Biogenic Amines in European Beers

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Agmatine and putrescine were always present in the 195 European beers analyzed. Agmatine was the prevailing amine (10.5 ± 5.8 mg/L), whereas putrescine levels fluctuated slightly (4.8 ± 2.3 mg/L). Spermine, spermidine, tryptamine, and β-phenylethylamine were not detected in every beer, and their levels were <2 mg/L. Histamine, in general, ranged from 0.5 to 1.1 mg/L, although relatively high levels were detected in some beers. Tyramine was present in each beer, and together with cadaverine, showed the highest fluctuations (non detected to 67.5 mg/L). Putrescine and polyamines could be considered as "natural" constituents of beers, whereas histamine, tyramine, and cadaverine would be "indicators" of microbial contamination during brewing. "Kriek" and "spontaneous fermentation" beers showed the highest values of tyramine and histamine. A significant relationship was found between pH and tyramine, histamine, and cadaverine in "spontaneous fermentation" beers. Beers should be avoided by patients receiving treatment with monoamine oxidase inhibitors, because tyramine levels found in beers were very variable and unpredictable.

Keywords: *Beers; biogenic amines; tyramine; histamine; alcoholic beverages; polyamines*

INTRODUCTION

Biogenic amines are commonly found in foods such as cheese, meat and fish products, wine, beer, and other fermented food (Stratton et al., 1991; Halász et al., 1994). These amines can have aliphatic, aromatic, or heterocyclic structures (Figure 1) and most of them can be generated by microbial decarboxylation of free amino acids (Brink et al., 1990). High levels of some biogenic amines may cause toxic effects with a wide variety of symptoms. Histamine can cause "histaminic intoxications" (Taylor, 1985; Stratton et al., 1991). Likewise, tyramine and β-phenylethylamine have been linked with food-induced migraines (Diamond, 1991; Vaughan, 1994). Recently, Peatfield (1995) reported that beer could be classified as a dietary precipitant of headache in patients with migraines. The symptoms of those direct toxic effects are usually self-limiting, and most individuals recover without complications. However, individuals receiving treatment with monoamine oxidase inhibitors (MAOIs) may be susceptible to serious complications, such as hypertensive crisis, when ingesting biogenic amines (Murray et al., 1988; Tailor et al., 1994).

It is very difficult to determine the exact toxicity threshold of biogenic amines. Brink et al. (1990) pointed out that it is important not to focus exclusively on the concentration of one particular amine when considering toxic levels for these amines because potentiator effects can exist. Factors such as amount of food consumed and amine content of other dietary food are also very important. Additive effects of consumption of a number of products (e.g., wine, cheese, and meat and fish products) during a short time span may result in biogenic amine intoxication, whereas consumption of each of these products alone does not cause any problem. No maximum or limits have been set for histamine or

