A Review of Biogenic Amines and Polyamines in Beer

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ABSTRACT

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The presence of biogenic amines and polyamines in foods and alcoholic beverages is important from both toxicological and technological points of view. High amounts of these compounds can lead to health problems and accurate methods of determination, as well as knowledge on where the compounds originate and how they can be controlled at the lowest levels, are important in the food and beverage industry. In brewing, the types of amines are dependent on the raw materials in the beverage, as well as the method of brewing, and any microbial contamination that may have occurred during the brewing process or during storage. Studies looking at biogenic amine and polyamine levels in various beers have been carried out by a number of researchers in Europe, Brazil, Canada and Cuba. This paper reviews the work from studies carried out previously and summarizes the values found by the various research groups. Methods of analysis for the amines including HPLC, HPTLC and enzyme immunoassays are reviewed.

Key words: Beer, biogenic amines, lactic acid bacteria, polyamines, tyramine.

INTRODUCTION

Biogenic amines (BAs) form a group of undesirable natural components widespread in foods and beverages, e.g. scombroid fish, meat and meat products, cheeses, vegetable products and wine (for reviews see[39,41]). Beer has also been reported as a possible health risk for some consumers due to BAs intake[9]. These high biogenic amine intake levels are not usually caused by a high amine content in individual beers, but rather by a very high beer consumption during a very short time interval. Although annual statistical beer consumption exceeds 100 L/capita/year in several countries, the consumption referred to here is several times higher in these particular individuals. Beer is now considered as a source of the dietary polyamines, putrescine, spermidine and spermine. These regulate cellular proliferation and differentiation[2,33].

BAs in foods and beverages arise mainly from the bacterial decarboxylation of the corresponding amino acids (Fig. 1). Thus, histamine (HI), tyramine (TY), tryptamine (TR), 2-phenylethylamine (PEA), agmatine (AGM) and cadaverine (CAD) are formed from histidine, tyrosine, tryptophan, phenylalanine, arginine and lysine, respectively. Putrescine (PUT) can arise both from ornithine and agmatine. Spermidine (SPD) and spermine (SPM) are formed biochemically from putrescine by the attachment of aminopropyl moieties catalysed by spermidine synthase and spermine synthase[45].

Excessive intake of dietary BAs can cause several deleterious effects. Psychoactive amines can affect the neural transmitters in the central nervous system. Vasoactive amines can act directly or indirectly on the vascular system as vasoconstrictors (namely TY) or vasodilators (HI).

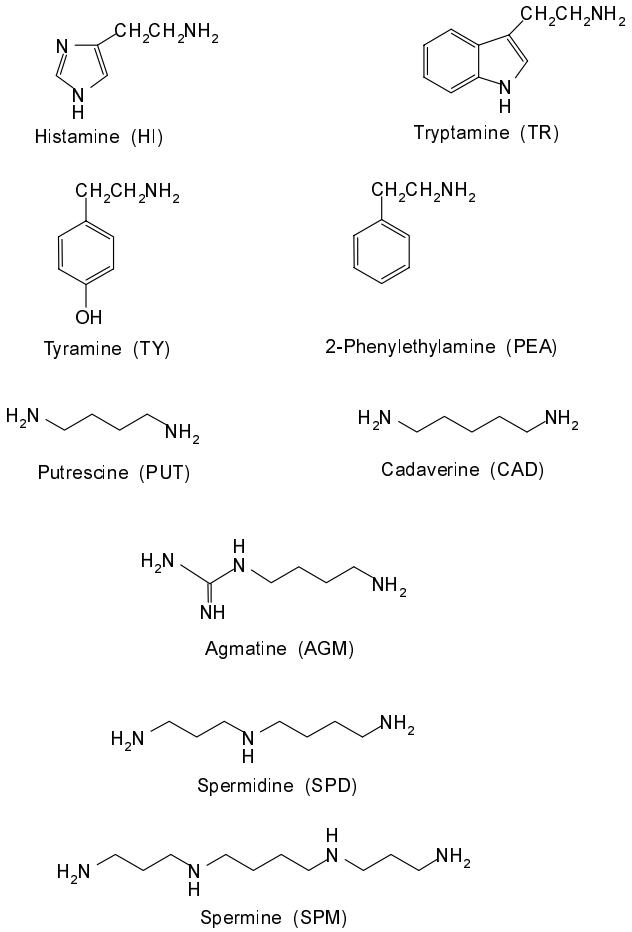

Fig. 1. Structures of some common biogenic amines.

