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Can biogenic amines cause ailments following the intake of edible mushroom meals?

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Abstract: Several toxicological centres have reported ailments, mainly digestive inconveniences, following the intake of provably edible mushroom species. The causes of such complaints have not been explained yet. We, therefore, tested levels of biogenic amines (BAs). Fruit bodies of widely consumed wild-growing species, *Imleria badia* and *Suillus variegatus* were stewed, then preserved by freezing or canning and stored for up to 12 months. Contents of six amines were determined in the fresh matter, in each step of preservation and during storage. Histamine (HIM) and cadaverine (CAD) were not detected at all. Putrescine (PUT) occurred in fresh fruit bodies at levels of 700–1 500 mg kg^{-1} dry matter (DM), however, its contents considerably decreased, particularly during stewing. Undesirable phenylethylamine (PEA) and tyramine (TYM) occurred at lower levels. Stewing, the technological step necessary in both the tested preservation treatments, reduced the contents of all the amines alike as sterilisation, whereas following storage showed a limited effect. PUT seems to be the only amine that could participate in the reported ailments.

Keywords: wild edible mushrooms; ailments following intake; preservation; storage

Biogenic amines (BAs), in the narrow sense of the term, are formed in foods primarily by bacterial decarboxylation of free amino acids under inappropriate conditions of storage and treatment. Monoamines histamine (HIM), tyramine (TYM), phenylethylamine (PEA) and tryptamine (TRM), diamines putrescine (PUT) and cadaverine (CAD) produced from histidine, tyrosine, phenylalanine, tryptophan, ornithine, and lysine, respectively, are the main compounds of this group. Their effects on human health, particularly of HIM, TYM and PEA, are detrimental at an elevated intake.

The toxic dose of either individual amines or their combinations strongly depends on the efficiency of the detoxification mechanisms of an individual, particularly on the activity of intestinal monoamine oxidase. Statutory limits have been only sporadic so far. Recommended upper limits, expressed per kg of food fresh matter, vary between 50 mg and 200 mg for HIM (dealing mainly with some risky sea fish species), 100–800 mg for TYM and 30 mg for PEA (Shalaby 1996). However, these limits have been applicable to healthy individuals. The risk of TYM increases markedly in patients treated with drugs inhibiting monoamine oxidase. Toxicological roles of PUT, CAD and TRM have been considered benevolently; nevertheless, they deplete a part of the monoamine oxidase capacity.

Whilst extensive data on BA contents and changes during the storage and processing of various foods have been available, information on edible mushrooms has been very limited. PUT was BA occurring in the

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highest content in fresh fruit bodies of 17 tested wild-growing species (Dadáková et al. 2009).

Wild-growing mushrooms have been a very popular delicacy in some European countries. For instance, mushroom picking has been a national hobby in the Czech Republic, with a statistical mean of 5.0–5.6 kg of fresh mushrooms per household yearly (Sisak et al. 2016). However, consumption is considerably higher in some individuals. A part of mushrooms is cooked in households and consumed immediately after being collected, the rest is preserved chiefly by air-drying, freezing, or canning. The mushroom mass has a very limited shelf life due to very high moisture content, relatively high protein level and extensive microbial load. Such conditions are convenient for considerable BA formation. Very high contents, namely of PUT were observed in raw *Imleria badia, Xerocomus chrysenteron,* and to a lesser degree in *Suillus variegatus* stored at 6 °C for 48 h (Kalač and Křížek 1997). In a survey (Jabłońska-Ryś et al. 2020) of 47 market samples of variously preserved 9 wild-growing and 3 cultivated species, PUT was the main BA. Pickling was the more effective method than drying as regards the BA level.

