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Concentrations of five biogenic amines in Czech beers and factors affecting their formation

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Biogenic amines in beer and in solid raw materials were determined by a high-performance liquid chromatographic method as *N*-benzamides. In a survey of 78 proprietary bottled bottom-fermented lager beers from 32 Czech breweries, mean concentrations were 0.55, 1.21, 6.85, 8.84 and 12.9 mg/l and standard deviations 1.08, 1.46, 5.19, 7.06 and 12.3 mg litre^{-1} for histamine, tryptamine, tyramine, putrescine and cadaverine, respectively. No significant differences were observed between beer types (pale/dark, original extract of wort 10%/12%) and amine concentrations. Testing three batches for changes in the amine concentration during the brewing process showed that the formation of amines occurred principally during the main fermentation. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Biogenic amines form a group of natural antinutritional and possibly toxic compounds widespread in foods (Smith, 1981; Askar & Treptow, 1986; Davídek & Davídek, 1995). They arise mainly from microbial decarboxylation of the corresponding amino acids or by transamination of aldehydes by amino acid transaminases. The most common diamines, cadaverine and putrescine, and the monoamines histamine, tyramine and tryptamine, are formed by microbial decarboxylation from lysine, ornithine, histidine, tyrosine and tryptophan, respectively.

Biogenic amines in low concentrations are essential for many physiological functions, while high concentrations may cause some deleterious effects. They function psychoactively and/or vasoactively. Psychoactive amines affect the neural transmitters in the central nervous system. The vasoactive amines act either directly or indirectly on the vascular system as vasoconstrictors (e.g. tyramine) or vasodilators (e.g. histamine). A scale of symptoms occurs following excessive oral intake of the biogenic amines. Toxicity strongly depends on the efficiency of detoxication, which may vary considerably between individuals and is affected by several factors. Normal intakes of the biogenic amines are metabolized in the intestinal tract by a fairly efficient detoxication system based on the activities of monoamine oxidase (EC 1.4.3.4) and diamine oxidase (EC 1.4.3.6) (Ten Brink *et al.*, 1990).

In non-fermented foods, the biogenic amines appear as a result of undesirable microbial activity. Very high levels of histamine are found in spoiled fish, especially from the scombroid group; a scale of the amines occurs in some well-matured cheeses, fish and meat products, as well as in some fruits and vegetables (e.g. spinach and tomato). Medium levels are usually observed in fermented products such as sauerkraut, soya sauce, wine and beer.

The occurrence and formation of biogenic amines, including spermidine and spermine formed from putrescine, were studied in beers produced in Italy (Cerutti *et al.*, 1985, 1987, 1989; Buiatti *et al.*, 1995), Spain (Izquierdo-Pulido *et al.*, 1989, 1991, 1994), Belgium (Dumont *et al.*, 1992), Germany (Donhauser *et al.*, 1992) and Cuba (Vidaud *et al.*, 1989).

The objective of the present work was to determine concentrations of five biogenic amines in Czech beers and the effects of technological factors on their formation during the brewing process. Beer is consumed in considerable quantities in the Czech Republic. Statistical consumption was about 165 litres per capita in 1994. Thus, beer could represent an important source of intake of these amines.

MATERIALS AND METHODS

Sampling

Samples of bottled bottom-fermented lager beers were taken from supermarkets within the Czech Republic from October 1994 to March 1995. Types and brands of

beers with massive levels of production and consumption were sampled.

Raw materials and semi-products of the brewing process were sampled in a brewery using traditional technology enabling control of a batch during the manufacturing process.

Amines determination

The amines were determined after their derivatization with benzoylchloride as *N*-benzamides by a high-performance liquid chromatographic (HPLC) method with isocratic elution. Two parallel analyses were necessary. Histamine and tyramine were determined by the first and the other three amines by the second. The analytical procedure was described in detail by Křížek and Hlavatá (1995).

