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Biogenic amine formation in bottled beer

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Abstract

Formation of six biogenic amines in bottled beers was investigated in seven laboratory experiments. Amines were determined as *N*-benzamides by micellar electrokinetic capillary chromatography. Formation of amines, during the initial 8-day storage of bottled beers from three breweries at 21 °C was very limited. However, statistically highly significant tyramine formation was observed in one experiment during 12-weeks' storage. Considerable increases of tyramine and to a lesser extent of histamine levels, were found in beers inoculated with mixed cultures of brewery lactic acid bacteria and stored until haze formation. Lactobacilli were much more effective amine producers than pediococci. Limited increases of putrescine and spermidine levels were observed only sporadically. Cadaverine contents were not affected; tryptamine levels were below the detection limit. Thus, tyramine and histamine levels can increase considerably in insufficiently pasteurised bottled beers.

Keywords: Biogenic amines; Tyramine; Histamine; Bottled beer; Lactic acid bacteria

1. Introduction

Beer has been commonly reported, among foods and beverages, to be a health risk for some consumers, resulting from biogenic amines (BAs) intake. Hypertensive crises were observed, after beer consumption, in patients treated with drugs inhibiting the detoxification enzyme, monoamine oxidase (MAO; EC 1.4.3.4; Lippman & Nash, 1990; Murray, Walker, & Doyle, 1988; Shulman, Tailor, Walker, & Gardner, 1997; Tailor, Shulman, Walker, Moss, & Gardner, 1994). The adverse effects were found both in tap and non-alcoholic beers and were caused by tyramine. Tyramine intakes exceeding 6 mg within a 4-h period or from beers containing over 10 mg tyramine per litre, have been considered as dangerous for such patients (Tailor et al., 1994). Beer was also observed to be a cause of headaches in migraine-susceptible consumers (Peatfield, 1995). Alcohol, and probably some other BAs present in beer, can potentiate tyramine effects. However, no risk was reported for healthy consumers.

Usual contents <1, <1, <1, 1, 1, 2, 5, 5 and 10 mg l^{-1} of spermidine, spermine, 2-phenylethylamine, tryptamine, histamine, cadaverine, putrescine, tyramine and agmatine, respectively, were reviewed in 16 original papers dealing with BAs levels and formation in beers from several European countries, Cuba and Brazil during the 1990s (Kalač & Křížek, in press). However, several times higher BA maximum contents have been found, e.g. 113 mg l^{-1} of tyramine (Tailor et al., 1994). Malt is a source of agmatine, putrescine, spermidine and spermine, while tyramine, histamine and cadaverine have been formed during the main fermentation by contaminating lactic acid bacteria.

In our previous work (Kalač, Hlavatá & Křížek, 1997) we observed increase of histamine and tyramine levels in bottled beer from a brewery during a 6-week storage period. The objective of the present work is to explain nature of this phenomenon.

2. Materials and methods

2.1. Testing of amines formation

The aim of determination of amine content changes in pasteurised beers was to observe whether amines can be

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formed chemically in closed bottles during the initial days after filling and pasteurisation. Three experiments were carried out to investigate the changes.

Bottles were taken after pasteurisation, stored in the dark at 21 °C, and amines were determined several times within an 8-day storage period. Three bottles were analysed simultaneously. All tested beers were bottom-fermented pale lagers with original wort extract 12% (w/w). Beers originated from three breweries: breweries A and B, have been known from our previous work, for low levels of amines in their beers, while brewery C had high levels. Our previous tests of histamine and tyramine formation in bottled beers (Kalač et al., 1997) were done with beers from brewery C.

The effects of histamine and tyramine precursor addition and storage time were tested in fourth experiment. Histidine or tyrosine was added in doses which approximately duplicated or triplicated the usual contents of those free amino acids (Basařová, Šavel, Janoušek, & Čížková, 1999). Bottled lager from the brewery A, taken from the filling operation prior to pasteurisation, was cooled in a refrigerator to prevent foaming, following tyrosine addition. Bottles of control variant were opened for the same time (2.5 min) as bottles in two further variants. L-Histidine (50 mg dissolved in 5 ml of redistilled water; Aldrich) or L-tyrosine (50 mg in the solid state due to its poor solubility in water; Aldrich) were added to each bottle (0.5 l) and bottles were immediately closed. Pasteurisation started approximately 15 min after bottle closing. Tyrosine was dissolved during pasteurisation. Bottles were then stored in the dark, at 21 °C for 12 weeks, and used for amine determination three times during the period. The initial amine contents were determined from five bottles, while two bottles from each variant were analysed during the experimental period.

