G. Gasarasi, M. Kelgtermans, K. J. Verstrepen, J. Van Roy, F. R. Delvaux and G. Derdelinckx

Occurrence of biogenic amines in beer: causes and proposals of remedies

Biogenic amines are biomolecules synthesized by eukaryotic and prokaryotic cells that can affect human health. Histamine and tyramine are the most important and can cause important troubles like headache and severe forms of allergy. Secondary biogenic amines can also be involved in the synthesis of nitrosamines in the stomach. In beer, the presence of biogenic amines is mainly due to the activity of contaminants that develop during fermentation but their synthesis was detected during malting too. The microorganisms involved are the Enterobacteria sp., possibly active at the early stage of the fermentation, and many lactic acid bacteria. Some strains of S. cerevisiae produce different biogenic amines, putrescine, spermidine and spermine mainly. Some speciality beers such as sour beers and beers produced by mixed cultures are characterized by a constitutive presence of biogenic amines due to the metabolism of the typical microflora involved. This paper demonstrates that it is possible to reduce the level of the biogenic amines significantly (up to 95 %) in speciality beers by keeping the process as traditional as possible. Mainly by assembling the freshly cooled wort with old beer containing lactic acid bacteria characterised by their lack of aminoacid decarboxylase activity.

BC 04 Brewery biology

(Deskriptors: biogenic amine, mixed culture, sour beer, speciality beer, spontaneous fermentation, Belgian Beer.

Deskriptoren: Biogene Amine, Mischkulturen, Spontangärungen, Bierspezialitäten, Belgische Biere)

1 Introduction

Biogenic amines are a typical class of amines; they are aliphatic, aromatic or heterocyclic compounds of low molecular weight produced by some biochemical activity of eukaryotic and prokaryotic cells (1, 6). These molecules, monoamines, diamines or polyamines, are characterized by a physiological activity, for example by increasing blood pressure, provoking headache, fever, transpiration or even vomiting. They belong to the class of non volatile amines and are well studied because of their potential role in medicine. In fact, they are polycationic compounds that are present in all biological materials and have been implicated in a wide variety of biological reactions including synthesis of DNA, RNA and proteins (18). With humans, biogenic amines can be active on the psyche also, by causing diseases of the nervous system (provoked by catecholamine not present in beer) or by influencing the circulatory system (blood-vessels) due to histamine and tyramine mainly, biogene amines present in beer sometimes.

In fermented drinks, the concomitant presence of ethanol and polyamines can accentuate the toxic effect of these biogenic amines. Moreover, these amines can react with nitrites to form N-nitrosamines in the foodstuffs (4, 7).

The synthesis of the biogenic amines results from the decarboxylation of the amino acids. The presence of free amino acid and certain bacteria capable of the decarboxylation are factors leading to the formation of the biogenic amines.

Authors: G. Gasarasi, M. Kelgtermans, K. J. Verstrepen, F.R. Delvaux, G. Derdelinckx, KUleuven, Centre for Malting and Brewing Science, Kasteelpark Arenberg, 22 B-3001 Heverlee, Belgium; J. Van Roy, Cantillon Brewery, Gheudestraat, 56, B-1090 Brussels, Belgium.

2 Biogenic amines in foods and fermented beverages (other than beer)

The concentration of biogenic amines in food and beverages is rather variable and dependent on the environment i.e. meat, fish, beer, water and wine. Cadaverine, putrescine and histamine may be produced in cells alive and "post mortem" from the decarboxylation of lysine, ornithine and histidine respectively. In meat and in fish, the decarboxylation may occur by two biochemical pathways : the endogenous decarboxylase enzyme naturally occurring in the tissue considered or exogenous enzymes released by various microorganisms associated with the environment, the storage and the processing procedure.

Official regulations are not well defined but there are recommendations or commercial agreements. Dutch wine importers for example refuse wine containing more than 3,5 mg/l of histamine while in Switzerland a maximum level of 10 mg/l has been set to comply with health criteria. Regarding legal aspects, the FDA (FDA recommendation/ 21 CFR 123) established a guidance level for histamine of 5 mg/100g (50 ppm) in case of fish; a maximum average content of 10 mg/100 g (100 ppm) was established by the EC for the acceptance of tuna and other fishes of the Scrombere-socidae families. It is expected that the EC will suggest for the future a maximum of 300 mg/kg for total biogenic amines in fish and fish products.