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TABLE I. Histamine content (mg/L) in beer.

Year	Country	Number of samples	Mean	SD	Range	References
1989	Spain	17	0.7	—	0.3–1.8	22
1989	7 European countries	48	1.4	—	0.3–17.0	22
	Spain	35 (common)	0.8	1.8	0.35–1.8	23
		20 (special)	1.2	1.4	0.3–4.9	
1991	Spain	30	0.7	0.4	—	14
1996	16 European countries	195	1.2	2.4	<0.3–21.6	18
1997	Czech Rep.	78	0.6	1.1	<0.3–4.7	24
1999	Brazil	91	0.2	0.3	<0.2–1.5	11

SD = standard deviation

TABLE II. Tyramine content (mg/L) in beer.

Year	Country	Number of samples	Mean	SD	Range	References
1989	Cuba	104	3.5–8.3[a]	—	1.2–9.3	47
1989	Spain	17	5.8	—	1.9–24.7	22
1989	7 European countries	48	6.1	—	1.5–46.8	22
1991	Spain	35 (common)	4.7	5.2	1.6–30.6	23
		20 (special)	7.7	7.7	1.9–24.8	
1995	Italy	16	4.3	—	0.6–17.2	3
1996	Spain	30	6.8	7.4	—	14
1996	16 European countries	195	6.5	9.0	0.5–67.5	18
1997	Czech Rep.	78	6.9	5.2	<0.3–22.5	24
1999	Brazil	91	2.2	4.8	0.3–36.8	11

[a] Mean values increased with increasing alcohol level.

A scale of symptoms can occur following the excessive oral intake of BAs. The particular risk strongly depends on detoxification efficiency, which can vary considerably between individuals and is affected by several factors. Normal intakes of BAs are metabolised in the intestinal tract by a fairly efficient detoxification system based on the activities of monoamine oxidase (MAO; EC 1.4.3.4) and diamine oxidase (DAO; EC 1.4.3.6).

The limited literature data on BAs content and formation in beer was reviewed by Stratton et al. in 1991[43]. The aim of this article is to review the information published since that review.

HEALTH RISKS OF BIOGENIC AMINES FOR BEER CONSUMERS

Hypertensive crises have been observed in patients treated with drugs inhibiting monoamine oxidase (used mainly in psychiatry) after beer consumption[31,35,40,44]. The adverse effects were observed from both draft and non-alcoholic beers and were attributed to TY. TY intake as low as 6 mg within a 4-hour period or beers with TY content over 10 mg/L were considered as dangerous for these particular patients[44]. Alcohol and probably some other BAs present in beer can potentiate TY effects. However, no risk was reported for healthy consumers.

Beer has been reported as a trigger for headaches with patients susceptible to migraines[36]. Histamine in alcoholic drinks was capable of triggering of allergic and allergic-like adverse responses, however, wine has been a more common source than beer[46]. Secondary amines (AGM, SPD and SPM) can react with nitrites to form nitrosamines, but no literature was found dealing with this particular reaction in beer.

BIOGENIC AMINES – CONTENT IN BEERS

Data on BAs in beers, analysed over the past few years has been collated chronologically in Tables I–IV. The results from surveys in several European countries, Brazil and Cuba are given for beers of different types. Although different analytical methods were used, the reported levels are comparable.

The nine determined amines can be divided into two groups. Group one includes PUT, SPD, SPM and AGM and can be considered as natural beer constituents primarily originating from the malt, whilst Group two, mainly HI, TY and CAD, usually indicate the activities of contaminating lactic acid bacteria during brewing[18].

