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TOXIKOLOGISCHE LITERATUR-RÜCKSCHAU: AGARITIN

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Seit einem Jahrzehnt bemühen sich Chemiker und Krebsforscher festzustellen, ob das im Kulturchampignon *Agaricus bisporus* enthaltene Hydrazinderivat "Agaritin" mutagen oder gar krebserregend ist. Mit Hilfe des Dokumentationsdienstes UNIDOC der Universitätsbibliothek Lausanne wurden 24 Publikationen zu diesem Thema erfasst, von denen 20 zur Auswertung für die vorliegende Rückschau geeignet waren.

Der CHEMIE des Agaritins sind 6 Arbeiten gewidmet (2, 11, 15, 16, 18, 20).

Mit dem NACHWEIS des Agaritins befassen sich die Veröffentlichungen Nr. 3, 6, 9, 11, 14 und 20. Die Hochleistungsflüssigchromatographie (HPLC) ist empfindlicher als die Hochleistungsdünnschichtchromatographie (HPTLC) und erlaubt, Agaritinmengen bis hinunter zu 6 Milliardstelgramm zu erfassen (9).

Der TOXIKOLOGIE widmen sich neun Veröffentlichungen, nämlich die Nummern 1, 4, 5, 7, 10, 13, 17, 18 und 19. Es geht daraus hervor, dass das Agaritin selbst nicht oder nur sehr schwach krebserregend ist (5, 13), wohl aber einige seiner Derivate, die durch chemischen oder biologischen Abbau des Agaritins entstehen (7, 14, 19). *Agaricus bisporus* besitzt ein Enzym, das diesen Abbau im lebenden Pilz beschleunigt (7), so dass die Pilze in verschiedenen Graden krebserzeugend sein können. Durch den Abbau des Agaritins wird die Mutagenität (im Bakterientest) um das 8-16fache gesteigert (19), die Krebserzeugung (bei Mäusen) um das 2-6fache (1).

Die HANDELSPILZE wurden in frischer, gefrorener, getrockneter und in Büchsen eingemachter Form untersucht (3, 6, 8, 10, 12, 14, 15, 16, 20). Es folgt aus diesen Untersuchungen, dass junge Pilze mehr Agaritin enthalten als alte (3), die frühen Ernten eines Kulturansatzes weniger als die späten Ernten (12), und dass auf synthetischem Kompost gewachsene Champignons stärker agaritinhaltig sind als die auf natürlichem Kompost gewachsenen (12). Der Agaritingehalt der Frischpilze wird als 400-700 mg/kg (6), 94-629 mg/kg (14) und 330-1730 mg/kg (8) angegeben. Im

Kühlschrank verlieren die Frischpilze in einer Woche 2-47% (6) oder bis 68% (8) ihres Agaritins. Eine andere Analyse zeigte, dass sie nur noch 0,33 mg/kg enthalten (6). Büchsenpilze enthalten 87% weniger Agaritin als Frischpilze (8), aber immerhin noch 1-55 mg/kg (14), während der Saft in der Büchse 3-103 mg/l Agaritin enthält (14). Nach Abbrühen enthalten die Pilze nur noch 1/3 (8) bis 2/3 (6) oder gar nur 5% (20) ihres Agaritins. Trockenpilze enthalten die höchsten Werte, nämlich 2,11-6,9 g/kg. Aufbewahren, Gefrieren und Abbrühen reduzieren zwar den Agaritingehalt, aber da dessen Abbauprodukte krebserzeugend sind, bedeutet das unter Umständen eine Erhöhung der Krebsgefahr, besonders dann, wenn lebende Pilze, die ja ein Abbau-Enzym enthalten (7) längere Zeit gelagert werden.

Die VERBREITUNG des Agaritins bei verschiedenen Pilzarten wurde 3 mal untersucht (10, 11, 20). In 43 essbaren Pilzen der Gattungen *Agrocybe, Amanita, Armillaria, Boletus, Calocybe, Cantharellus, Clitopilus, Coprinus, Craterellus, Flammulina, Hirneola, Hydnum, Hygrophorus, Kuehneromyces, Lactarius, Langermannia, Leccinum, Lentinus, Lepista, Leucoagaricus, Lycoperdon, Macrolepiota, Marasmius, Morchella, Pleurotus, Russula, Sarcodon, Stropharia, Suillus, Tricholoma, Tuber, Volvariella* und *Xerocomus* wurde kein Agaritin festgestellt, wohl aber in 11 *Agaricus*-Arten, nämlich *A. arvensis, augustus, bisporus, bitorquis, campester, edulis, excellens, macrosporus, niveolutescens, perrarus, silvicola, subperonatus* und *vaporarius* (20). Nur 5 *Agaricus*-Arten, die aber aus andern Gründen für die industrielle Kultur kaum in Frage kommen, enthalten nicht mehr feststellbare Mengen oder gar kein Agaritin, nämlich *A. haemorrhoidarius, langei, meleagris, silvaticus* und *xanthoderma* (20).