The sources of biogenic amines in daily food can be fish, meat, cheese, wine, beer and even water; other fermented meals like sauerkraut are also potential origins (1). The importance of each of them should be considered with regard to the amount of food consumed, the presence of other amines in the food or other dietary components and the consumption of alcohol or the use of medicine. Regarding current food, attention is necessary with cheeses which involve fermentation in their production process (Table 1). Indeed, some of them for example Roquefort can contain amounts as high as 2000 mg of histamine per kg. Only seldom the normal daily consumption will be higher than 200 g cheese pro day.

In wine, the presence of biogenic amines is also a topic of concern (10, 11, 12, 13). Their concentration is low after the alcoholic fermentation and increases in most wines during malolactic fermentation to a very variable extent due to lactic acid bacteria

Table 1 : Biogenic amines in cheese and microorganisms involved

Type of cheese	Microorganisms involved	Main biogenic amines detected	Average of concentration (mg/kg)
Young Gouda cheese	no	Not detected	Not detected
Cottage cheese Fresh cheese	lactic acid bacteria	Depend on the microflora of the milk	Nd –150
Emmenthal cheese Appenzeller cheese	Propionic bacteria	Cadaverine Tyramine	200 – 250
Münster cheese Romadur cheese	Yellow- and red cultures	Putrescine / Tyramine Phenylethylamine	70 – 1500 2000 – 4000
Camembert Brie	Penicillium camemberti	Tyramine	50 - 200
Roquefort	Penicillium roquefortii	Histamine	up to 2300

Table 2 : Biogenic amines encountered in must and wine (8)

Biogenic Amine	White must	White wine	Red must	Red wine
Histamine (mg/l)	nd	5.1 – 16.5	nd – 0.3	6.1 – 15.2
Tyramine (mg/l)	nd – 0.4	nd – 0.4	nd	nd – 6.0
Putrescine (mg/l)	nd	1.0 – 7.6	1.1 – 8.2	1.3 – 27.2
Cadaverine (mg/l)	nd – 0.6	< 0.17 – 1.1	nd – 1.3	0.6 – 2.7
Phenylethylamine (mg/l)	nd – 0.9	nd – < 0.47	nd – < 0.47	nd – < 0.47

activity (Table 2). The major biogenic amines present are histamine, tyramine and putrescine. Other amines such as methylamine, ethylamine, phenylethylamine, isoamylamine and diaminopentane (cadaverine) which are present in grape-must are produced but also degraded during vinification.

lonvaud-Funei (10) indicates that compiling many results, she was forced to conclude that there is no general rule for the evolution and presence of biogenic amines in wines. Some wines contain most biogenic amines while others contain none. In a study conducted on 135 wines (principal components analysis), it was clearly shown that the content in biogenic amines is related to several parameters which are also of importance by the production of some speciality beers. These parameters are related to :

- the presence of bacteria in wine which have the enzymatic equipment necessary for the reactions of biogenic amine synthesis (decarboxylase and transport systems);
- that aminoacids are present in sufficient amount in the wine after the alcoholic fermentation;
- that the contact time of wine with yeast lees is sufficient to allow autolysis and the liberation of peptides and aminoacids;
- that the pH-value of the environment is suitable since it acts not only on the biological activity but is also a selective factor; indeed, at high pH-levels, biogenic amines are always produced at high amounts and since white wines are generally more acidic than red wines they contain less biogenic amines; further, pH changes can induce structural changes of histidine decarboxylase (14);
- the type of winemaking and whether it involves malolactic fermentation or not (some authors connect malolactic fermentation to presence of biogenic amines in wine).

In fact, regarding fermented beverages, wine is much better studied than beer regarding the presence of biogenic amines; indeed, biogenic amines are constitutive compounds in most of wines while in beer their presence is generally linked to the presence of contaminants like Enterobacteria sp and lactic acid bacteria or to the use of inappropriate strains of S. cerevisiae. The quantity of biogenic polyamines as putrescine, spermidine and spermine synthesized by S. cerevisiae vary considerably and the concentration in the medium seems to linked to the strain and the culture conditions (17). Putrescine derives from the decarboxylation of ornithine, an aminoacid involved in the metabolism of arginine while spermidine and spermine are more complex structures resulting from successive condensation reactions with decarboxylated S-adenosylmethionine.

3 Biogenic amines in beer

The content of biogenic amines in beer is generally of minor concern since their occurrence is, in theory, not expected . Indeed, most of lager beers contain from only small quantities down to not detectable amounts, of biogenic amines (Table 3). Their presence is generally linked to the malting process (16) or to the presence of contaminants during the beer production process but as already indicated, S. cerevisiae sp. can be potential sources too (18).