Agmatine (Table IV), a compound not routinely determined probably due to its minimal adverse effects for man, was the prevailing amine in the beers tested, with mean values around 10 mg/L[11,14,18]. TY and PUT (Tables II and III) were amines at relatively high levels, while the levels of the others, including HI, have commonly been lower and are probably of lower toxicological importance. Thus, TY has been the main biogenic amine identified in beer that causes the adverse effects described earlier and high levels were reported by Tailor et al.[44].

Similar levels of BAs were found in both alcoholic and non-alcoholic beers[3,11,18,24], suggesting that the production methods for non-alcoholic beers do not remove amines. No significant correlations were found between TY or HI levels and alcohol content, or extract of original wort[22,24]. However, higher levels of the both amines were observed in beers with a high total acidity[22], which indicated activity of lactic acid bacteria.

Spontaneously fermented Belgian beers and top-fermented beers showed the highest levels of TY and HI and

TABLE III. Polyamine (putrescine, spermidine and spermine) content (mg/L) in beer.

	Year	Country	Number of samples	Mean	SD	Range	References
Putrescine	1992	Belgium	11	4.6	—	2.6–8.4	8
	1995	Italy	16	2.9	—	1.4–4.2	3
	1996	Spain	30	4.5	1.7	—	14
	1996	16 European countries	195	4.8	2.3	1.5–15.2	18
	1997	Czech Rep.	78	8.8	7.1	<0.3–30.7	24
	1999	Brazil	91	3.9	1.4	0.9–9.8	11
	2002	Poland	27	1.8	0.8	0.6–3.6	42
Spermidine	1992	Belgium	11	0.3	—	<0.2–0.8	8
	1995	Italy	16	0.7	—	0.3–1.4	3
	1996	Spain	30	0.8	1.0	—	14
	1996	16 European countries	195	0.7	1.0	<0.3–6.8	18
	1999	Brazil	91	0.7	0.8	<0.2–6.0	11
Spermine	1995	Italy	16	ND	—	<0.2	3
	1996	Spain	30	0.2	0.4	—	14
	1996	16 European countries	195	0.3	0.7	<0.3–3.9	18
	1996	Brazil	91	0.3	0.5	<0.2–2.1	11
	2002	Poland	27	8.4	3.6	1.2–15.2	42

TABLE IV. Cadaverine, tryptamine, 2-phenylethylamine and agmatine content (mg/L) in beer.

	Year	Country	Number of samples	Mean	SD	Range	References
Cadaverine	1992	Belgium	11	3.2	—	<0.15–13.3	8
	1995	Italy	16	—	—	0.2–0.8	3
	1996	Spain	30	0.7	0.4	—	14
	1996	16 European countries	195	2.4	6.1	<0.3–39.9	18
	1997	Czech Rep.	78	12.9	12.3	<0.3–49.1	24
	1999	Brazil	91	0.5	0.4	<0.2–2.6	11
Tryptamine	1995	Italy	16	<1.0	—	ND–2.6	3
	1996	Spain	26	1.6	2.1	—	14
	1996	16 European countries	195	0.4	1.0	<0.3–5.4	18
	1997	Czech Rep.	78	1.2	1.5	<0.3–9.7	24
	1999	Brazil	91	0.5	1.5	<0.35–10.1	11
2-Phenylethyl-amine	1995	Italy	16	1.0	—	0.5–1.6	3
	1996	Spain	30	0.3	0.1	—	14
	1996	16 European countries	195	0.4	0.8	<0.3–8.3	18
	1999	Brazil	91	0.1	0.2	<0.2–1.7	11
Agmatine	1996	Spain	30	9.3	4.5	—	14
	1996	16 European countries	195	10.5	5.8	0.5–40.9	18
	1999	Brazil	91	10.9	7.0	2.1–46.8	11
	1999	Belgium	150	—	—	4.0–30	32

also of PEA and TR[10,32]. These types of beers represent the highest toxicological risk for patients treated with MAO inhibitors. Loret et al.[32] have proposed a safety limit of 20 mg/L for the sum of the TY + HI + CAD + PEA contents.

Low levels of TY, HI, PUT, CAD, PEA and TR, but not exceeding 0.5 mg/L of the individual amines, were observed in a survey of local Nigerian ginger beer and beers produced from sorghum, maize and plantain[29].

The question of whether increased BAs levels affect the sensorial quality of beer is still unresolved.