There exists an unresolved question about the reasons of digestive inconveniences following sometimes the consumption of provably edible mushroom species. In Switzerland, ailments resulting from edible species were the most frequent reasons to contact a toxicological information centre during 1995–2009 (Schenk-Jaeger et al. 2012) and an emergency department in Bern during 2001–2017 (Keller et al. 2018). Similarly, in Poland, with the high consumption of wild-growing species, 87.5% of 457 cases in adults with clinical symptoms of poisoning in a hospital were caused by the ingestion of identified edible species (Gawlikowski et al. 2015). Within 443 identified mushroom poisonings in emergency departments of the Province of Parma, Italy, during 1996–2016, 24.4% of cases were due to edible species, mostly the popular *Boletus edulis* (Cervellin et al. 2018). In a recent four-year report from a Turkish emergency department, 34% of 168 mushroom poisonings surprisingly originated from cultivated species (Vişneci et al. 2019). Unfortunately, information on species and detected harmful substances has been mostly missing in these reports. Contamination of wild-growing mushrooms with toxic look-alike species cannot be thus excluded. There arises a question: Can BAs be among the poisoning causes? Recent reviews on poisoning caused by mushrooms do not remark on this group at all (Govorushko et al. 2019; Nieminen and Mustonen 2020).

The objective of this study was, therefore, to determine changes in BA contents during commonly used preservation treatments, freezing and canning, in two widely consumed wild-growing mushroom species with the previously detected high level of some amines in raw fruit bodies.

MATERIAL AND METHODS

Sampling and preservation treatments. Fruit bodies of both *I. badia* (formerly *Xerocomus badius*) and *S. variegatus* were collected from a coniferous forest during a day in September 2014 and in September 2017. Many tens of young and fully developed, non-injured complete fruit bodies (cap and stipe) were cleaned and cut up vertically into four equal portions 2–4 h after being collected. Each of the portions was then separately cut into slices about 2 mm thick, and the slices within each portion were mixed. One of the four portions was used for the determination of the initial level of BAs [after freeze-drying (Alpha 1–4 DL; Christ, Germany) of a proportion of about 500 g]. At the same time, a part (3 × 10 g) of the second portion of fresh slices was used for the determination of initial dry matter (DM).

The remaining two portions were used for usual preservation treatments:

i) Frozen storage of stewed mushrooms: the slices were stewed with added tap water (one-fifth of mushroom weight) in an open pan for 15 min. Approximately the same weight of water was evaporated. DM content was determined, and a part of the stewed mushrooms was freeze-dried for the determination of the amines (time 0). Six and twelve polypropylene cups of 150 mL in volume were filled up with 140 g of cooled stewed mushrooms in 2014 and 2017, respectively, closed and stored in a freezer mat at –18 °C (Comfort; Liebherr, Germany). The storage period was up to 6 months and 12 months in 2014 and 2017, respectively. Three cups were sampled on each of the sampling dates.

ii) Canned mushrooms: the slices stewed together with those for the previous variant were used. Nine and fifteen glass jars of 180 mL in volume with twist-off caps were filled up with 150 g of hot stewed slices in 2014 and 2017, respectively, closed and sterilised in a boiling water bath for 30 min. The jars were then stored for 48 h at a laboratory temperature of 21–22 °C to enable the germination of bacterial spores. The second sterilisation followed under the same conditions as the first one. The jars were then stored at 21–22 °C in dark for up to 6 months and

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Table 1. Parameters of the used analytical method determined by 10 replicates of an internal matrix material (freeze-dried *Imleria badia*)

Parameter	Tyramine (TYM)	Phenylethylamine (PEA)	Tryptamine (TRM)	Putrescine (PUT)
Content (mg kg^{-1} DM) (mean ± SD; n = 10)	52.7 ± 1.4	205 ± 4.7	43.5 ± 1.4	726 ± 14.5
LOQ (mg kg^{-1} DM)	3.1	2.3	2.5	2.2
Recovery (%)	71	79	89	85
Repeatability (%)	2.7	2.3	3.3	2.0

DM – dry matter; LOQ – limit of quantification; contents of histamine (HIM) and cadaverine (CAD) were below limits of quantification (1.9 mg kg^{-1} and 1.9 mg kg^{-1} DM, respectively)

12 months in 2014 and 2017, respectively. DM content and initial contents of the amines after stewing were the same as in the previous variant. The contents were determined also in the mushroom matter after the second sterilisation (time 0). Three jars were sampled on each of the sampling dates.