Histamine and tyramine were determined in 35 ml of beer degassed under vacuum, to which 1,7-diaminoheptane was added as an internal standard. The mixture was alkalized with sodium hydroxide solution, benzoylchloride was added followed by sonication. The procedure continued with acidification to pH 6.0 with perchloric acid, addition of a phosphate buffer and sodium chloride and mixing. The *N*-benzamides formed were extracted into 10 ml of diethyl ether; 5 ml of the extract were evaporated with a warm air stream and the residue was dissolved in 0.4 ml of mobile phase (71% (v/v) methanol in water). A 10 μl aliquot of the extract was then injected for HPLC analysis.

Cadaverine, putrescine and tryptamine were determined in 40 ml of degassed beer. A solution of 1,6-diaminohexane was used as an internal standard. The following procedure was the same as described above, but only 1 ml of diethyl ether extract was evaporated and dissolved in 0.3 ml of 63.5% (v/v) methanol in water. The injected volume was again 10 μl.

Separation of *N*-benzamides was carried out using a high-performance liquid chromatograph HPP 4001 (Laboratorní Přístroje, Prague) on an HPLC column SGX-C_{18} 3 mm×150 mm at 15 MPa, a flow rate 0.5 ml min^{-1} at 254 nm. The peak areas were integrated using an Apex integrator (Data Apex, Prague). The retention times were 10.15 and 14.95 min for histamine and tyramine, respectively, and 6.50, 7.35 and 8.40 min for putrescine, cadaverine and tryptamine, respectively.

A modified procedure was used for determination of the amines in solid raw materials and spent grain; 12–30 g of material was twice shaken with diluted perchloric acid and centrifuged. Supernatants were dissolved in perchloric acid and used for derivatization of the amines.

All the chemicals used were of analytical grade. Chromatographic determination was carried out within a week after derivatization. The samples for HPLC analyses were stored at −15°C.

The analytical data were calculated from the calibration values for the individual amines designed for concentration ranges of 5–25 mg $litre^{-1}$. Samples were diluted for higher amine concentrations. The correlation coefficients for the amine calibration curves ranged between 0.997 and 0.999. The detection limits were very similar for all the amines, with values of about 0.3 mg $litre^{-1}$.

Reproducibility of the analytical procedure was tested by parallel analyses of seven samples from one bottle of beer. Relative standard deviations were 2.7%, 7.6%, 8.5%, 11.2% and 11.4% at mean concentrations of 35.2, 10.5, 5.85, 21.5 and 1.51 mg $litre^{-1}$ for cadaverine, putrescine, histamine, tyramine and tryptamine, respectively.

RESULTS AND DISCUSSION

Uniformity of the amine concentrations within a batch was tested by parallel analyses of seven bottles from one batch. Relative standard deviations were 4.4%, 6.0%, 9.4%, 11.4% and 12.5% at mean concentrations of 32.8, 8.44, 0.88, 6.30 and 1.14 mg $litre^{-1}$ for cadaverine, putrescine, histamine, tyramine and tryptamine, respectively. The values of the deviations are comparable with those observed in testing the reproducibility of the analytical procedure and those reported by Izquierdo-Pulido *et al.* (1993) using determination by ion-pair liquid chromatography with postcolumn derivatization. We thus used one bottle as a representative sample of a batch of beer.

Uniformity of the amine concentrations in a type of beer from a brewery in different batches was tested in three types of widely consumed bottled beer. Five batches of each type of beer were sampled at 2-week intervals. The results are given in Fig. 1. There are differences in the amine concentrations among batches up to 70–150% of the maximum values. Comparable differences among batches were reported by Donhauser *et al.* (1992). However, absolute concentration levels seem to be characteristic of a brewery and (to a limited extent) of the different types of beers produced within this brewery. Similar conclusions were reached for histamine and tyramine in Spanish beers (Izquierdo-Pulido *et al.*, 1989), and for tyramine, cadaverine and, to a limited extent, tryptamine in German beers (Donhauser *et al.*, 1992).

A survey of the amine occurrence was carried out with 78 samples of different brands of bottled beers from 32 Czech breweries. Numbers of samples correspond to the status of a type of beer in the Czech market. Comprehensive statistical data are given in Table 1, distribution of the amine concentrations in Fig. 2.

Analysis of variance of relationships between beer types (pale/dark, original extract of wort 10%/12%, w/w) and amine concentrations showed no significant differences.