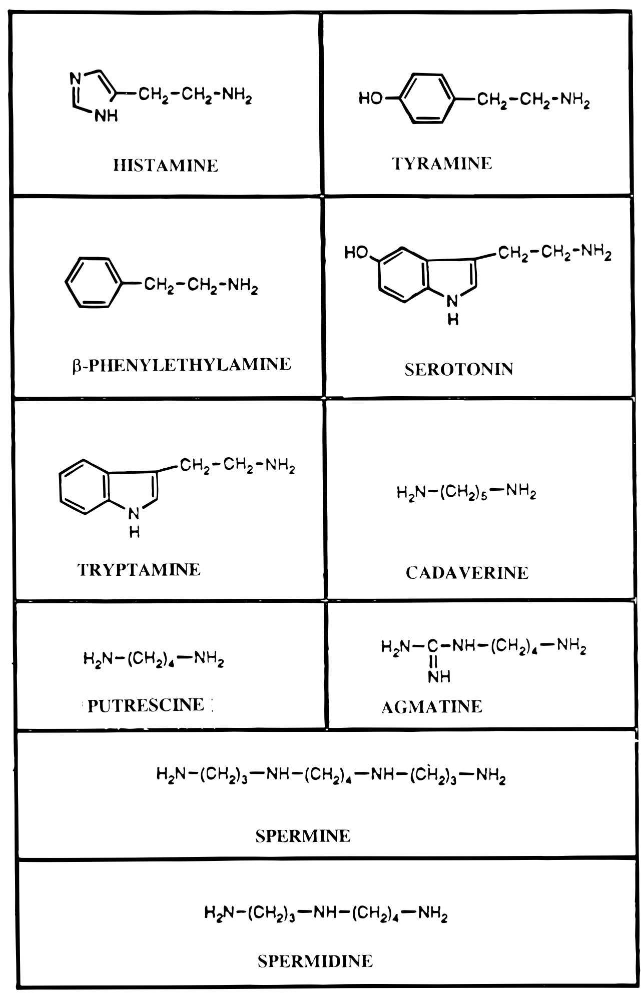

Figure 1. Chemical structures of biogenic amines commonly found in foods.

tyramine levels in beers. Limits of 10−100 mg histamine/100 g in foods and 2−10 mg histamine/L in wines

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have been suggested (Stratton et al., 1991). Threshold values of 100−800 mg/kg for tyramine and 30 mg/kg for β-phenylethylamine have been reported (Brink et al., 1990). Tailor et al. (1994) suggested that tyramine levels of >10 mg/L in beers should be considered unsafe for patients taking MAOIs. Furthermore, formation of nitrosamines is another reason for avoiding accumulation of biogenic amines in foods. Secondary amines, such as putrescine, spermine, and spermidine, can react with nitrite to form carcinogenic nitrosamines (Scanlan, 1983).

Despite the potential biological relevance of biogenic amines in foods, a relatively limited number of studies have attempted to measure the levels of these amines in beers, particularly compared with studies of biogenic amines in wines. Because beer is generally consumed in greater amounts than wine, it has been suggested that beer might be more of a hazard to the consumer (Hannah et al., 1988). Histamine and tyramine are the most frequently studied amines in beers (Zee et al., 1981a; Cerutti et al., 1985; Izquierdo-Pulido et al., 1989; Donhauser et al., 1993). However, information on the levels of diamines and polyamines in beers is still lacking. In view of the possible harmful effects of these amines, their concentrations in foods deserve careful investigation. This study was conducted to provide data on the contents of biogenic amines in European beers. Particular emphasis was placed on obtaining data on the most commonly consumed beers, such as "lager" and "pils" types, and on beers made with unusual brewing processes, such as "spontaneous fermentation" beers.

EXPERIMENTAL PROCEDURES

Samples. The sampling plan was designed to include beers that are representative of those currently manufactured in Europe. A total of 195 samples of bottled or canned beers were purchased from commercial outlets in Germany, Austria, Belgium, Bulgaria, Czech Republic, Denmark, Spain, France, Great Britain, Greece, The Netherlands, Ireland, Italy, Portugal, Switzerland, and the former Yugoslavia.

Biogenic Amines. Histamine, tyramine, β-phenylethylamine, tryptamine, serotonin, cadaverine, putrescine, agmatine, spermine, and sperimidine were determined by a reversed-phase liquid chromatography method (Izquierdo-Pulido et al., 1993). The method is based on ion-pair chromatography partition and involves a post-column reaction with orthophthalaldehyde (OPT) to form fluorescent derivatives with amines. Treatment of beer before injection required only decarbonation and filtration through a 0.45-μm filter.

All reagents were of analytical grade except HPLC reagents that were LC grade. Biogenic amine standards were purchased from Sigma Chemical Co. (St. Louis, MO).

Other Analytical Parameters. Alcoholic content (AC), original gravity (OG, the back-calculated solute of the wort from which the beer was fermented), and real extract (E, the concentration of the total solutes expressed as %w/w) were determined automatically with a Technicon beer analyzer (Technicon International Division, 1979). Total acidity (TA, total nonvolatile acidity expressed as lactic acid in % w/w), pH, and real degree of fermentation (RDF, the percentage of original gravity which has been fermented) were determined by the ASBC methods (ASBC, 1987). The RDF was calculated with the following formula: $RDF(\%) = [100(1 - E/OG)]/[(1 - (0.005161 - E)]$.