Analytical methods. DM content of mushroom fruit bodies differs both among and within species, mainly due to different weather conditions preceding the picking. Thus, amine contents are expressed per DM of freeze-dried fruit bodies in this study.

All analyses of the amines were performed in triplicate. All used chemicals were of analytical or higher grades.

A procedure using freeze-dried samples was developed and described in detail (Dadáková et al. 2009). Briefly, a freeze-dried powdered sample was extracted with 0.6 M perchloric acid with added heptane-1,7-diamine as an internal standard. The extracted amines were derivatised with dansyl chloride at pH 9.2. The dansyl derivatives were extracted with heptane, an aliquot was then evaporated to dryness. The solids were dissolved in acetonitrile.

The calibration curve was prepared by the same procedure. Instead of the mushroom extract, 1 mL of the standard solution of amines was used. The solution was prepared by the dissolution of 400 mg of each of the six amines (HIM, TYM, PEA, TRM, PUT and CAD) per L of 0.6 M perchloric acid and 100 µL of the internal standard solution was added. The following steps were the same as in the sample analysis.

The derivatised amines were determined using an Agilent rapid resolution liquid chromatograph (RRLC) with a Zorbax Eclipse XDB-C18 column [50 mm × 4.6 mm internal diameter (i.d.), 1.8 µm particle size], equipped with a RRLC in-line filter 0.2 µm (Agilent Technologies, USA) under the described conditions (Dadáková et al. 2009).

The parameters of the analytical method were determined by 10 replicates using the internal matrix material of freeze-dried *I. badia* (Table 1).

Statistical methods. All analytical data were processed using the programme ChemStation for LC 3D Systems (Agilent Technologies, USA). The tools of MS Office Excel were used for calibration and calculation of parameters of the used analytical method. The statistical significance of changes in amine contents during storage (between time 0 and the final month) was tested by regression analysis and t-test using statistical tools of MS Office Excel. Statistical significance was tested at a level of $P < 0.05$.

RESULTS AND DISCUSSION

DM contents of the analysed species varied around 10 g in 100 g fresh weight, the levels typical of fresh mushroom fruit bodies. The contents expressed per fresh matter are thus about ten times lower than the following data on the amine contents expressed per DM.

Only BAs with quantifiable contents are given in Tables 2–5.

In the analysed fresh fruit bodies, PUT occurred at the highest levels, between 700 mg kg^{-1} and 1 500 mg kg^{-1} DM (Tables 2–5). No regulations for PUT in foods have been established so far. Rauscher-Gabernig et al. (2012) proposed maximum tolerable levels between 140 mg kg^{-1} and 180 mg kg^{-1} (original matter) for sauerkraut, fish, and cheese. The highest contents, up to about 150 mg kg^{-1} fresh matter found in the analysed mushrooms, should be thus taken into consideration if evaluating possible detrimental health effects. Moreover, the content of PUT can increase considerably in fresh fruit bodies even under proper storage conditions (Kalač and Křížek 1997). Both the tested preservation treatments, however, decreased

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the PUT level as compared with the initial content in the fresh matter.

Biologically active PEA was the amine with the second highest content in *I. badia* (between 200 mg kg^{-1} and 280 mg kg^{-1} DM); however, it was not detected in *S. variegatus.* Both TYM and TRM were present at levels below 100 mg kg^{-1} DM. HIM was not detected, likewise CAD. The values fit well with previous reports (Kalač and Křížek 1997; Dadáková et al. 2009). HIM, the most detrimental amine, was, however, detected at high levels in dried (types of drying unspecified) *B. edulis*, *I. badia* and *S. variegatus* (Jabłońska-Ryś et al. 2020).