In der folgenden CHRONOLOGISCHEN ZUSAMMENSTELLUNG erscheinen die Arbeiten in der Form, wie sie in der Datenbank von UNIDOC erscheinen, d.h. mit einer englischen Zusammenfassung. Nur die Zitationen wurden den Gepflogenheiten der Mycologia Helvetica angepasst.

1 Toth, B., D. Nagel, K. Patil, J. Erickson and K. Antonson, 1978: TUMOR INDUCTION WITH THE N ACETYL DERIVATIVE OF 4 HYDROXYMETHYL PHENYLHYDRAZINE A METABOLITE OF AGARITINE OF AGARICUS BISPORUS. - Cancer Res. 38: 177-180.

N'-Acetyl-4-(hydroxymethyl)phenylhydrazine was administered as a 0.0625% solution in drinking water continuously for the life span of Swiss mice, from 6 wk of age. Compared to that in untreated controls, in treated animals the lung tumor incidence rose from 15 to 34% in females and 22 to 48% in males, whereas the incidence of blood vessel tumors increased from 8 to 32% in females and 5 to 30% in males. Histopathologically, the tumors were classified as adenomas and adenocarcinomas of the lungs and angiomas and angiosarcomas of the blood vessels. The commonly eaten mushroom *A. bisporus* contains β-N-(γ-L(+)-glutamyl)-4-hydroxymethylphenyl-

hydrazine, which under certain conditions yields 4-hydroxymethylphenylhydrazine and L-glutamic acid. Since 4-hydroxymethylphenylhydrazine is relatively unstable, its acetyl derivative was synthesized for this study. The possible environmental significance of the findings is discussed.

2 Wallcave, L., D. Nagel, C. R. Raha, H. S. Jae, S. Bronczyk, R. Kupper and B. Toth, 1979: AN IMPROVED SYNTHESIS OF AGARITINE. - J. org. Chem. 44: 3752-3755.

L-Glutamic acid 5-(2-(4-(hydroxymethyl)phenyl)hydrazide) (agaritine), a compound present in *Agaricus bisporus*, the commercial edible mushroom, was synthesized for the bioassay of its possible tumorigenic properties. The mixed anhydride derived from 1-benzyl N- (benzyloxycarbonyl)-L-glutamate and ethyl chloroformate reacted with 4-carboxyphenylhydrazine to form the benzyl ester of N-(benzyloxycarbonyl)-L-glutamic acid 5-(2-(4-carboxyphenyl)hydrazide). Reduction of 3 with BH3/THF gave the corresponding 4-(hydroxymethyl) phenyl derivative which on hydrogenolysis in THF over Pd/C gave agaritine. The overall yield from agaritine was 25%, some 25-fold higher than previously obtained.

3 Chiarlo, B., E. Cajella and C. Acerbo, 1979: THE PRESENCE OF AGARITINE IN A MUSHROOM AGARICUS BISPORUS COMMONLY CULTIVATED IN ITALY. - Fitoterapia 50: 111-114.

The presence of β-N-(γ-L(+)glutamyl)-4-hydroxymethylhydrazine (agaritine) (a potential carcinogen) in *A. bisporus* was ascertained by HPTLC (high performance TLC) on silica gel (BAW 63:10:27, Rf = 0.19; piridine/aniline/water 9:1:4, Rf = 0.62), mixed HPTLC with agaritine and UV absorption spectra (maxima at about 237 nm in phosphate buffer at pH 7). The amounts of agaritine, semiquantitatively evaluated, were higher in young mushrooms.

4 Toth, B., and D. Nagel, 1981: STUDIES OF THE TUMORIGENIC POTENTIAL OF 4 SUBSTITUTED PHENYL HYDRAZINES BY THE SUB CUTANEOUS ROUTE. - J. Toxicol. Environ. Health 8: 1-10.