Formation of amines in pasteurised beers inoculated with brewery lactic acid bacteria was tested in a further three experiments. Bottled lagers produced in brewery A were taken after pasteurisation and divided into three variants: control bottles opened only for the same time as the other two variants and closed again, and variants inoculated with cultures either of pediococci or lactobacilli. The bacteria were isolated from contaminated keg beers. Different mixed cultures containing various species of lactobacilli or pediococci were used in the individual experiments. The bottles were stored in the dark in an incubator at 28 °C until a slightly visible bottom haze appeared after 14–16 days. Amines were then determined simultaneously in three or five bottles.

2.2. Analytical methods

Biogenic amines were derivatised by benzoylchloride in 40 ml of beer sample. The amines were thus converted to N-substituted benzamides, which were extracted in the further step by diethylether; after evaporation of the solvent they were dissolved in methanol.

Analyses were carried out on Spectraphoresis 2000, a fully automated system for capillary zone electrophoresis, equipped with a multi-wavelength UV–VIS scanning detector (Thermo Separation Products, Fremont, CA, USA). Separations were achieved using a plain fused silica capillary column, 43 cm total length (36 cm effective length to the detector) and 75 μm inner diameter (CElect FS75 CE column, Supelco, Bellefonte, PA, USA). Data processing was performed using Spectacle and PC 1000 CE software v. 3.0. The procedure was described in detail by Křížek and Pelikánová (1998). This paper also described the method for determination of the detection limits. Detection limits were 0.6, 0.8, 0.8, 0.8, 1.0 and 4.1 mg l^{-1} for cadaverine, putrescine, tyramine, tryptamine, histamine and spermidine, respectively.

Reproducibility of the analytical procedure was tested by parallel analyses of seven beer samples from one bottle. Relative standard deviations were 10.8, 12.7, 10.4 and 12.8% at mean contents 28.8, 6.7, 22.7 and 17.6 mg l^{-1} for tyramine, putrescine, cadaverine and spermidine, respectively. Histamine and tryptamine levels were below the detection limits.

2.3. Statistical methods

Differences in amine contents, during beer storage and between the variants, were tested by *t*-test with different sample variances using the Microsoft Excel Tools Pack. Level $P < 0.05$ was used as a minimum limit of significance. Values below the detection limits were used for the calculations as halves of the limits.

3. Results and discussion

Five amines were detected in measurable levels. Tryptamine contents were always below the detection limit. Spermine content could not be determined as its peak interfered with a large peak of an unidentified compound.

Changes in amine contents in beers from three breweries stored at room temperature for 8 days are given in Table 1. None of the breweries have had any problems with beer durability during at least a 3-month storage period. Thus, efficiency of pasteurisation has been good.

Assessing amine content dynamics, both the relatively high relative standard deviations of determination, mainly at low amine contents, and changes in amine formation and degradation are notable. Degradation by lactic acid bacteria has not yet been described in beer but has been known for histamine and tyramine, e.g. in fermented salami (Leuschner & Hammes, 1998; Leuschner, Heidel, & Hammes; 1998). Spermidine and spermine can be formed from putrescine.

Table 1
Changes in amine contents (mg l^{-1}) in stored beer from three breweries

Beer from brewery	Storage time (days)	Histamine	Tyramine	Putrescine	Cadaverine	Spermidine
A	0	ND	10.5	5.4	2.1	5.2
	4	1.2	6.0	5.1	1.4	ND
	6	1.0	12.3	4.9	1.8	7.9
	8	2.0	16.6	5.8	1.5	8.4
B	0	2.2	1.1	7.9	0.7	ND
	3	1.6	1.0	7.3	0.9	ND
	6	2.3	1.5	6.1	0.7	ND
	8	3.0	3.6	7.6	1.3	ND
C	0	9.7	102	9.5	48.1	ND
	5	9.3	101	11.5	48.6	ND
	8	8.8	105	11.9	50.7	ND

ND, content below the detection limit.

Increase of histamine content from the non-detectable level to 2.0 mg l^{-1} in beer from brewery A was the only significant difference ($P<0.05$) observed within three experiments of eight-day storage of the bottled beers. Thus, chemical formation of amines during the initial stage of pasteurised beer storage can be assessed as very limited.

A different situation was observed for changes of tyramine levels during 12-weeks' storage of bottled beer. Tyramine contents increased with prolonged time of storage, significantly, with critical P levels 0.002, 0.0001 and 0.04 and r^2 values 0.7167, 0.8658 and 0.2679 for the variants with tyrosine addition, histidine addition and control, respectively. These results indicate a possibility of tyramine formation in a chemical way; therefore no sensorial changes of beer due to microbial spoilage were observed. However, supplementation with tyrosine as the precursor of tyramine had no effect. Similar information was reported by Izquierdo-Pulido, Mariné-Font, and Vidal-Carou (2000) who did not observe a direct relationship between free tyrosine level in wort and tyramine formation during fermentation. Levels of free tyrosine or histidine thus do not seem to be critical factors in tyramine or histamine formation in bottled beer.

