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Formation of biogenic amines in four edible mushroom species stored under different conditions

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Wild growing mushrooms (*Boletus badius*, *Boletus chrysentereon* and *Boletus variegatus*) and cultivated common mushroom (*Agaricus bisporus*) were stored at 6°C or 20°C as intact fruiting bodies, wet slices and stewed slices for at least 2 days. Biogenic amines were determined as *N*-benzamides by high-performance liquid chromatography. No detectable levels of histamine and tyramine were observed. Concentrations of putrescine were up to 1600 mg kg^{-1} dry matter and of cadaverine up to 165 mg kg^{-1} dry matter in mushrooms of consumable quality. These levels seem to be safe for human consumption. No unambiguous effect of storage temperature on the amine levels was observed. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Wild growing mushrooms are a very popular delicacy in several countries of Central and Eastern Europe. Collecting mushrooms has become a 'national hobby' in the Czech Republic.Their annual consumption may exceed 10 kg in some individuals.

Because of the very low dry matter content of the fruiting bodies (approximately 10%) and a chemical composition suitable for undesirable changes by both endogenous and microbial enzymes, mushrooms should be stored at low temperatures and consumed as soon as possible after harvesting. Cookery books and similar literature give warnings against the consumption of old and thawed fruiting bodies, prolonged storage and reheating of dishes containing mushrooms and the simultaneous consumption of mushrooms and alcohol. However, the causes of the health risks remain unclear.

The formation of biogenic amines seems to be a process which could participate in the undesirable changes. Yen (1992) reported notable increases in the content of six biogenic amines during storage of straw mushroom (*Volvariella volvacea* (Bull. ex Fr.) Sing.) at 25°C versus 4°C.

Biogenic amines form a group of antinutritional compounds. Increased intake of these substances can exert psychoactive and/or vasoactive effects. Histamine and tyramine seem to be the most biologically active. The amines arise mainly from microbial decarboxylation of the corresponding amino acids, the diamines putrescine and cadaverine and the monoamines histamine and tyramine being formed from ornithine, lysine, histidine and tyrosine, respectively. Biogenic amines are widespread in foods (Smith, 1981; Askar & Treptow, 1986; Ten Brink *et al.*, 1990; Davídek & Davídek, 1995).

The objective of the present work was to determine changes in the concentrations of four common biogenic amines in widely consumed species of wild edible mushrooms stored under various household conditions. The cultivated common mushroom was taken as a comparative material.

MATERIALS AND METHODS

Samples

Fruiting bodies in different stages of development were collected during the autumn in 1994 and 1995 from coniferous woods around the town of České Budějovice. Species of the genus *Boletus* (*Xerocomus*) were tested: namely, *Boletus badius* Fr., *Boletus chrysentereon* (Bull.) Quél. and *Boletus variegatus* Sow. ex Fr. Common mushroom (*Agaricus bisporus* (Lange) Imbach), strain X-20, was taken from a mushroom plant.

The treatments and storage of the mushrooms simulated different household conditions. The fruiting bodies were cleaned as for culinary purposes and divided into three parts. The first part was left as the intact bodies; the second part was cut into slices and left in the wet

state; the third part was stewed by boiling for 10 min in the proportions 0.70 kg of sliced mushrooms to 0.05 kg of water. The cooled down material was then sampled again. The three variants were stored in a refrigerator at 6°C and in the dark at 20°C. Both sliced variants were kept in beakers in layers 8–10 cm thick.

Analytical methods

Mushroom dry matter was determined in duplicate by oven-drying at 105°C for 6 h.

The amines (putrescine, cadaverine, histamine and tyramine) were determined as the *N*-benzamides, after derivatization with benzoylchloride, using high-performance liquid chromatography with isocratic elution. Two parallel analyses were carried out. Histamine and tyramine were determined by the first analysis and putrescine and cadaverine by the second analysis. The procedure has been described in detail by Křížek (1991).

The amines were extracted from 20 g of sliced mushrooms with diluted perchloric acid under shaking for 60 min. Recovery of extraction was 95.4% and 89.5% for putrescine and cadaverine, respectively. Differences between the parallel determinations did not exceed 12%.

The results are presented as mean values from duplicate analyses.

RESULTS AND DISCUSSION

No detectable concentrations of histidine and tyramine were observed in this study, although Yen (1992) reported concentrations of up to hundreds of milligrams per kilogram (wet basis) in *Volvariella volvacea* stored at 25°C for 5 days. A partial explanation for this may be in the different contents of precursor amino acids in different mushroom species.

Dry matter contents and putrescine and cadaverine concentrations in the initial materials are given in Table 1. Levels of putrescine were considerably higher than those of cadaverine. A similar pattern was observed by Yen (1992). Differences in the amine concentrations may be affected by mushroom species, the proportions of young and old fruiting bodies and also by weather conditions, which may affect, for example, the Enterobacteriaceae population (Simon-Sarkadi *et al.*, 1994).