All the analyses were carried out in duplicate, and the time interval from purchase to analysis was short. Once the sample arrived at the laboratory, it was opened and quickly decarbonated. One fraction was immediately analyzed for AC, OG, ER, TA, and pH. The other fraction was kept at −20 °C until the time of biogenic amine analysis.

Statistical Methods. Biogenic amine concentrations in beers were not normally distributed. Data were evaluated by the Kruskall-Wallis test and Spearman's coefficient correlation for nonparametric data. If significant group differences were identified, differences between individuals were evaluated by the Dunn's test. Significance was assigned at a level of $p \leq 0.05$. Analyses were performed with the statistics package SPSS for Windows 6.0.1. (SPSS Inc., Chicago, IL).

Table 1. Biogenic Amine Contents (mg/L) in European Beers

biogenic amine	mean ± SD[a]	range	median	percentiles P_5	P_{25}	P_{75}	P_{95}
histamine	1.2 ± 2.4	nd[b]−21.6	0.7	0.0	0.5	1.1	3.5
tyramine	6.5 ± 9.0	0.5−67.5	3.2	1.4	2.4	5.6	27.8
β-phenylethylamine	0.4 ± 0.8	nd−8.3	0.4	0.0	0.3	0.5	1.2
tryptamine	0.4 ± 1.0	nd−5.4	0.0	0.0	0.0	0.3	2.5
cadaverine	2.4 ± 6.1	nd−39.9	0.5	0.3	0.4	1.1	15.8
putrescine	4.8 ± 2.3	1.5−15.2	4.4	2.0	3.1	5.8	9.5
agmatine	10.5 ± 5.8	0.5−40.9	10.0	2.8	6.5	13.5	20.0
spermine	0.3 ± 0.7	nd−3.9	0.0	0.0	0.0	0.3	1.8
spermidine	0.7 ± 1.0	nd−6.8	0.5	0.0	0.3	1.0	1.8

[a] Mean values ± standard deviation. [b] nd, not detected (detection limit for biogenic amines was 0.30 mg/L).

RESULTS AND DISCUSSION

Biogenic amine contents in beers regardless of origin are indicated in Table 1. Agmatine and putrescine were always present in all beers. Agmatine had the highest average content, with 50% of the samples having values of >10 mg/L. The fluctuation of putrescine levels was minimal, with 50% of the samples in the range 3.1−5.8 mg/L, and no sample with levels >16 mg/L. Spermine and spermidine were not always present, but when they were, their contents were generally low. Previous work (Zee et al., 1981b; Izquierdo-Pulido et al., 1994) has shown that the polyamines agmatine, spermine, and spermidine are already present in raw materials, such as malt and hop. Therefore, these amines can be considered as "natural" constituents of beer. Polyamine formation during beer fermentation has not been observed (Dumont et al., 1992; Izquierdo-Pulido et al., 1994). Putrescine can be also considered a natural constituent of beer because of its raw material origin; however, this amine has been related to spoilage of several food products, such as fish and meat (Veciana-Nogués et al., 1996). No putrescine formation has been reported during beer fermentation, and only Zee et al. (1981b) found putrescine formation in wort inoculated with a strain of *Lactobacillus brevis*, but not during brewing.

β-Phenylethylamine and tryptamine, in general, ranged from not detected to 0.5 mg/L, whereas serotonin was detected in only three samples and at very low levels (<1 mg/L); this is the first time that the presence of serotonin in beers has been reported. Little information is available for these amines. Tryptamine formation was observed during fermentation, but at low levels (Izquierdo-Pulido et al., 1996). Although β-phenylethylamine could be formed by heat decarboxylation of phenylalanine during the severe heat treatment applied to the malt in the manufacturing of some dark beers (Cerutti et al., 1985), we did not find particularly high levels of this amine in dark beers.