4-Methylphenylhydrazine hydrochloride (4-MPH) was administered to randomly bred Swiss mice as 26 weekly s.c. injections of 140 μg/g of body wt and N'-acetyl-4-(hydroxymethyl)phenylhydrazine (AMPH) as 26 weekly s.c. injections of 500 μg/g. As a solvent control, physiological saline was also given as 26 weekly s.c. injections of 0.01 ml/g. The 4-MPH treatment induced a significant incidence (24%) of fibrosarcomas in males. In the 4-MPH-treated females and some AMPH-treated male mice, a few soft-tissue tumors were observed but their appearance could not be related to treatment. 4-MPH is formed under special experimental conditions from 4-hydroxy-

methylphenylhydrazine, which is an in vitro breakdown product of agaritine, an ingredient of the cultivated mushroom *Agaricus bisporus*.

5 Toth, B., C. R. Raha, L. Wallcave and D. Nagel, 1981: ATTEMPTED TUMOR INDUCTION WITH AGARITINE IN MICE. - Anticancer Res. 1: 255-258.

Agaritine, a constituent of the cultivated mushroom of commerce *Agaricus bisporus*, was administered at concentrations of 0.0625 and 0.03125% in drinking water daily for life to randomly bred Swiss mice. Consumption of the chemical resulted in no detectable carcinogenic action under the experimental conditions; however, a substantial number of animals developed convulsive seizures.

6 Ross, A. E, D. Nagel and B. Toth, 1982: OCCURRENCE STABILITY AND DECOMPOSITION OF BETA-N-GAMMA-L DEXTROGLUTAMYL-4-HYDRO-XYMETHYLPHENYL HYDRAZINE AGARITINE FROM THE MUSHROOM AGARICUS BISPORUS. - Food Chem. Toxicol. 20: 903-908.

A chromatographic technique was developed that could separate β-N(γ-L(+)-glutamyl)-4-hydroxymethylphenylhydrazine (agaritine) from all other components in 10-500 µl samples of mushroom extracts. Locally purchased mushrooms contained mean levels of 0.4-0.7 mg agaritine/g. The agaritine content of the mushrooms had decreased by 2-47% after 1 wk of storage in a domestic refrigerator and by 36-76% after 2 wk of such storage. Canned mushroom soup and canned mushrooms did not contain detectable agaritine; a sample of frozen mushrooms contained a mean level of 0.33 mg/g and a batch of fresh mushrooms lost approximatively 32% of their agaritine content on cooking. In mice given 3 mg agaritine by gavage, agaritine was detected in all parts of the gastrointestinal tract 15 min after dosing, but none was detectable in the gut after 3 h. The enzyme γ-glutamyltranspeptidase derived from pig's kidney was capable of decomposing agaritine to glutamic acid and 4-(hydroxymethyl) phenylhydrazine, and had 9-fold such activity as an enzyme isolated from mushrooms.

7 Ross, A. E., D. Nagel and B. Toth, 1982: EVIDENCE FOR THE OCCURRENCE AND FORMATION OF DIAZONIUM IONS IN THE AGARICUS BISPORUS MUSHROOM AND ITS EXTRACTS. - J. Agric. Food Chem. 30: 521-525.

N-β-((+)-γ-Glutamyl)-4-(hydroxymethyl)phenylhydrazine (agaritine), a component of the common cultivated commercial mushroom *A. bisporus*, is hydrolyzed to the 4-(hydroxymethyl)benzenediazonium ion, a carcinogen in mice, by an enzyme system present in the mushroom. This system, together with others, may be responsible for a 0.6-ppm level of the 4-(hydroxymethyl)benzenediazonium ion in the mushroom. A 2nd diazonium ion is generated in acid extracts of *A. bisporus* from a precur-

sor of unknown structure in the mushroom.