In most of beers, the concentration of biogenic amines is linked to the presence of a microbial contamination by Gram negative

Table 3 : Biogenic amines encountered in beers (2, 3, 5, 7, 9, 15)			
Biogenic Amine	European lager Beers	American lager Beers	local Speciality Beers
Histamine (mg/l)	Nd – 2.5 (Nd : not detected)	Nd – 2.5	Nd – 10
Tyramine (mg/l)	Nd – 45	Nd – 40	Nd – 80
Putrescine (mg/l)	Nd – 7.5	Nd – 5.0	Nd – 50
Cadaverine (mg/l)	Nd – 5.0	Nd – 2.5	Nd – 50
Spermidine (mg/l)	Nd – 5.0	Nd – 5.0	Nd – 5.0
Spermine (mg/l)	Nd – 3.0	Nd – 3.0	Nd – 5.0

Table 4 : Assumed relation between contaminants and the presence of specific biogenic amines in beer						
	Histamine	Putrescine	Spermidine	Spermine	Cadaverine	Tyramine
Gram negative bacteria Enterobacteria aerogenes E. coli Klebsiella sp.	+	+	+/-	+/-	+	+
Gram positive bacteria lactobacillus brevis lactobacillus sp. Streptococcus sp. leuconostoc sp. Pedicoccus sp.	+	+	nd	nd	nd	+
Saccharomyces sp S. cerevisiae Other Saccharomyces sp	nd	+	+	+	nd	nd
non-Saccharomyces yeasts Pichia sp. Candida sp. Brettanomyces sp. Dekkera sp.	data and references available are insufficient					

Table 5 : Biogenic amine content of a lambic wort inoculated by air exposure during 15 hours (spontaneous fermentation)									
Time of fermentation	**Temp**	**Extract °C**	**pH Plato**	**Histamin mg/l**	**Putrescine mg/l**	**Spermidine mg/l**	**Spermine mg/l**	**Cadaverine mg/l**	**Tyramine mg/l**
After cooling (15 hours)	19.5	12.1	5.30	< 0.2	15	< 0.3	< 0.5	30	3.1
5 days	15.5	12.0	4.66	< 0.2	32	< 0.3	< 0.5	30	3.2
20 days	15.0	5.0	4.13	< 0.2	41	< 0.3	< 0.5	37	3.9
45 days	11.8	3.9	3.96	< 0.2	41	< 0.3	< 0.5	37	4.0
300 days	11.0	1.9	3.85	< 0.2	48	< 0.3	< 0.5	39	64

bacteria as Enterobacteria sp (E.coli , Enterobacter sp.) and by Gram positive bacteria as lactobacillus sp., Streptococcus sp. and Pediococcus sp. The trial to establish a definitive link between beer contaminants and the potential presence of typical biogenic amines is hazardous (Table 4). Indeed, the aminoacid decarboxylating activity seems to be strain specific in case of lactic acid bacteria (10); it may be that the activity is randomly distributed within the species as for example in lactobacillus sp., Pediococcus sp. and leuconostoc sp. in case of wine. Virkajarvi and al.(20) observed the synthesis of biogenic amines (mainly cadaverine and putrescine, but not histamine or spermine) by contaminating deliberately an immobilized primary fermentation bioreactors system by Klebsiella terrigena. Concerning flavour, they observed an increase of the DMS concentration (above the threshold value) but no change in concentration of higher alcohols and esters. In consequence, it is noticeable that immobilized systems are also susceptible to contamination by microrganisms synthesizing biogenic amines.

Nevertheless, some speciality beers which involve mixed cultures (such as Belgian lambic and Gueuze beers, Flanders sour beers) or specific lactic acid bacteria strains can be characterized, like with most wines, by a constitutive presence of biogenic amines. An analogous evolution with some deviation characterizes most of sour beers produced by using an uncontrolled mixed culture as starter or by practising the spontaneous fermentation technique. Regarding human health it is important, as for wine, to improve the production methods (10, 11).

Our research focused on the traditional Gueuze and lambic beers typical in the country of Brussels. They are characterized by

Table 6 : Biogenic amine content of a lambic wort inoculated by air exposure during 15 hours (spontaneous fermentation) and consecutive assembling with old beer containing a typical mixed culture of Streptococcus thermophylus and lactobacillus delbrueckii.