Histamine was not detected in 16% of the samples and fluctuated from 0.5 to 1.1 mg/L in 50% of the beers; however, relatively high levels were found in some beers. Tyramine was found in all the beers analyzed, and tyramine and cadaverine were the amines with the highest fluctuations. Raw materials could contribute to the final content of histamine, tyramine, and cadav-

Table 2. Biogenic Amine Contents (mg/L) in Different Types of European Beers

type of beer	n	agmatine[a]	putrescine	tyramine	cadaverine	histamine
top-fermented						
ale	18	10.7 ± 4.5[b] (1.1−15.7)	5.7 ± 2.0[d] (2.6−9.7)	5.0 ± 3.9[e] (1.9−17.4)	0.9 ± 1.2[g] (tr-4.2)	0.6 ± 0.9[i] (0.5−2.0)
stout & porter	20	9.8 ± 4.4[b] (5.2−10.8)	3.8 ± 1.9[d] (2.4−8.2)	4.1 ± 2.1[e] (1.1−9.1)	0.7 ± 0.6[g] (nd-1.9)	1.0 ± 0.8[i] (nd-3.2)
Weissbier	13	7.0 ± 2.5[b] (3.0−10.7)	4.8 ± 1.6[d] (2.4−6.7)	10.3 ± 12.3[e] (1.3−33.6)	4.8 ± 7.0[g] (0.4−17.7)	0.9 ± 0.5[i] (tr-2.4)
Kriek	9	2.2 ± 1.7[c] (1.1−3.4)	4.5 ± 0.7[c] (3.5−5.1)	22.5 ± 13.4[f] (6.7−36.4)	6.3 ± 6.0[g] (1.9−15.2)	5.6 ± 5.8[k] (1.6−14.0)
Trappsite	7	8.9 ± 5.8[b] (3.2−19.4)	5.9 ± 2.5[d] (3.2−9.1)	3.6 ± 1.0[e] (1.8−4.5)	0.8 ± 0.6[g] (tr-1.9)	1.0 ± 0.6[i] (nd-1.6)
bottom-fermented						
lager	36	10.2 ± 4.9[b] (0.9−27.2)	4.1 ± 1.9[d] (1.5−9.7)	4.9 ± 4.7[e] (1.6−20.9)	0.8 ± 1.3[g] (nd-6.2)	0.7 ± 0.5[i] (nd-2.6)
pils	44	13.3 ± 6.7[b] (5.1−40.9)	5.1 ± 1.4[d] (2.6−8.8)	5.6 ± 7.5[e] (0.5−46.8)	2.0 ± 5.6[g] (nd-31.4)	1.0 ± 2.2[i] (nd-17.0)
Dortmünder	9	11.2 ± 2.7[b] (6.1−13.4)	3.8 ± 0.8[d] (3.0−5.6)	3.1 ± 1.8[e] (1.4−7.6)	0.3 ± 0.2[g] (nd-0.5)	0.6 ± 0.4[i] (nd-1.3)
Bock	12	12.9 ± 7.9[b] (0.4−21.5)	5.5 ± 3.8[d] (2.1−12.4)	3.6 ± 2.2[e] (2.1−10.2)	0.7 ± 0.6[g] (nd-1.5)	0.9 ± 0.8[i] (nd-2.9)
spontaneous fermentation						
Lambic & Gueuze	12	8.9 ± 5.3[b] (1.0−18.8)	6.4 ± 4.6[d] (2.8−15.2)	21.3 ± 20.5[f] (0.8−67.6)	10.0 ± 12.9[h] (0.4−39.9)	5.8 ± 6.2[k] (tr-21.6)
nonalcoholic	15	7.1 ± 4.6[b] (2.6−16.8)	3.1 ± 1.3[d] (1.6−4.7)	6.2 ± 8.6[e] (2.1−31.5)	1.0 ± 1.9[g] (nd-5.3)	0.6 ± 0.8[i] (nd-3.2)