About 90% of samples contained less than 2 mg $litre^{-1}$ of histamine and nearly 80% of samples had a tryptamine level below 2 mg $litre^{-1}$, while concentrations of

10 mg litre^{-1} for tyramine and putrescine were exceeded in nearly 30% of samples. The maximum levels determined in the beers tested were 5.91, 6.59, 22.5, 30.7 and 49.1 mg litre^{-1} for histamine, tryptamine, tyramine, putrescine and cadaverine, respectively.

These findings compare well with data reported for numerous European and Cuban beers (Cerutti *et al.*, 1985, 1987; Izquierdo-Pulido *et al.*, 1989; Vidaud *et al.*, 1989; Donhauser *et al.*, 1992; Dumont *et al.*, 1992; Buiatti *et al.*, 1995). However, increased levels of putrescine were observed in Czech beers. The concentrations most often reported in European beers are within the range 0.2–8.0 mg litre^{-1}, whereas the upper value was exceeded in about one-third of our samples.

The observed amine concentrations can be considered as virtually harmless for most beer consumers despite the fact that alcohol inhibits the activity of both monoamine oxidase and diamine oxidase. However, the situation is different for patients being treated with antidepressive drugs that inhibit monoamine oxidase. A single dose of 5–6 mg of tyramine or histamine may have a deleterious effect. Even non-alcoholic beers must be considered unsuitable for these patients, as recommended previously by Murray *et al.* (1988).

The second part of our work dealt with factors affecting amine concentrations during the brewing process and storage of bottled beers. Three batches were checked during brewing, from raw materials to the final

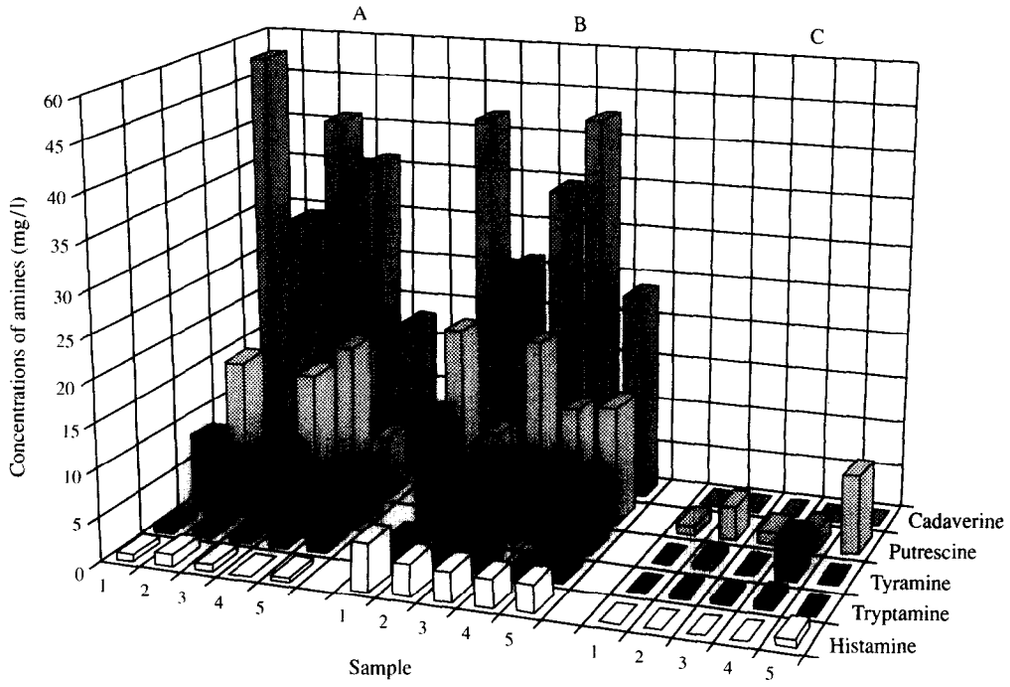

Fig. 1. Amine concentrations (mg litre^{-1}) in five batches of three types of beer: A, 10%, pale; B, 10%, dark; C, 12%, pale.