8 Liu, J. W., R. B. Beelman, D. R. Lineback and J. J. Speroni, 1982: AGARITINE CONTENT OF FRESH AND PROCESSED MUSHROOMS AGARICUS BISPORUS. - J. Food. Sci. 47: 1542-1548

Agaritine concentrations were determined in fresh and processed mushrooms using HPLC (high performance liquid chromatography). Agaritine concentrations (percentage of fresh weight) of fresh mushrooms varied considerably among 14 lots from 10 different sources; the range was 0.033-0.173% and the average was 0.088%. Postharvest storage for 5 days at 2° and 12° C reduced original agaritine content by as much as 68%. Freezing, storage for 30 days at -25° C and subsequent thawing reduced agaritine by approximatively 74%. Bisulfite treatment prior to freezing and freezing rate had no significant effect on agaritine content. Canning operations reduced agaritine levels by as much as 87%; approximatively 66% was removed during blanching and the remainder by thermal processing and storage.

9 Speroni, J. J. and R. B. Beelman, 1982: HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF AGARITINE IN CULTIVATED MUSHROOMS. - J. Food Sci. 47: 1539-1541.

A sensitive, high performance liquid chromatographic method is described for quantification of agaritine, a naturally occurring phenylhydrazine derivative isolated from *Agaricus bisporus*. Freeze-dried mushrooms were extracted with methanol, evaporated to dryness, the residue resuspended in 0.005 N NaH_2PO_4, pH 4.25 and subsequently passed through a C18 reverse-phase SepPak. A mobile phase of 0.005 N NaH_2PO_4, pH 4.25, was pumped through a Partisil; SCX cation-exchange column at 0.6 ml/min. Agaritine was monitored at 237 nm, and linear standard curves were observed over the range 0-2 µg. Recoveries of agaritine standards averaged > 90%, while the lower limit of detectability was 0.006 µg. Co-chromatography and UV scans indicated that agaritine from mushroom extracts is the major component absorbing at 237 nm at the retention volume of authentic agaritine.

10 Sterner, O., R. Bergman, E. Kesler, G. Magnusson, L. Nilsson, B. Wickberg, E. Zimerson and G. Zetterberg, 1982: MUTAGENS IN LARGER FUNGI 1. 48 SPECIES SCREENED FOR MUTAGENIC ACTIVITY IN THE SALMONELLA MICROSOME ASSAY. - Mutat. Res. 101: 269-282.

Specimens of large fungi (mushrooms) were screened for mutagenic activity by the Salmonella/microsome assay, with strains TA98, TA2637 and TA100. Out of 48 spp. tested, 37 exhibited a significant but for the most part weak activity. The activity observed in the presence of S9 mix was typically between 0 and 50% of that without,

and in no case was the activity increased in the presence of microsomal enzymes. Six metabolites reported to occur in some of the species included in this investigation were also tested. Significant mutagenic activity was found with isovelleral from *Lactarius* sp., agaritine (3) from *Agaricus bisporus* and related species and β-nitraminoalanine from *A. silvaticus*. Isovelleral may be a major mutagen in some of the sharp-tasting and mutagenic *Russulaceae* species. *A. bisporus* (cultivated specimen) was weakly mutagenic toward all 3 strains of S.typhimurium used, and agaritine was weakly active toward TA2637 alone. This fungus might contain other mutagenic materials as well. β-nitraminoalanine was not found in the particular collection of *A. silvaticus* tested here. The mutagenicity observed for the fungus in this work may therefore be due to other metabolites. Even though many species found to be mutagenic are used as (human) food, it seems premature to make specific recommendations about eventual health risks. Further information is needed about the chemistry and toxicology of the active compounds as well as about the effects of various methods used in preparing mushrooms for food.

11 Speroni, J. J., 1982: STUDIES ON AGARITINE IN CULTIVATED MUSHROOMS: QUANTITATIVE ANALYSIS, NATURAL DISTRIBUTION, AND THERMAL DESTRUCTION KINETICS. - Ph.D. dissertation. Pennsylvania State Univ., University Park, USA, PA.

91 pp., AVAIL: Univ. Microfilms Int., Order No. DA8228942.

12 Speroni, J. J., R. B. Beelman and L. C. Schisler, 1983: FACTORS INFLUENCING THE AGARITINE CONTENT IN CULTIVATED MUSHROOMS AGARICUS BISPORUS. - J. Food Prot. 46: 506-506.

Agaritine concentrations were determined in fresh mushrooms grown from various spawn strains on several compost types and harvested at different phases of the cropping cycle. A wild spawn strain produced mushrooms with approximatively 2 times the agaritine content of 7 other, more commercially important types. Mushrooms harvested from a synthetic compost produced significantly higher amounts of agaritine than 5 other compost types. Mushrooms harvested later in the cropping cycle were more likely to have higher agaritine levels than earlier harvested mushrooms. Agaritine was also present in the mycelium of *A. bisporus* growing in liquid culture, but at much lower levels than present in the fruiting bodies.