[a] Mean ± standard deviation and range in parentheses (nd, not detected; tr, trace). Means in the same column bearing a common superscript letter are not different ($p \geq 0.05$).

erine (Zee et al., 1981b; Izquierdo-Pulido et al., 1994). However, relatively high levels of those amines should be considered as indicators of microbial contamination during brewing because their formation has been related to the presence of contaminating microorganisms, such as lactic acid bacteria (Zee et al., 1981b; Halász et al., 1994; Izquierdo-Pulido et al., 1996).

Levels of biogenic amines in the different types of beers are indicated in Table 2. Only data of the prevailing amines, such as agmatine, putrescine, tyramine, cadaverine, and histamine, were included. Beers are classified in the two classical groups, top- and bottom-fermented, a differentiation based on whether yeast floats or sinks by the end of fermentation. Also, two more groups were added. One group included the "spontaneous fermentation" beers (i.e., "Lambic" and "Gueze") in which fermentation is carried out by many different microorganisms and yeast (including the genera *Saccharomyces* and *Brettanomyces*), and the other group included "nonalcoholic beers".

Agmatine levels were the same in all the different types of beers except for "Kriek" beers, which had agmatine levels (2.2 ± 1.7 mg/L) below the levels of the other beer types. Agmatine was the prevailing amine in most types of beers. Only "Kriek", "Weissbier", and "spontaneous fermentation" beers had average tyramine levels that were higher than average agmatine levels. No differences in putrescine levels were observed among the different types of beers, and average levels of putrescine fluctuated in a relatively narrow range (3.1−6.4 mg/L). Donhauser et al. (1993) also reported that fluctuations in putrescine values were minimal in German beers.

The most important differences among types of beers were found for tyramine, cadaverine, and histamine, even though a high variability in their levels was observed. "Kriek" and "spontaneous fermentation" beers showed the highest average values of tyramine and histamine. "Spontaneous fermentation" beers also showed the highest average values of cadaverine. Moreover, high levels of tyramine and cadaverine were found in "Weissbier" beers. Production of all the beers studied involves lactic fermentation, which could be implicated in amine formation. Dumont et al. (1992) also found high levels of cadaverine and histamine in "Gueuze" beers but no tyramine data were reported in their work. On the other hand, "spontaneous fermentation" as well as "Kriek" beers are also characterized by the use of a relatively high proportion of unmalted wheat and unmalted barley, respectively. Few data are available about the influence of unmalted cereal on biogenic amine contents. Zee et al. (1981b) reported that the partial substitution of malt by adjuncts, such as barley and rice, resulted in lower amine contents in fermented worts. In contrast, the opposite trend was observed in the beers just cited.

All bottom-fermented beers showed similar levels of amines, and no differences were found among the different types of beers for any amine. "Lager" and "pils" beers, the most common beers, showed relatively low average levels of tyramine, cadverine, and histamine; however, relatively high levels of those amines were found in particular beers. Some authors have reported that high levels of biogenic amines may be specific to certain breweries (Donhauser et al., 1993; Izquierdo-Pulido et al., 1996). The last group of beers, the "nonalcoholic", did not have significantly lower amounts of biogenic amines than the majority of regular beers, indicating that methods used to produce them do not remove amines. Our results on "nonalcoholic" beers are in agreement with those of Buiatti et al. (1995).

If beers were observed as a function of their country of origin, Belgian beers showed, on an average, high levels of tyramine, histamine, and cadaverine. This fact can be explained because an important number of beers analyzed from this country were "spontaneous fermentation" and "Kriek" beers. German beers showed a wide range of tyramine and cadaverine levels, mainly because of "Weissbier" beers, whereas some particular "pils" Spanish beers showed relatively high levels of tyramine.

Some authors (Zee et al., 1981a; Cerutti et al., 1985; Donhauser et al., 1993) have identified relationships between biogenic amines and other analytical parameters, such as alcoholic content, OG, RDF, TA, and pH.