Table 1. Amine concentrations (mg litre^{-1}) in bottled beers tested

Beer type:	12%[a], pale	12%[a], dark	10%[a], pale	10%[a], dark	Non-alcoholic	Diet	Low-energy
No. of samples:	22	5	25	11	7	5	3
Histamine							
$\bar{x}$	0.59	0.41	0.30	0.73	0.77	1.19	0.30
s_x	1.14	0.41	0.58	1.69	1.26	1.65	0.52
Tryptamine							
$\bar{x}$	0.99	0.56	1.23	1.23	1.42	1.65	2.77
s_x	1.10	0.61	1.49	1.31	1.82	1.66	3.42
Putrescine							
$\bar{x}$	7.74	6.67	9.45	10.40	7.75	9.46	11.50
s_x	5.72	3.23	6.21	8.46	8.92	11.90	11.90
Cadaverine							
$\bar{x}$	12.50	12.60	12.10	13.90	13.00	14.70	14.30
s_x	13.30	12.70	12.60	14.50	11.10	13.70	13.50
Tyramine							
$\bar{x}$	7.32	6.08	5.77	7.09	6.16	12.70	4.68
s_x	5.60	3.68	5.12	4.22	4.91	7.00	3.89

[a]Original extract of wort (%, w/w).

beers, in a brewery using the traditional technology of bottom-fermentation lager beer production.

Concentrations of the amines in the raw materials and spent grain are given in Table 2. Tryptamine occurred only in brewer's yeast. Concentrations of the other amines usually varied among the batches. Relatively high levels of histamine, putrescine and tyramine were found in the malt meals. Putrescine levels of about 40 mg kg^{-1} were reported by Izquierdo-Pulido *et al.* (1993). The decarboxylating enzyme activity of microflora is considered to cause amine formation during barley malting (Dumont *et al.*, 1992). High concentrations of histamine and tyramine were found in hop extracts compared to dried hops and hop granules. Data in the literature are controversial. While Smith (1981) reported levels of 30–40 and 120–160 mg kg^{-1} for histamine in hops and hop extracts, respectively, no histamine was detected in hops by Izquierdo-Pulido *et al.* (1993) and no amines in hop extract by Cerutti *et al.* (1985).

The amine concentrations in bottom-fermentation brewer's yeast (*Saccharomyces cerevisiae* var. *uvarum*) can be used only as an approximation due to different cell counts. The wide range in concentrations may also arise because the yeasts in the first batch (X1) were used only once, while the others (X2 and X2) were used three times, for fermentation. However, Izquierdo-Pulido *et*