BIOGENIC AMINES IN RAW MATERIALS

Data on BAs levels in malt, hops or hop derivatives (pellets, extract) as well as pitching yeast is very limited[13,19,24]. No amines should be present in production water. PUT, SPD, SPM and AGM are present in malt and yeast at higher levels than in hops. The levels of the other amines are also low in these raw materials. Taking into account the low levels of hops and yeast in beer, it can be said that malt is the main source of the four aforementioned amines in beer. Please note that a portion of these amines is removed in the spent grain[24].

HI, PEA, TR and CAD levels were seen to increase slowly during a five-day barley germination, and a increase of 3–5.5 mg/kg/day was observed for PUT, SPD, SPM and AGM. The TY increase was lower[19]. Malting conditions such as germinating intensity, kiln temperature and barley variety will all affect the final amine levels in malt[13]. Amines were not detected in the rice used as an adjunct cereal[19].

BIOGENIC AMINES – FORMATION DURING BREWING

The greatest TY[6,12,19,23,24], HI[12,24] and CAD[24] formation was observed during the main fermentation. Levels of PUT[13,24] and AGM[13] decreased starting from the mashing process. The bottom yeast *Saccharomyces cerevisiae* var. *uvarum* did not appear to produce TY or HI during fermentation and moreover, yeast recycling for several fer-

mentations did not influence amine levels[16]. Also wild yeasts were not found to produce TY, while a significant positive relationship was observed between amine formation and lactic acid bacteria[17].

Bacteria isolated during beer fermentation and distinguished by TY and TR formation were identified as *Pediococcus* spp., mainly *P. damnosus.* No *Lactobacillus* spp. were isolated. TY formation was negligible at *Pediococci* spp. counts below 4×10^3 colony-forming units (CFU) per mL, while at over 1×10^5 CFU/mL, TY levels ranged between 15–25 mg/L[17]. Thus, TY content has been proposed as a reliable indicator of the level of *Pediococcus* spp. contamination during a beer fermentation[15]. Washing the pitching yeast with phosphoric acid was an effective way to reduce the number of *Pediococci* and consequently reduce the TY content of the beer[17]. However, species of the genus *Lactobacillus* also participate in BAs formation. *Lactobacillus frigidus, L. brevissimilis* and *L. brevis* have been reported as amine-forming beer contaminants[7].

Different strains of lactic acid bacteria found in foods have been reported to differ widely in their ability to produce BAs[4]. This situation could also exist in brewery lactic acid populations and may explain the differences existing in TY, and also HI contents, among breweries and among batches within one type of beer from the same brewery.

A direct relationship was not observed between the free tyrosine levels in wort and TY formation during fermentation. Content of this amino acid does not seem to be a critical factor in TY formation[20].

Amines can also be formed by the activity of lactic acid bacteria during the storage of beer in bottles, cans or kegs prior to consumption. Secondary fermentation in some bottles of special beers resulted in elevated BAs levels[32]. However, most of the beers studied were pasteurised. A significant increase in TY and HI contents that was observed in one pasteurised beer stored for several weeks[24], was probably due to under-pasteurisation. Considerable increases of TY, and to a lesser extent HI, were found in adequately pasteurised beers that were inoculated post pasteurisation with mixed *Lactobacillus* spp. or *Pediococcus* spp. isolated from spoiled beer, and stored at 28°C until haze formation commenced. *Lactobacilli* were reported to be more effective amine producers than *Pediococci.* Minimum changes in PUT, CAD and SPD levels were observed[25].

Lactobacillus paracasei isolated from a bottled wheat beer produced no HI, PUT or CAD during cultivation in malt medium at 30°C for 7 days, or in beer in opened bottles. In contrast, *Lactobacillus buchneri* cultivated as a negative control, increased the HI content in beer from 15 to 65 mg/L, while the PUT and CAD content did not change[49]. The HI content in beer has been proposed as a good indicator of brewing hygiene since it does not originate from the malt[13].

