ELSEVIER

Food Chemistry 52 (1995) 199–202

0308-8146/95/$9.50

Analytical Food, Nutritional and Clinical Methods Section

Determination of biogenic amines in alcoholic and non-alcoholic beers by HPLC

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(Received 13 December 1993; revised version received and accepted 18 February 1994)

Eight biogenic amines (histamine, tyramine, tryptamine, 2-phenyl-ethylamine, putrescine, cadaverine, spermine and spermidine) in alcoholic and non-alcoholic beers have been determined by HPLC. The results obtained ranged between 0·1 and 17·2 ppm. Only six amines were found because histamine and spermine were not present in detectable amounts. Spermidine was detected in all samples (0·3–1·4 ppm) despite absence of references in the literature about the content of this amine in beers. Non-alcoholic beers did not have significantly lower amounts of biogenic amines than alcoholic beers.

INTRODUCTION

Biogenic amines have been found to occur during production of foods such as wines, beers, cheeses, fishery products and other fermented foods (Maga, 1978). Amino acid decarboxylation is the main mode of biosynthesis of these amines (Rice & Koehler, 1976). Many microorganisms in fact contain amino acid decarboxylases and therefore can produce a variety of amines from natural amino acids (Lovenberg, 1974). These compounds are in general vasoactive and can cause modification of blood pressure. Some of the effects of amines are well known, such as severe headache, hypertension, renal intoxication and, in more severe cases, intracerebral haemorrhage and death (Kuhn & Lovenberg, 1982; Antila, 1983). This is especially true when they are taken in combination with ethanol which is a monoamine oxidase inhibitor (Blackwell *et al.*, 1964).

In view of the possible harmful effects of these amines, their concentrations in foods deserve careful investigation. The levels of biogenic amines in beer in some studies have been evaluated (Stratton *et al.*, 1991). Since beer is generally consumed in greater amounts than wine, it has been suggested that beer might be more of a hazard to the consumer (Hanna *et al.*, 1988). Histamine has been found in the concentration of 2·6–4·7 mg/litre in Swedish beer, 3·2–15 mg/litre in Danish beer and 20 mg/litre in French beer. The European beers, particularly the Dutch and German beers, contain the highest amounts of histamine (7·3 and 6·7 mg/litre, respectively). Tyramine levels have been also surveyed in beers from several different countries and appear to occur at higher levels than histamine (Zee *et al.*, 1981). Moreover, tyramine has also been found in non-alcoholic beers, indicating that the methods used to produce it do not remove this amine. In this paper the authors have investigated the biogenic amine contents in alcoholic and non-alcoholic beers using a fast HPLC method for the simultaneous determination of eight biogenic amines in cheese: histamine, tyramine, tryptamine, 2-phenyl-ethylamine, putrescine, cadaverine, spermidine and spermine (Moret *et al.*, 1991).

MATERIALS AND METHODS

Reagents and samples

Tryptamine (Tryp), 2-phenyl-ethylamine (2-Phe), putrescine (Put), cadaverine (Cad), histamine (His), tyramine (Tyr), spermidine (Spd), spermine (Spm), 1,7-diaminoheptane (1,7-DH) and dansyl chloride (>99%) were obtained from Fluka (Buchs, Switzerland). Amines, except for 1,7-diaminoheptane, were purchased as hydrochloride salts, and the results were corrected on the basis of their purity and referred to the free base. Orthophosphoric acid and anhydrous sodium carbonate were from Merck (Darmstadt, Germany). HPLC grade solvents (Carlo Erba, Milan, Italy) and water purified with a Milli-Q system (Millipore, Bedford, MA, USA) were used throughout. Beer samples were obtained from commercial sources.

Standard solutions

A stock standard solution was prepared by adding an accurately weighed amount of each amine (*c.* 100 mg) to a 100 ml volumetric flask and diluting to volume

with water. Five working standard solutions at different concentrations were prepared from the stock solution, adding a fixed and known amount of internal standard before dilution. Fresh dilute standard solutions must be prepared weekly because some amines are subject to decomposition.