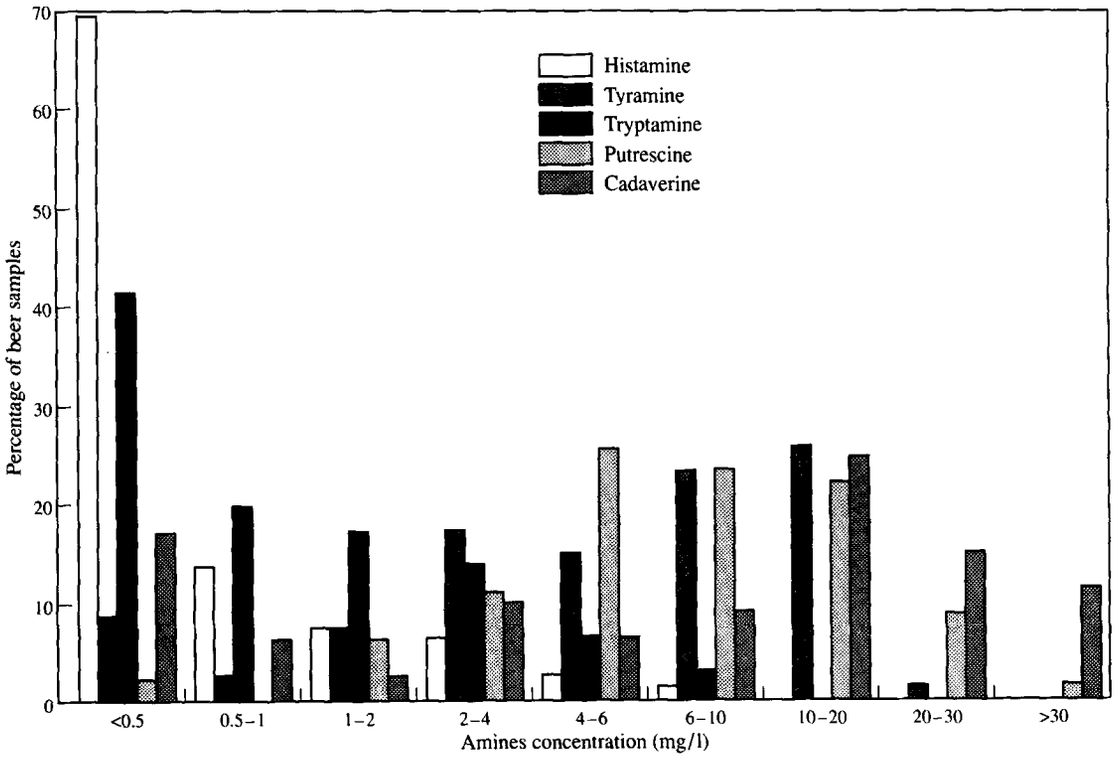

Fig. 2. Distribution of amine concentrations among the assortment of Czech beers tested.

Table 2. Amine concentrations (mg kg^{-1}) in used raw materials and spent grain

Raw material	Batch	Histamine	Tyramine	Tryptamine	Putrescine	Cadaverine
Malt meal	X1	11.20	23.90	ND	60.90	24.20
	X2	6.75	28.50	ND	84.00	9.39
	X3	17.30	20.10	ND	71.10	5.18
Hop	X2	6.91	15.60	ND	23.90	7.81
	X3	5.49	12.30	ND	13.70	2.35
Hop granules	X1	3.53	12.70	ND	20.80	0.66
	X2	8.15	11.40	ND	21.30	7.34
	X3	7.73	9.55	ND	20.00	4.47
Hop extract	X1	53.10	228.00	ND	5.43	7.51
Spent grain	X1	ND	29.30	ND	29.20	0.81
	X2	ND	ND	ND	10.70	2.50
	X3	1.35	32.30	ND	10.90	1.39
Yeasts	X1	2.06	24.10	4.33	2.14	12.50
	X2	11.80	17.40	13.50	13.70	22.20
	X3	8.33	17.70	16.90	69.30	38.40

ND, not detectable.

al. (1995) reported that *Saccharomyces cerevisiae* var. *uvarum* did not produce histamine and tyramine during fermentation, and recycling the yeast did not influence the formation of these biogenic amines.

Concentrations of the amines in spent grain varied widely but should not cause any health problem during feeding to farm animals.

Similar results and trends were obtained within the three batches of beer, therefore mean values are presented in Fig. 3. Regardless of amine occurrence in the raw materials, only putrescine was detected in the raw materials gathered prior to boiling. Its concentration decreased before cooling of the wort. The decrease may be due to spermidine and spermine formation. Measurable levels of histamine, tyramine and cadaverine were formed during the main fermentation. The effects of maturation, filtration and pasteurization were limited. Tryptamine was detected only in beer after the main fermentation. These results are comparable with those reported by Cerutti *et al.* (1989), Donhauser *et al.* (1992), Dumont *et al.* (1992) and Izquierdo-Pulido *et al.* (1991). Contaminating lactic acid bacteria (*Lactobacillus* and *Micrococcus* spp.) may participate in the amine formation during the brewing process (Šavel, 1980; Donhauser *et al.*, 1992; Straub *et al.*, 1995). Thus, amine concentrations can be considered as a marker of microbial contamination of a brewery.

The final steps before bottled beer consumption are handling and storage. Two common types of beers were therefore stored in the dark at temperatures of 6°C and 20°C for 6 weeks and tested for amine concentration changes at 2-week intervals. The results are given in Table 3 and 4. Tryptamine concentrations were negligible. The findings may be considered only as initial information. Levels of cadaverine and probably putrescine did not change during storage. Concentrations of tyramine increased gradually at both temperatures, as did histamine. The rapid increase in histamine concentration during the initial 2-week period in the pale beer stored at 6°C is surprising. These changes should be therefore studied in detail.