13 Toth, B. and H. Sornson, 1984: LACK OF CARCINOGENICITY OF AGARITINE BY SUB CUTANEOUS ADMINISTRATION IN MICE. - Mycopathologia 85: 75-80.

Agaritine (A), an ingredient of the cultivated mushroom of commerce *Agaricus bi-*

sporus, was administered by s.c. injection of 2 groups of randomly bred Swiss mice. In the 1st group the animals of both sexes were treated at a 100 µg/g body wt basis 5 times at weekly intervals, while in the 2nd group the mice received a single A treatment of 100 µg/g body wt for females and 50 µg/g body wt for males. The administration of the compund resulted in no detectable carcinogenic effect in the animals. Since some of the breakdown products of A were shown to be carcinogenic in mice and the mushroom itself was found to be mutagenic, the field is discussed in the light of the obtained results.

14 Fischer, B., J. Lüthy und C. Schlatter, 1984: GEHALTSBESTIMMUNG VON AGARITIN IM ZUCHTCHAMPIGNON (AGARICUS BISPORUS) MITTELS HOCHLEISTUNGSFLÜSSIGCHROMATOGRAPAHIE (HPLC). - Z. Lebensm. Unters. Forsch. 179: 218-223.

A procedure is described for the determination of agaritine in the commercial mushroom *Agaricus bisporus* by high performance liquid chromatography (HPLC). Agaritine was extracted from the mushroom sample with methanol and the filtered extract diluted with phosphate buffer. An aliquot of this solution was used directly for the HPLC- separation on a cation exchange column (Partisil SCX) with 0.5 mM phosphate buffer (pH 1.8) as mobile phase and u.v. monitoring at 237 nm. The agaritine content in fresh mushrooms was found to be in the range of 94-629 mg/kg fresh weight. Canned mushrooms contained 1-55 mg/kg drained weight with 3-103 mg/l in the liquid. The highest agaritine values were found in dried commercial mushrooms amounting to 2.110-6.905 g/kg.

15 Speroni, J. J., S. K. Sastry and R. B. Beelman, 1985: THERMAL DEGRADATION KINETICS OF AGARITINE IN MODEL SYSTEMS AND AGARITINE RETENTION IN CANNED MUSHROOMS. - J. Food Sci. 50: 1306-1311.

The effects of heating and pH on agaritine degradation in buffer, mushroom puree and canned mushrooms were investigated. Ampules containing agaritine with citric-phosphate buffer or agaritine with mushroom puree buffered with the same salts were heated for selected time intervals at temperatures ranging from 101-133° C. Additionally, cans containing quartered mushrooms with brine and buffer were still-retorted using five thermal process schedules with equivalent lethalities for Clostridum botulinum (Fo values). In both the buffer and puree systems, agaritine degradation could be modeled by first-order kinetics. In the canning study mushrooms processed for 68 min at 115° C contained about 23% less agaritine than those processed for 11 min at 127°C.

16 Sastry, S. K., R. B. Beelman and J. J. Speroni, 1985: A THREE-DIMENSIONAL FINITE ELEMENT MODEL FOR THERMALLY INDUCED

CHANGES IN FOODS APPLICATION TO DEGRADATION OF AGARITINE IN CANNED MUSHROOMS. - J. Food Sci. 50: 1293-1299.

A three-dimensional finite element model was developed for simultaneous solution of heat and mass transfer equations in domains of irregular shape. The model was tested for thermal destruction of agaritine (a naturally occurring phenylhydrazine derivative) in canned mushrooms. Model predictions and experimental data were in good agreement and indicated that high temperature-short time (HTST) treatments tended to favor higher agaritine retention within cans than low temperature-long time treatments. Results also indicate the presence of a post-processing concentration gradient between solid and liquid phases. The model can be adapted to other situations involving thermally induced changes in irregular-shaped particulate foods.

17 Lawson, T. and Y. Chauhan, 1985: METABOLISM OF ARYLHYDRAZINES BY MOUSE LIVER MIXED-FUNCTION OXIDASES IN-VITRO. - J. Agric. Food Chem. 33: 218-219.