Instrumentation

HPLC determinations of biogenic amines were performed with a Varian liquid chromatograph (Palo Alto, CA, USA) Model 9010 and a Rheodyne Model 7161 manual injector with a 10 μl loop. The detector was a Varian Model 9050 UV-VIS spectrophotometer set at 254 nm. An IBM Personal System/2 Model 30 286 computer and an Epson LX-400 printer were used for data acquisition and registration. The column was a reversed-phase Spherisorb 3STG (15 cm × 4·6 mm i.d.; particle size 3 μm), with a Spherisorb 5 ODS-2 guard column (Phase separations, Queensferry, UK). The three solvent reservoirs contained the following eluents: (A) acetonitrile, (B) 0·01 M dipotassium hydrogenphosphate buffer solution, adjusted to pH 7 with orthophosphoric acid (85%), and (C) water. The elution programme consisted of the gradient system shown in Table 1, with a flow-rate of 0·8 ml/min. Before use, the eluents were filtered through a 0·22 μm Durapore filter (Millipore) and degassed under vacuum. The eluted dansylamines were detected by monitoring the UV absorbance at 254 nm.

Biogenic amines extraction

Degassed beer (10 ml; under vacuum) introduced into a separating funnel were added with 50 μl of aqueous solution of 1,7-diaminoheptane (concentration of 1 mg/ml), saturated with anhydrous sodium carbonate and submitted to extraction using 10 ml of a mixture *n*-butanol-chloroform 1:1 (v/v). The whole mixture was blended with a horizontal orbital motion blender for 30 min. After decanting, 1 ml of organic phase was introduced into a screw-capped tube and added with two drops of 1 M hydrochloric acid. The solution was then evaporated to dryness at room temperature with a Uniequip System (Uniequip, Martinsried, Münich, Germany). The residue was finally dissolved with 1 ml of saturated sodium bicarbonate and 1 ml of dansyl chloride reagent 5 mg/ml in acetone). The sealed test-tube was immediately mixed for 30 s using a Vortex mixer. The reaction mixture was then left for 1 h at 40°C with occasional shaking. Finally, the mixture was evaporated at 40°C with the Uniequip System and the residue was dissolved with 1 ml of acetonitrile for HPLC analysis. After centrifugation (or filtration) the sample was injected and the chromatographic conditions were according to Moret *et al.* (1991).

Table 1. HPLC elution program for amine analysis

Time (min)	A (%)	B (%)	C (%)
0·0	65	35	0
1·0	65	35	0
5·0	80	20	0
5·1	80	0	20
6·0	90	0	10

A, Acetonitrile; B, phosphate buffer (pH 7); C, water. Flow rate, 0·8 ml/min.

RESULTS AND DISCUSSION

The recovery of biogenic amines was determined by an addition of known amount of amine standard solution to a sample (no. 11). The recoveries obtained calculating concentrations before and after standard addition were compared. The data, as means of three values, are shown in Table 2. We can observe good recoveries for all amines except for spermidine and spermine (65·1 and 52·8%, respectively).

In Fig. 1 are shown the chromatograms of alcoholic and non-alcoholic beers. Of the eight biogenic amines assayed for 16 beer samples (among which are four non-alcoholic beers), six amines (tryptamine, 2-phenylethylamine, putrescine, cadaverine, tyramine and spermidine) were found in detectable amounts. Table 3 shows biogenic amine levels in the beer samples.

Table 2. Recovery test

Amine	Amount determined in sample (mg per 100 g)	Amount of standard added (mg per 100 g)	Amount found in sample (mg per 100 g)	Recovery (%)
Tryp	nd	5·7	5·5	96·5
2-Phe	0·7	5·1	5·4	93·1
Put	2·5	3·2	4·9	86·0
Cad	0·5	3·4	3·2	82·0
His	nd	6·2	5·6	90·3
Tyr	1·7	7·5	8·8	95·6
Spd	0·4	3·2	2·8	65·1
Spm	nd	3·5	1·9	52·8
IS	nd	3·9	3·8	97·4

nd, Not detectable; IS, internal standard (1,7-diaminoheptane). Results are means of three analyses of a beer sample which underwent the whole analytical procedure.