The observed increase of histamine contents in the fourth experiment (Table 2) from the initial non-detectable level to 1.7–1.8 mg l^{-1} was not assessed, as such levels are not a risk for healthy consumers. Significant increase ($P=0.0001$) of spermidine was also found in the control variant. However, this is the only observed increase of that amine during beer storage.

The most considerable formation of tyramine and histamine was observed in beers inoculated with mixed cultures of pediococci or lactobacilli (Table 3). Significantly increased tyramine levels were produced by lactobacilli activity in experiments I and II. Similarly, lactobacilli caused the highest increase of histamine in experiments II and III and of putrescine in experiment II. Significant formation of the amines by pediococci activity was found only for tyramine in experiment II and histamine and spermidine in experiment III. However, amine levels were lower than those caused by lactobacilli. Thus, lactobacilli seem to be the main factor affecting amine formation during bottled beer storage.

As reported by Izquierdo-Pulido, Font-Fabrégas, Carceller-Rosa, Mariné-Font, and Vidal-Carou (1996), bacteria isolated during beer fermentation with abilities to produce tyramine and tryptamine, were identified as *Pediococcus* spp., mainly *P. damnosus*. No lactobacilli were isolated. Tyramine formation was negligible at pediococci counts below 4×10^3 CFU ml^{-1}, while, at

Table 2
Changes in amine contents (mg l^{-1}) during storage of control variant beer (C) and beer with added (100 mg l^{-1}) histidine (HIS) or (100 mg l^{-1}) tyrosine (TYR)

Storage time (weeks)	Histamine			Tyramine			Putrescine			Spermidine		
	C	HIS	TYR	C	HIS	TYR	C	HIS	TYR	C	HIS	TYR
0	ND			1.3			2.3			8.8		
4	1.8	ND	ND	7.1	9.1	10.9	2.3	2.4	2.5	9.1	9.5	10.2
8	1.8	1.8	1.8	15.8	13.0	14.2	2.2	2.6	2.4	14.3	4.9	7.9
12	ND	1.7	1.7	8.7	14.4	13.6	2.8	2.8	2.6	18.8	13.4	12.5

Table 3
Amine contents (mg l^{-1}) in control beer and beer inoculated with lactic acid bacteria stored until haze formation in three repeated experiments I–III

Experiment	Variant	Histamine	Tyramine	Putrescine	Cadaverine	Spermidine
I	Control	ND	7.4	2.8	0.9	17.2
	Pediococci	ND	8.6	3.5	0.7	23.7
	Lactobacilli	ND	54.3	3.5	0.9	17.6
II	Control	ND	1.0	4.0	ND	4.4
	Pediococci	ND	5.0	4.0	ND	ND
	Lactobacilli	16.1	24.5	7.6	ND	4.9
III	Control	ND	6.7	4.1	1.5	4.4
	Pediococci	2.5	8.0	4.5	1.6	7.3
	Lactobacilli	11.7	6.7	4.2	1.2	5.5

over 1×10^5 CFU ml^{-1}, tyramine levels ranged between 15 and 25 mg l^{-1}. Washing of pitching yeast with phosphoric acid was effective for eliminating pediococci and consequently reducing tyramine levels in beer. However, species of the genus *Lactobacillus* also participate in amine formation. *Lactobacillus frigidus*, *L. brevissimilis* and *L. brevis* were reported as amine-forming beer contaminants (Donhauser, Wagner, & Geiger, 1992).

We found only one paper dealing with amine-forming activities of lactic acid bacteria isolated from bottled beer. *Lactobacillus paracasei* from wheat beer produced no histamine, putrescine or cadaverine during either cultivation in malt-medium at 30 °C for 7 days or in beer in opened bottles. In contrast, *L. buchneri* cultivated as a negative control, increased histamine contents in beer from 15 to 65 mg l^{-1} during 7 days, while putrescine and cadaverine levels did not change (Zimmermeier, Vogt, & Kunz, 1998).

In conclusion, the results prove that considerable levels of tyramine and histamine can be formed in bottled beers by lactic acid bacteria, mainly by lactobacilli, surviving insufficient pasteurisation. Amines formation can also be expected, under similar conditions, in cans and kegs. Increased levels of amines can be suspected in beers contaminated by lactic acid bacteria during production, notwithstanding bacteria elimination by filtration and their inactivation by pasteurisation. Amine level can thus be used as an attractive indicator of poor microbial status of the brewing procedure. High amine contents can be expected in some wheat beers, in which lactic acid bacteria form part of the fermentation microflora. Moreover, contaminated yeasts can be a source of amine-producing bacteria in bottle-conditioned beers.

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