The metabolism of a group of arylhydrazines and arylhydrazides (found in the mushroom Agaricus bisporus and exhibits carcinogenicity) by mouse liver mixed-function oxidases was studied in vitro. The arylhydrazines, 4-methylphenylhydrazine hydrochloride and the N-acetyl derivatives of 4-methylphenyl- and 4-(hydroxymethyl) phenylhydrazine, were all readily metabolized. Their metabolism was inhibited by metyrapone and CO. Agaritine, the arylhydrazide (N-β-((+)-γ-glutamyl)-4-(hydroxymethyl)phenylhydrazine) (A), was poorly metabolized. The small amount of metabolism of A was not inhibited by metyrapone of CO, suggesting that the breakdown represented nonspecific decomposition, not metabolism.

18 Toth, B.1986: HYDRAZINES IN EDIBLE MUSHROOM OCCURRENCE CARCINOGENESIS CHEMISTRY AND ENVIRONMENTAL IMPLICATIONS.

SO UICC (UNION INTERNATIONALE CONTRE LE CANCER, INTERNATIONAL UNION AGAINST CANCER).

14TH INTERNATIONAL CANCER CONGRESS, BUDAPEST, HUNGARY, AUG. 21-27, 1986.

(No summary available)

19 Friederich, U., B. Fischer, J. Lüthy, D. Hann, C. Schlatter and F. E. Würgler, 1986: THE MUTAGENIC ACTIVITY OF AGARITINE A CONSTITUENT OF THE CULTIVATED MUSHROOM AGARICUS BISPORUS AND ITS DERIVATIVES DETECTED WITH THE SALMONELLA MAMMALIAN MICROSOME ASSAY AMES TEST. - Z. Lebensm. Unters. Forsch. 183: 85-89.

Purified agaritine (N'-(γ-L(+)-glutamyl)-p-hydroxymethyphenylhydrazine) isolat-

ed from *Agaricus bisporus*, p-hydrazinobenzoic acid (its presumptive precursor) and some agaritine-degradation products were tested for mutagenic activity with the Salmonella/mammalian microsome assay (Ames test). Consistent with the literature, agaritine showed a distinct direct-acting mutagenicity with the strain TA1537 (30 revertants/µmol) and with TA97. Incubation of agaritine at alkaline pH increased the mutagenic effect. Pre-incubation of agaritine with γ-glutamyl transferase (GT) during 10 h at room temperature (pH 8.2) even enhanced the mutagenicity by a factor of 8 to 16 depending on the strain. In accordance with this finding, synthetic p-hydroxymethylphenylhydrazine (the presumptive product of the GT catalyzed degradation) showed also a distinct direct-acting mutagenicity, but the increase was only about 3- to 6-times compared with agaritine. The hypothetical ultimate mutagenic metabolite of agaritine, the p-hydroxymethylbenzenediazonium ion, a compound occurring naturally in *A. bisporus*, showed the highest mutagenic activity (with TA1537 approximately 300 to 1000 revertants/µmol).

20 Stijve, T., R. Fumeaux and G. Philippossian, 1986: AGARITINE, A P-HYDROXYMETHYLPHENYLHYDRAZINE DERIVATIVE IN CULTIVATED MUSHROOMS (AGARICUS BISPORUS), AND IN SOME OF ITS WILD-GROWING RELATIVES. - Dtsch. Lebensm. Rundsch, 82: 243-248.

Agaritine is a naturally occurring 4-hydroxyphenylhydrazine derivative of glutamic acid found in *Agaricus bisporus* Lange, the cultivated mushroom of commerce in the Western hemisphere. Selective methods for the determination of agaritine in crude methanol extracts by both high performance liquid chromatography and thin-layer chromatography are described.

Agaritine concentrations were estimated in both fresh and processed mushrooms. Fresh mushrooms contain up to 0.065% of the hydrazine derivative, ant this level decreases with the age of the carpophore. Drying and canning operations reduce agaritine levels: as much as 95 percent may be removed by thermal processing and subsequent storage.

The presence of agaritine seems to be limited to *Agaricus* species. It was not encountered in 43 edible mushrooms from other genera.

Screening the genus *Agaricus* for an agaritine-free, easily cultivable mushroom was unsuccessful: eleven wild-growing, good edible species were also found to contain the compound, whereas five others, which lack the ability to biosynthesize agaritine, are either unedible, difficult to cultivate, or reported to produce potential harmful nitroamino acids.