Overall, the analysed fresh species belong among raw food materials with very high levels of PUT as compared with vegetables and other food items of plant origin (Kalač 2014). The differences in BA composition and content may be caused both by mushroom species, as apparent between *S. variegatus* and *I. badia,* and microbial load and activity, notably of putrefactive bacteria.

Stewing, the technological step necessary in both the tested preservation treatments, decreased the contents of all amines (Tables 2–5). The greatest reduction was observed in TRM in *I. badia* (Tables 2, 4). A part of the amines could be released from tissues into juice; however, it seems to be retained in the preserved mushrooms. Overall, stewing appears to be an important factor of BA reduction.

The effects of frozen mushroom storage (Tables 2, 3) on changes in the amine contents should be considered chiefly by the experiments in 2017 with storage periods up to 12 months. All possibilities occurred – stability, decrease and increase. Nevertheless, the changes were significant ($P < 0.05$) in only several cases and on the whole, they were not too noticeable.

Double sterilisation of canned mushrooms (Tables 4, 5) caused a certain increase of the amines (except of TRM) in *I. badia* harvested in 2014 (Table 4) as compared with the level in the stewed matter, while a decrease was observed in *I. badia* harvested in 2017 (Table 4) and in *S. variegatus* (Table 5). The following storage up to 12 months brought significant ($P < 0.05$) changes in several cases, nevertheless of a limited extent. The storage did not affect the contents of biologically active TYM and PEA.

Overall, stewing and sterilisation show to be the main treatments decreasing the contents of amines, whereas the following storage has a limited effect. Proper treatment of mushrooms thus keeps the amines at levels mostly safe for healthy consumers.

Unfortunately, the nature of changes in BA during the culinary processing of food materials has not been explained yet. It has been accepted that the amines occur as basic cations bound as salts and in conjugates. There may be supposed a reaction between primary amino groups, mainly of diamines, with reducing sugars under thermal treatments such as stewing or sterilisation of mushrooms, leading to a decrease of the amine contents. However, according to limited available data, the

Table 2. Changes in biogenic amine (BA) contents during stewing and following storage of frozen sliced *Imleria badia* fruit bodies harvested in 2014 and 2017 [mg kg^{-1} dry matter (DM)] (mean ± SD; $n = 3$)

Parameter	Tyramine (TYM)	Phenylethylamine (PEA)	Tryptamine (TRM)	Putrescine (PUT)
2014				
Original matter	59.1 ± 4.1	208 ± 8.1	40.9 ± 0.7	696 ± 16.1
After stewing (time 0)	48.3 ± 0.6	136 ± 2.7	27.7 ± 1.4	533 ± 12.7
After storage for 3 months	61.7 ± 4.1	154 ± 3.8	26.4 ± 0.5	600 ± 24.8
After storage for 6 months	65.1 ± 3.3	156 ± 2.9	29.9 ± 0.5	635 ± 16.2
Significance of storage (α)	0.004	0.004	0.054	0.001
2017				
Original matter	97.4 ± 0.1	277 ± 3.4	96.8 ± 3.8	1 070 ± 64.3
After stewing (time 0)	81.9 ± 1.6	204 ± 0.9	44.1 ± 0.1	798 ± 21.0
After storage for 3 months	80.7 ± 3.3	195 ± 2.5	55.5 ± 1.7	811 ± 16.8
After storage for 6 months	81.6 ± 1.6	201 ± 4.3	55.6 ± 1.1	804 ± 36.0
After storage for 9 months	77.3 ± 4.1	180 ± 1.8	63.9 ± 1.2	795 ± 20.3
After storage for 12 months	79.1 ± 4.3	185 ± 5.1	66.4 ± 2.5	842 ± 24.2
Significance of storage (α)	0.69	0.61	0.32	0.003