ANALYTICAL METHODS

Methods for the measurement of biogenic amines in foods are not numerous and only a few of these methods have been applied to beer. The first procedures developed focused on the most toxicologically important amines – HI and TY. Izquierdo-Pulido et al.[23] used two extraction methods. HI was extracted to n-butanol after sample alkalisation, re-extracted with hydrochloric acid and determined spectrofluorimetrically as a fluorescent complex with *o*-phthaldialdehyde (OPA). Extraction of TY similarly originated in an alkaline sample and was carried out with ethyl acetate. After re-extraction with hydrochloric acid, the tyramine complex with α-nitroso-β-naphtol was measured using spectrofluorimetry. As other biogenic amines are also of interest, the same authors[21] developed a comprehensive and more selective method. Ten amines – HI, TY, serotonin, PEA, TR, CAD, PUT, AGM, SPD and SPM – were separated in one run using ion-pair chromatographic partition on a reversed-phase column. After separation, the amines were detected as fluorescent derivatives with *o*-phthaldialdehyde. Determination limits of the method ranged from 0.3 to 0.4 mg/L, except for serotonin and SPM, which were slightly higher.

Though post-column derivatization is more common when using *o*-phthaldialdehyde, Petridis et al.[37] described a simple and selective HPLC method for beers and other beverages with pre-column OPA derivatization. Recoveries for HI, TY, PUT, CAD, TR and PEA were higher than 95%. The detection limits of the BAs ranged between 5–25 μmol/L.

In recent years, Arlorio et al.[1] have combined the extraction pre-separation step with ion-pair chromatography. The extraction steps were carried out by either butanol or an ionic exchanger. The authors concluded that many interfering compounds were eliminated by the pre-separation. However, this procedure appears to be laborious and detection limits were not given.

Alternatively to the detection of post-column derivatives, another approach was the separation of derivatives prepared prior to high performance liquid chromatography. Buiatti et al.[3] separated eight amines (HI, TY, PEA, TR, CAD, PUT, SPD and SPM). Amine derivatives with dansyl chloride were eluted from a reversed-phase column by an acetonitrile/water solution at pH 7. The gradient of acetonitrile concentration in the mobile phase was 65–90% during 0–6 min. For detection, the UV detector was set at 254 nm.

Derivatives with dansyl chloride were also separated by a high performance thin-layer chromatographic method (HPTLC). Microwave assisted dansylation reduced analysis time and the detection limits of the amines ranged between 0.28–0.39 ng[30].

Another agent was used for pre-column derivatization of beer amines by Křížek and Hlavatá[27]. Benzoyl chloride forms stable derivatives with biogenic amines and these derivatives were extracted from beer samples adjusted to pH 6.0 by a phosphate buffer solution. The determination limit was 0.3 mg/L. More recently, the reagent *p*-nitrobenzyloxycarbonyl chloride (PNZ-Cl) was used for pre-column derivatization of biogenic amines in fermented beverages and vinegars[26]. This method was applied to the determination of PEA, TR, PUT, CAD, HI, TY, SPD, SPM and serotonin. Recoveries of BAs ranged from 78% to 93%.

Another technique for beer amine determination is the amino acid analyser. This procedure employs a potassium

citrate buffer system and a post-column ninhydrin reaction[13].

HI in beers has also been determined by capillary zone electrophoresis with amperometric detection[48]. This method did not require any sample clean-up procedures, but used a detector not ranked among standard detector types.

Very promising techniques involve enzyme immunoassay methods or selective enzyme biosensors. For the determination of HI in beers a competitive enzyme immunoanalysis has been developed[5]. This method is very selective and not affected by either HI, TY or their various derivatives. The proof limit of this method is 7 ng/mL. A more versatile enzyme sensor for the simultaneous determination of three BAs (HI, TY and PUT) was designed by Lange and Wittmann[28]. However, the lower detection limits with this sensor (range between 5–10 mg/kg) appeared to lack sensitivity.

CONCLUSIONS

There is currently much work ongoing globally as researchers try to better understand the impact of biogenic amines and polyamines in our foods[34,38]. It is clear that in beer, bacterial contamination is a key source of these compounds and that this is a source that can be controlled to ensure that the beer meets all quality and safety standards. In addition better methods of analysis for these compounds are being developed which allows for better monitoring.

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