Time of fermentation	Temp	Extract °C	pH Plato	Histamin mg/l	Putrescine mg/l	Spermidine mg/l	Spermine mg/l	Cadaverine mg/l	Tyramine mg/l
After cooling (15 hours	19.5	12.1	4.73	< 0.2	2.0	< 0.3	< 0.5	2.2	3.8
15 days	16.0	10.2	4.02	< 0.2	5.9	< 0.3	< 0.5	3.1	4.9
35 days	13.6	3.9	3.94	< 0.2	8.2	< 0.3	< 0.5	5.1	4.9
60 days	11.1	2.7	3.94	< 0.2	8.1	< 0.3	< 0.5	5.0	5.0

spontaneous fermentation, in other words the wort is inoculated naturally by the environmental air by cooling, after boiling in presence of overaged hops, in a large open vessel during 1 night (about 15 hours); the wort is then introduced in used Port or French wine casks. These containers are just cleaned with hot water and steamed before reuse; they are neither lined nor waxed. The evolution of the biogenic amine content of a traditional inoculated wort is very variable from brewery to brewery and from brew to brew due to the environment (i.e. age of casks, outside temperature); both temperature and pH seem to play the most important role; worts introduced in casks at pH-levels higher than 5.0 and further cooled during storage below 15°C (due to the environmental conditions of the cellar) before the start of the Saccharomyces phase of the fermentation (normally after seven days or more) can show very high biogenic amine contents.

The target of our research is still to reduce the biogenic amine content in lambic by keeping the traditional production process as unchanged as possible. Until now, we carried out several experiments requiring a minimal accommodation of the actual process and based our work on suggestions published by lonvaud-Funei (10) and by Pretorius (11) although we didn't use any modified microorganisms. First of all, as already mentioned by lonvaud, we confirm that, in case of sour beers or beers produced by spontaneous fermentation, the addition of 2000 mg of lactic acid pro litre of wort in order to lower the pH-value and to inhibit the development of Enterobacteria sp. reduces only partially the biogenic amines synthesis. Certainly not at a satisfactory level (unpublished results). Further, we tested at lab scale several pure cultures of lactic acid bacteria isolated from different origins and assessed them regarding their biogenic amine production activity. We selected finally two strains, Streptococcus thermophylus and lactobacillus delbrueckii . The reason for the choice of Streptococcus sp. is linked to its ability to colonize wort as soon as the temperature drops below 50°C while lactobacillus delbrueckii is a familiar contaminant of breweries; furthermore, both are homofermentative lactic acid bacteria and did not show any detectable biogenic amine production activity.

The handling suggested is easy : the last brew of the season (at the end of April), cooled at the air, is inoculated with 20 l/cask of pure cultures of Streptococcus thermophylus and lactobacillus delbrueckii produced at the lab. The inoculation rate is about 10^8 cells/Ml. After the Saccharomyces sp. fermentation phase, the casks are closed and a secondary lactic acid fermentation took place; after four months, the concentration reached 23.4 g/l of lactic acid.

This sour beer was assembled in two steps at a rate of 5% to the brews produced during the next season (beginning in October). First 2.5 % was added at 50°C, the other 2.5% were added when the temperature of the wort reached 20°C.

After 60 days, the total amount of biogenic amines is 14 mg/l, putrescine and tyramine mainly and the concentration does not seem to increase further after 15 weeks of fermentation in casks (Table 6).

Casks containing a mixture of freshly brewed wort cooled by contact with the environmental air (spontaneous fermentation) and 5% of old beer produced in presence of the cultures of Streptococcus thermophylus and lactobacillus delbrueckii were characterized the following properties:

- absence of the typical DMS flavour of young lambic which was normally due to the presence of Enterobacteriacae at the early stage of the lambic fermentation;
- palatable body and pleasant mouthfeel after 5 weeks of fermentation without the strong acidity generally linked to the presence of acetic acid bacteria;
- ropiness at early stage of the fermentation (5 – 9 weeks).

Unfortunately at present we are unable to describe the evolution of the microflora present at each stage but this research is going on. The reason for a reduced biogenic amine content in lambic produced by the modified traditional way is probably due to the application of an adapted starter culture. It is assumed that either this technology completely suppresses the endogenous bacteria activity responsible for the production of the biogenic amines or that it degrades the biogenic amines produced by undesirable strains (10).