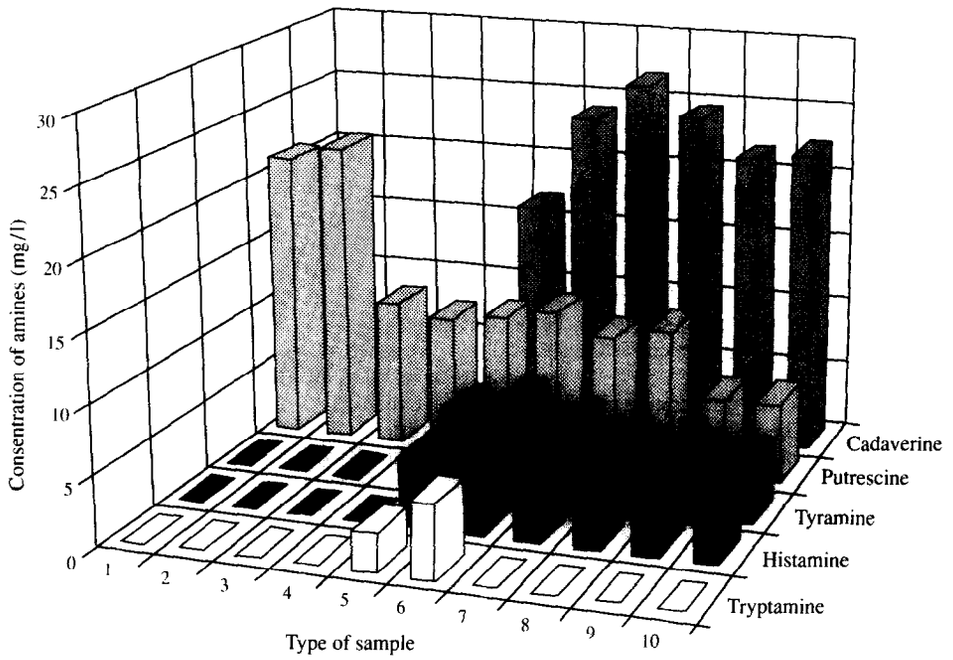

Fig. 3. Changes in amine concentrations (mg litre^{-1}) during the brewing process of three batches of a pale beer with original extract of wort (10%, w/w). 1, raw materials gathered prior to boiling: 2, hopped wort after boiling; 3, hopped wort prior to cooling; 4, hopped wort after cooling; 5, pitched wort; 6, beer after the main fermentation; 7, beer at the end of maturation; 8, filtered beer; 9, beer prior to pasteurization; 10, bottled beer.

Table 3. Changes of amine concentrations (mg litre^{-1}) in a bottled pale beer with original extract of wort (10%, w/w) stored at different temperatures

Time (weeks)	Histamine		Tyramine		Putrescine		Cadaverine	
	6°C	20°C	6°C	20°C	6°C	20°C	6°C	20°C
0	1.19		0.31		16.00		28.00	
2	1.57	3.04	0.78	0.33	8.95	9.81	24.50	24.9
4	18.10	—	2.85	1.68	10.50	10.20	23.90	24.8
6	19.00	2.29	3.45	2.30	10.00	10.60	22.60	27.2

Table 4. Changes of amine concentrations (mg litre^{-1}) in a bottled dark beer with orginal extract to wort (10%, w/w) stored at different temperatures

Time (weeks)	Histamine		Tyramine		Putrescine		Cadaverine	
	6°C	20°C	6°C	20°C	6°C	20°C	6°C	20°C
0	3.50		0.52		13.20		24.90	
2	1.70	3.02	0.33	0.61	11.70	12.70	26.10	27.0
4	4.69	5.67	1.29	1.54	10.40	15.20	23.60	30.4
6	2.80	14.50	7.89	8.24	10.40	11.90	23.00	27.5

CONCLUSIONS

The concentrations of biogenic amines in the assortment of Czech bottled beers tested compare well with values reported from several European countries. No significant differences were observed between type of beer (colour and original extract of wort) and amine concentrations. The concentrations found seem to be harmless for most beer consumers. Most of the amines were formed during the main fermentation. Tyramine and histamine concentrations may increase during longer-term storage of bottled beer.

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