Table 3. Alcoholic Content (AC), Original Gravity (OG), Degree of Fermentation (DF), Total Acidity (TA), and pH of European Beers

type of beer	*n*	AC (% v/v)	OG (% w/w)	DF (%)	TA (% w/w)[a]	pH
top-fermented						
ale	18	4.3−7.3[b]	10.2−18.3	58.9−69.7	0.16−0.59	3.72−4.52
stout & porter	20	3.0−10.5	7.9−22.2	51.0−70.5	0.13−0.37	3.95−4.65
Weissbier	13	4.8−7.1	11.4−16.3	62.8−70.0	0.15−0.43	3.85−4.73
Kriek	9	4.0−7.9	12.0−17.1	41.5−70.8	0.53−0.96	3.22−3.57
Trappiste	7	6.9−9.7	12.3−19.6	66.4−78.9	0.30−0.54	3.69−4.59
bottom-fermented						
lager	36	3.4−8.7	8.5−23.0	56.9−73.2	0.10−0.48	4.00−4.74
pils	44	3.6−7.5	8.0−17.3	55.3−78.9	0.10−0.43	3.93−4.95
Dortmünder	9	4.7−5.5	11.1−12.6	65.4−69.0	0.15−0.36	4.12−4.72
Bock	12	3.5−7.7	9.7−18.4	56.3−69.9	0.20−0.50	4.08−4.73
spontaneous fermentation						
Lambic & Gueuze	12	3.2−7.5	11.4−18.0	33.1−69.9	0.24−1.04	3.24−4.47
nonalcoholic	15	<1	8.5−12.6	57.0−65.3	0.12−0.30	4.00−4.65

[a] Expressed as lactic acid. [b] Range.

Spearman's correlation coefficients (r) and coefficients of determination (r^2) were calculated between amine contents and the aforementioned analytical parameters among the different types of beers (Table 3). Several significant correlations were found; however, the r^2 values were always <25%. Thus, a definitive relationship could not be established between amines and any of the analytical parameters. "Spontaneous fermentation" beers were a peculiar case, because a significant relationship was found for tyramine, histamine, and cadaverine with respect to pH ($r^2 \geq 60\%$; $p \leq 0.05$). Thus, as the pH values were lower, higher levels of those amines could be found. In acidic media, amino acid decarboxylation is favored, which results in the formation of an alkaline amine and carbon dioxide (Zee et al., 1981a). Also, lower pH levels are indicative of increased bacterial activity, especially of lactic acid organisms that could be implicated in amine formation.

Levels of histamine and tyramine found in beers were too low to produce direct toxicological effects. Following the generally recommended guideline of a 6 mg maximum allowable tyramine ingestion with in a 4-h period (Tailor et al., 1994), we calculated that 9.4% of the analyzed beers could provoke toxicological effects in patients taking irreversible MAOIs after consumption of 330 mL (regular size can volume). If consumption of two cans (660 mL) is considered, the percentage is 15.3%, and for three cans (990 mL), the percentage reaches 24.7%. "Pils" and "lager", the most popular beers, in general showed low levels of tyramine; however, up to 20% of samples showed tyramine levels >10 mg/L, which was reported an unsafe level for beers (Tailor et al., 1994).

The tyramine levels in beers found in our study were very variable, and one cannot predict whether the ingestion of a beer would result in an adverse reaction in patients taking MAOIs. Therefore, beers should be included among the prohibited foods for patients receiving treatment with MAOIs. Furthermore, the relative contribution of other pressor agents (e.g., tryptamine, β-phenylethylamine, and histamine) and the possible potentiation by other amines (e.g., cadaverine and putrescine) is currently not known. "Kriek" and "spontaneous fermentation" beers would be associated with a higher risk of provoking a dangerous rise in systolic blood pressure of patients receiving MAOIs than other beers, because these two types of beer have the highest levels of tyramine and cadaverine.

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