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Table 3. Changes in biogenic amine (BA) contents during stewing and following storage of frozen sliced *Suillus variegatus* fruit bodies harvested in 2014 and 2017 [mg kg^{-1} dry matter (DM)] (mean ± SD; n = 3)

Parameter	Tyramine (TYM)	Putrescine (PUT)
2014		
Original matter	23.1 ± 0.5	1 040 ± 5.6
After stewing (time 0)	20.9 ± 0.2	925 ± 47
After storage for 3 months	–	763 ± 37.6
After storage for 6 months	23.2 ± 0.9	781 ± 24.6
Significance of storage (α)	0.30	0.06
2017		
Original matter	26.3 ± 0.1	1 520 ± 39.4
After stewing (time 0)	21.6 ± 0.5	1 180 ± 56.1
After storage for 3 months	20.3 ± 1.1	1 180 ± 50.7
After storage for 6 months	19.0 ± 1.1	1 080 ± 42.7
After storage for 9 months	25.0 ± 4.0	1 120 ± 11.8
After storage for 12 months	25.1 ± 1.9	1 110 ± 19.9
Significance of storage (α)	0.02	0.07

contents of glucose and arabinose in mushrooms are relatively low (Kalač 2016).

Microbial, in particular bacterial, contamination shows to be a potentially serious cause of alimentary ailments following the consumption of provably edible species. Fresh mushrooms are an ideal medium for microbial growth due to high water activity, relatively high protein content and neutral pH. Fruit bodies of wild-growing mushrooms are exposed to various weather conditions and particularly to attacks of numerous pests, namely insects, snails, and animals. Infection with various microorganisms could be thus supposed. Unfortunately, literature data have been very scarce. Venturini et al. (2011) determined a microbial load in 226 samples of 14 wild-growing species from Spanish retail markets and supermarkets. The total microbial counts ranged from 4.4 log colony-forming units (CFU) per g fresh matter to 9.4 log CFU per g fresh matter with the prevailing genus *Pseudomonas*. *Listeria monocytogenes* was detected in 11.5%, *Yersinia enterocolitica* in 1.8% of the analysed samples. No pathogens were isolated from 176 samples of 7 cultivated species.

As results from medical evaluations (Schenk-Jaeger et al. 2012; Gawlikowski et al. 2015; Cervellin et al. 2018; Keller et al. 2018), ailments caused by edible mushrooms have been generally limited to nausea, vomiting, stomach ache and diarrhoea starting during several hours after the ingestion. Gastroenteritis can be induced also by the high consumption of poorly digestible mushroom mucus (slime from the cap, e.g. from some *Suillus* spp.) (Prager and Goos 1984) or from the low ac-

Table 4. Changes in biogenic amine (BA) contents during stewing, double sterilisation and following storage in glass jars of sliced *Imleria badia* fruit bodies harvested in 2014 and 2017 [mg kg^{-1} dry matter (DM)] (mean ± SD; n = 3)

Parameter	Tyramine (TYM)	Phenylethylamine (PEA)	Tryptamine (TRM)	Putrescine (PUT)
2014				
Original matter	59.1 ± 2.1	208 ± 18.1	40.9 ± 0.7	696 ± 16.1
After stewing	48.3 ± 0.6	136 ± 2.7	27.7 ± 1.4	533 ± 12.7
After sterilisation (time 0)	52.1 ± 1.6	159 ± 2.6	23.1 ± 0.9	584 ± 17.8
After storage for 3 months	56.9 ± 1.5	155 ± 8.5	30.7 ± 1.5	596 ± 13.8
After storage for 6 months	60.5 ± 1.9	161 ± 6.8	32.9 ± 1.3	589 ± 8.3
Significance of storage (α)	0.23	0.65	0.009	0.86
2017				
Original matter	97.4 ± 0.1	277 ± 11.4	96.8 ± 3.8	1 070 ± 53.4
After stewing	81.9 ± 1.1	204 ± 0.9	44.1 ± 0.1	797 ± 21.0
After sterilisation (time 0)	73.9 ± 3.1	197 ± 5.3	37.5 ± 0.7	768 ± 36.2
After storage for 3 months	80.0 ± 2.8	201 ± 5.5	42.4 ± 2.3	810 ± 15.0
After storage for 6 months	79.0 ± 1.1	195 ± 7.1	38.1 ± 1.5	813 ± 37.1
After storage for 9 months	81.9 ± 1.8	207 ± 5.9	49.9 ± 1.1	922 ± 21.5
After storage for 12 months	74.9 ± 1.1	189 ± 2.3	40.0 ± 0.9	885 ± 35.0
Significance of storage (α)	0.69	0.62	0.32	0.003

