced in a similar way. The level of agaritine reported in various fresh samples of *A. bisporus* has been in the range 100 to 1700 mg/kg fresh weight, with average values commonly between 200 and 500 mg/kg (Levenberg 1961, 1964, Daniels *et al.* 1961, Kelly *et al.* 1962, Liu *et al.* 1982, Ross *et al.* 1982b, Speroni and Bellman 1982, Speroni *et al.* 1983, Fischer *et al.* 1984, Stijve *et al.* 1986, Hashida *et al.* 1990, Sharman *et al.* 1990, Andersson *et al.* 1994). Although we cannot tell whether the two fresh mushroom samples analysed by us contain agaritine levels comparable to newly harvested mushrooms, or whether the analysed mushrooms already had started to lose agaritine due to degradation during storage, the levels detected, 212 and 229 mg/kg, respectively, were within average values normally reported.

Reduction in agaritine content during blanching/cooking has been reported by three investigators. Liu and co-workers (1982) found a reduction in agaritine content by 57 and 75%, respectively, in two different strains of *A. bisporus* blanched in boiling water for 5 min. Similarly, when two fresh samples of *A. bisporus* containing 290 and 295 mg/kg agaritine, respectively, were blanched for 5–7 min, the agaritine content in the mushrooms was reduced to 181 and 169 mg/kg, respectively. After blanching the brine

contained 116 and 134 mg/kg agaritine, respectively, giving a total amount of 297 and 303 mg/kg in mushrooms plus brine (Fischer *et al.* 1984). In agreement with these two observations, Japanese investigators have reported a significant decrease in agaritine content of *A. bisporus* boiled at 100 °C for 10 min (Hashida *et al.* 1990).

Heat-treatments during the canning process (high temperatures during a short time) is used to tackle the challenge of destroying undesired micro-organisms. During this treatment agaritine is both extracted from the mushroom and thermally degraded (Sastry *et al.* 1985, Speroni *et al.* 1985). Removal of agaritine by an initial blanching would be especially desirable because any breakdown products of agaritine formed during this thermal process would not accumulate in the can. Furthermore, if one of the breakdown products of agaritine proves to be carcinogenic, it seems more desirable to optimize the blanching operation to remove agaritine from the mushrooms before retorting and using high temperature–short time processing for the conservation process.

Table 4 summarizes published measurements on agaritine content of canned *A. bisporus*. No agaritine was found in the first report on canned *A. bisporus* (Ross *et al.* 1982b), but the detection limit was not given in this study. The other published studies indicate that canned products contain low agaritine levels, usually less than 10% of the agaritine level commonly detected in fresh mushrooms (Liu *et al.* 1982, Stijve *et al.* 1986, Hajslova *et al.* 1998). This conclusion is supported by the present investigation in which the average content was found to be 17.2 ± 7.5 mg/kg agaritine in canned *A. bisporus*. Two samples of fresh *A. bisporus* purchased on the market by us contained more than 200 mg agaritine per kg mushrooms.

A comparison of the agaritine concentration in mushrooms and brine from canned products in the present and earlier studies (table 1 and 2, and 4, respectively), indicate a near-equilibrium situation in agaritine levels between solid and liquid phases. This equilibration probably occurs during can storage.

Table 5 summarizes available reports on agaritine levels in miscellaneous types of non-canned *A. bisporus* products on the market. Some of the products analysed have been shown to contain high levels of agaritine, whereas other products, particularly processed products, contain low levels. Two of the previously analysed food products are similar to the products reported by us in table 3. Both of these products were mushroom soups that contained low agaritine levels—in both cases under the detection limit (Ross *et al.* 1982b, Sharman *et al.* 1990). Our three samples of mushrooms soup contained different amounts of agaritine—1.8, 12.0 and 62.8 mg/kg.

Besides being detected in 10 different *Agaricus* species (Levenberg 1964), agaritine has also been found in shiitake (*Lentinus edodes*), but at very low levels, 0.82 mg/kg (Hashida *et al.* 1990). Other workers did not find any agaritine in shiitake (Stijve *et al.* 1986). With such low quantities of agaritine in fresh *L. edodes*, it came as no surprise that we were unable to detect agaritine in a Swedish sample of canned shiitake (results not shown).

It is concluded that the level of agaritine in the cultivated mushroom can be reduced more than 10 times during the wet canning process, resulting in low agaritine levels in canned products.

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Table 4. Reported levels of agaritine in canned Agaricus bisporus.

Agaritine content (mg/kg fresh weight)[a]			
Mushrooms	Brine	Number of samples	Reference
Undetectable	Undetectable	3	Ross *et al.* (1982)
29 (23–35)	34 (25–43)	2	Stijve *et al.* (1986)
65 (40–90)	115 (80–150)	2	Liu *et al.* (1982)
31 (1–64)	? (3–104)	11	Fischer *et al.* (1984)
28 (20–33)	9 (6–11)	12	Sharman *et al.* (1990)
Undetectable	—	—	Hashida *et al.* (1990)
17.2 (< 3–30)	11.0 (< 3–30)	35	This report

[a] Range within parentheses.

Table 5. Reported levels of agaritine in miscellaneous types of Agaricus bisporus *products.*

Type of product	Agaritine content (mg/kg fresh weight)	Reference
Frozen mushrooms	330	Ross *et al.* (1982b)
Mushrooms sautéed in olive oil at 300 °C for 7 min	300	Ross *et al.* (1982b)
Sliced mushrooms and mushroom powder used as ingredients for dehydrated soups	1000–2500[a]	Stijve *et al.* (1986)
Pasta sauce (n = 4)	1.6–15.3	This report
Mushroom soup	0	Ross *et al.* (1982b)
Mushroom soup	< 5	Sharman *et al.* (1990)
Mushroom soup (n = 3)	1.8–62.8	This report
Mushroom sauce	3.9	This report

[a] In mg/kg dry weight.

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