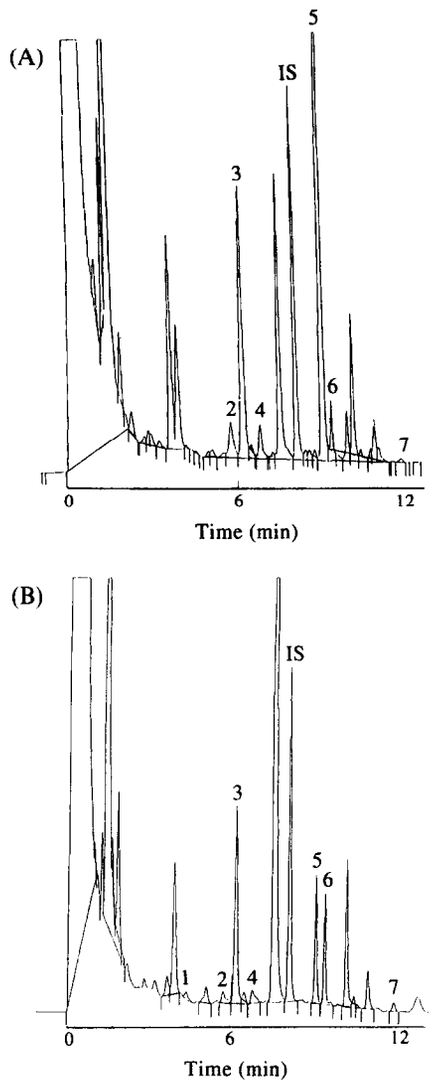

Fig. 1. Chromatograms of alcoholic beer (A) and non-alcoholic beer (B). For chromatographic conditions, see Materials and Methods. 1, Tryptamine; 2, 2-phenylethylamine; 3, putrescine; 4, cadaverine; IS, international standard; 5, tyramine; 6, spermidine; 7, spermine.

Tyramine and putrescine have been found in all samples in accordance with the data of literature and their contents were higher than other amines in almost all beers. Tyramine content particularly covered a wide range (0·6–17·2 ppm) whereas spermidine was detected in all samples (0·3–1·4 ppm), although there are no literature reports about the content of this amine in beers. Non-alcoholic beers did not have significantly lower amounts of biogenic amines than alcoholic beers, which was not anticipated.

CONCLUSIONS

The biogenic amine content in samples investigated (alcoholic and non-alcoholic beers) are not very high and the results obtained ranged between 0·1 and 17·2 ppm. In all beers the putrescine (1·4–4·2 ppm) and tyramine (0·8–17·2 ppm) contents were higher than other biogenic amines. We have not observed differences between alcoholic and non-alcoholic beers; this suggests that amine content in beers could be principally related to the particular working conditions of each plant (brewery) as well as factors such as fermentation, sanitation or contamination of pitching yeast. Other factors, such as raw materials (malt and hop) could also influence the presence of amines in beers.

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Table 3. Concentrations of biogenic amines in analysed beers (ppm)

	Tryp	2-Phe	Put	Cad	His	Tyr	Spd	Spm
Alcoholic								
1	nd	1·6	3·3	0·6	nd	17·2	0·5	nd
2	1·0	1·0	3·5	0·6	nd	3·0	0·5	nd
3	0·2	1·2	3·9	0·3	nd	1·8	1·1	nd
4	0·1	1·4	2·9	0·7	nd	1·6	0·6	nd
5	0·8	0·7	3·0	0·3	nd	3·8	0·5	nd
6	0·1	1·1	2·9	0·2	nd	0·8	0·5	nd
7	1·8	0·7	2·7	0·4	nd	2·0	0·9	nd
8	0·3	1·4	3·1	0·6	nd	9·2	0·5	nd
9	nd	1·4	4·2	0·3	nd	1·3	0·5	nd
10	nd	0·9	3·3	0·2	nd	0·6	0·6	nd
11	nd	0·9	2·7	0·4	nd	2·8	0·3	nd
12	2·6	1·5	3·1	0·8	nd	15·9	0·4	nd
Non-alcoholic								
13	nd	0·6	2·6	0·3	nd	1·8	1·4	nd
14	nd	0·5	1·4	0·2	nd	2·2	0·5	nd
15	2·0	1·3	2·5	nd	nd	2·3	0·8	nd
16	nd	nd	2·0	nd	nd	1·3	0·9	nd

nd, Not detectable.

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