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Table 5. Changes in biogenic amine (BA) contents during stewing, double sterilisation and following storage in glass jars of sliced *Suillus variegatus* fruit bodies harvested in 2014 and 2017 [mg kg^{-1} dry matter (DM)] (mean ± SD; n = 3)

Parameter	Tyramine (TYM)	Putrescine (PUT)
2014		
Original matter	23.1 ± 0.5	1 040 ± 5.6
After stewing	20.9 ± 0.2	925 ± 7.2
After sterilisation (time 0)	15.1 ± 0.4	712 ± 7.0
After storage for 3 months	17.7 ± 0.5	884 ± 11.5
After storage for 6 months	16.4 ± 0.6	865 ± 9.5
Significance of storage (α)	0.33	0.079
2017		
Original matter	26.3 ± 0.1	1 520 ± 39.4
After stewing	21.6 ± 0.5	1 180 ± 56.1
After sterilisation (time 0)	18.9 ± 0.4	1 080 ± 29.1
After storage for 3 months	15.5 ± 0.6	998 ± 35.5
After storage for 6 months	16.7 ± 0.9	1 020 ± 34.6
After storage for 9 months	20.7 ± 0.8	965 ± 40.7
After storage for 12 months	23.2 ± 0.3	1 030 ± 42.1
Significance of storage (α)	0.008	0.097

tivity of trehalase in the small intestine mucosa, an enzyme converting disaccharide α,α-trehalose to glucose (Arola et al. 1999). Trehalose is a by far prevailing sugar in mushrooms, however, its levels vary very widely among species. The contents above 10 g (100 g)$^{-1}$ DM are not rare, levels around 40 g (100 g)$^{-1}$ DM were reported in several species (Kalač 2016). Maldigestion of trehalose in some individuals, e.g. those with untreated celiac disease, can cause abdominal symptoms similar to those of lactose maldigestion and intolerance.

The main toxic substances occurring in mushrooms are proteins/peptides able to promote cytotoxic effects acting on specific targets. For instance, two novel ribotoxin-like proteins, enzymes already studied in other organisms for their toxicity, were recently discovered in *B. edulis*. Proper cooking is thus necessary for their destruction (Landi et al. 2021).

CONCLUSION

The contents of BAs with possible detrimental health effects differed between the tested widely consumed wild-growing species. In the raw state, HIM was not detected in any species, TYM occurred at levels considered as safe. Attention should be paid to the contents of PEA in *I. badia*. Nevertheless, the contents of both the determined BAs decreased during the tested preservation treatments. However, the highest observed levels of PUT in fresh fruit bodies and, in particular, its easy production, mainly under improper storage conditions, should be taken into consideration. PUT thus cannot be excluded from causes of ailments following edible mushroom intake. For a generalising evaluation, our results observed in two species should be verified for further edible species.

Contamination of wild-growing edible mushrooms with pathogenic bacteria and enterotoxins seems to be the major potential factor. Fruit bodies aged, mushy or damaged by pests should be excluded. Conditions convenient for microbial growth and activity, such as elevated temperature and high humidity, should be minimised, and effective thermal treatment should follow the collection of fruit bodies as soon as possible. The handling of mushroom meals should respect their highly perishable nature.

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