No detectable amines in *Agaricus bisporus* can be attributed to testing of homogeneous young fruiting

Table 1. Initial dry matter and amine concentrations in fresh and stewed mushrooms

	Initial DM (%)	Amine concentration (mg kg^{-1})			
		Putrescine		Cadaverine	
		FM	DM	FM	DM
Fresh mushrooms					
Boletus badius (1994)	7.94	80.4	1010	4.0	50.4
Boletus badius (1995)	7.91	65.5	828	9.4	119
Boletus chrysentereon	7.70	49.6	644	ND	ND
Boletus variegatus	7.64	43.2	565	ND	ND
Agaricus bisporus	8.92	ND	ND	ND	ND
Stewed mushrooms					
Boletus badius (1994)	9.39	137	1460	7.9	84.1
Boletus badius (1995)	8.98	46.8	521	ND	ND
Boletus chrysentereon	9.03	58.8	651	8.5	94.1
Boletus variegatus	9.19	69.2	754	7.7	83.9
Agaricus bisporus	12.60	ND	ND	ND	ND

FM, fresh matter; DM, dry matter; ND, not detectable.

Table 2. Concentrations of putrescine (mg kg^{-1} dry matter) in mushrooms after 48 h storage at different temperatures

	Intact fruiting bodies		Slices		Stewed slices	
	6°C	20°C	6°C	20°C	6°C	20°C
Boletus badius (1994)	558	430	1630	955[a]	674	1240
Boletus badius (1995)	1160	1380[a]	1170	1300[b]	1020	273[a]
Boletus chrysentereon	754	527	628	5050[b]	679	562
Boletus variegatus	539	580	500	573	597	625
Agaricus bisporus	ND	368	ND	116	ND	33.4

ND, not detectable.
[a]Limit of consumability.
[b]Spoiled mushrooms.

Table 3. Concentrations of cadaverine (mg kg^{-1} dry matter) in mushrooms after 48 h storage at different temperatures

	Intact fruiting bodies		Slices		Stewed slices	
	6°C	20°C	6°C	20°C	6°C	20°C
Boletus badius (1994)	43.0	44.2	74.8	76.9[a]	ND	60.2
Boletus badius (1995)	165	103[a]	82.4	74.4[b]	51.1	23.1[a]
Boletus chrysentereon	ND	ND	11.4	1020[b]	27.2	35.4
Boletus variegatus	ND	133	ND	ND	64.7	ND
Agaricus bisporas	ND	36.9	ND	ND	ND	ND

ND, not detectable.
[a]Limit of consumability.
[b]Spoiled mushrooms.

bodies. No clear tendency in amine concentration changes as a result of stewing can be observed. Yen (1992) reported decreases of about one-third and one-half for putrescine and cadaverine, respectively, during cooking of *Volvariella volvacea* in water for 5 min. However, information on the character of the losses (extraction to water or volatilization) is lacking.

Concentrations of putrescine and cadaverine in all the tested mushrooms after 2 days of storage are given in Tables 2 and 3, respectively. This storage time is usually recommended as an upper limit. Analysis of variance, using the *F*-criterion, showed that the effects of mushroom species, treatment and storage temperature on the amine levels were insignificant.

Examples of changes in putrescine and cadaverine concentrations during 7 days of storage are given in Tables 4 and 5, respectively. The fluctuation in putrescine concentrations may be partially attributed to the formation of spermidine and spermine from the amine.

A meal of wild growing mushrooms may contain up to about 40 g of dry matter. Thus, from the highest observed concentrations in mushrooms of consumable quality a consumer could ingest about 65 and 7 mg of putrescine and cadaverine, respectively. Although no limits of maximal intake are known for these amines, the calculated intake may be considered as safe. Moreover, putrescine in lower doses plays a positive role as an essential substance for growth and cell proliferation (Bardócz, 1993; Bardócz *et al.*, 1995).

Table 4. Changes in putrescine concentrations (mg kg^{-1} dry matter) in *Boletus chrysentereon* during prolonged storage

Storage time (days)	Intact fruiting bodies		Slices		Stewed slices	
	6°C	20°C	6°C	20°C	6°C	20°C
2	754	527	629	5050[b]	679	562
3	711	832[b]	936	—	838	497[b]
4	437	—	834	—	720[a]	—
7	—	—	1630[b]	—	790[b]	—

ND, not detectable.
[a]Limit of consumability.
[b]Spoiled mushrooms.

Table 5. Changes in cadaverine concentrations (mg kg^{-1} dry matter) in *Boletus chrysentereon* during prolonged storage

Storage time (days)	Intact fruiting bodies		Slices		Stewed slices	
	6°C	20°C	6°C	20°C	6°C	20°C
2	ND	ND	11.4	1020[b]	27.2	35.4
3	ND	ND[b]	ND	—	ND	68.4[b]
4	ND	—	ND	—	22.1[a]	—
7	—	—	2000[b]	—	293[b]	—

ND, not detectable.
[a]Limit of consumability.
[b]Spoiled mushrooms.

CONCLUSION

The tested species of widely consumed edible mushrooms contain putrescine and cadaverine in levels which could be assessed as safe. Thus, biogenic amines do not seem to be the agents causing some complaints following consumption of inappropriately treated edible mushrooms.

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