4 Conclusions

In general, the presence of biogenic amines in beer is linked to microbial contaminants such as Enterobacteria sp. and lactic acid bacteria. Some strains of S. cerevisiae are also biogenic producers but of putrescine, spermidine and spermine only. Some speciality beers as sour beers produced by mixed cultures and as Brussels lambic are traditionally characterized by a constitutive presence of biogenic amines. We studied a procedure to reduce the presence of these molecules by keeping the traditional process as unchan-

ged as possible. Founding partially our work upon research carried out to improve the quality of French wines, we were able to limit the production of biogenic amines during the early stage of the fermentation below 5% of the concentration formed in traditional lambics. Final results will have to be established but we do not expect an increase of more than 10 mg/l due to the activity of lactic acid bacteria on the aminoacids liberated by the yeast autolysis. The procedure proposed is readily applicable at industrial scale and implicates only a very little adaptation of the traditional process. It is essential to keep the traditional production procedure of beers such as lambic characterised by the typical Brettanomyces sp., Dekkera sp. and other wild yeasts flavours alive at an economical viable scale. Finally, since consumers and connoisseurs appreciate the beer types characterized by the "Brett" flavours and by the mouthfeel of lactic acid and accept to pay for it, it is important that they have healthy beers at their disposal. Further, these beers are also an excellent substrate to study the ecology and the metabolism of lactic acid bacteria and yeasts in mixed cultures and in beverages stored for a long time (several months and even years).

5 Zusammenfassung

G. Gasarasi, M. Kelgtermans, K. J. Verstrepen, J. Van Roy, F. R. Delvaux und G. Derdelinckx: Auftreten von biogenen Aminen in Bier: Ursachen und Vorschläge zur Vermeidung – Monatsschrift für Brauwissenschaft 56, Nr. 3/4, 68-75, 2003

BC 04 Brauereibiologie

Biogene Amine sind Biomoleküle von eukaryotisch oder prokaryotischen Zellen die die menschliche Gesundheit beeinflussen können. Histamin und Tyramin sind davon die beiden wichtigsten Verbindungen. Sie können bedeutende gesundheitliche Nebenwirkungen haben wie Kopfschmerzen oder starke Formen von allergischen Reaktionen. Sekundäre biogene Amine können ebenfalls an der Synthese von Nitrosaminen im Magen beteiligt sein. Das Vorhandensein von biogenen Aminen in Bier ist vor allem auf die Aktivität von kontaminierenden Mikroorganismen zurückzuführen, die sich während der Gärung entwickeln. Ihre Bildung konnte während des Mälzens ebenfalls beobachtet werden. Die daran beteiligten Mikroorganismen sind Enterobacteria Sp., die möglicherweise während der Angärung aktiv sind und viele Lactobacillus-Arten. Einige Stämme von Saccharomyces cerevisiae bilden unterschiedliche biogene Amine, vor allem Putrescin, Spermidin und Spermin. Einige Bierspezialitäten wie z.B. Sauerbiere und Biere, die mit vermischten Kulturen hergestellt werden, sind charakterisiert durch das Vorhandensein von biogenen Aminen, die aus den an der Gärung beteiligten Mikroorganismen stammen. Dieser Beitrag stellt dar, dass es möglich ist, den Gehalt von biogenen Aminen deutlich (bis zu 95%) in Bierspezialitäten dadurch zu reduzieren, dass man den Prozess so traditionell wie möglich erhält. Vor allem das Vermischen der frisch abgekühlten Würze mit altem Bier, das Milchsäurebakterien enthält, welche sich durch ihren Mangel an Aminosäure-Decarboxylase-Aktivität auszeichnen, bringt eine Reduzierung.

G. Gasarasi, M. Kelgtermans, K. J. Verstrepen, J. Van Roy, F. R. Delvaux et G. Derdelinckx: Existence d'amines biogènes dans la bière : causes et propositions de remèdes – Monatsschrift für Brauwissenschaft 56, Nr. 3/4, 68-75, 2003

BC 04 Biologie brassicole

Les amines biogènes sont des biomolécules synthétisées par des cellules eucaryotes et procaryotes qui peuvent nuire à la santé publique. L'histamine et la tyramine, qui sont les plus importantes, peuvent provoquer des troubles tels que maux de tête et des formes graves d'allergies. Les amines biogènes secondaires peuvent également être impliquées dans la synthèse de nitrosamines dans l'estomac. Dans la bière, la présence d'amines biogènes est due à l'activité des contaminants qui se développent pendant la fermentation; leur synthèse à également été détectée pendant le maltage. Les micro-organismes impliqués sont les *Enterobacteria sp.*, probablement actifs en début de la fermentation et beaucoup de bactéries d'acide lactique. Quelques souches de *S. cervisiae* produisent différentes amines biogènes, la putriscine, la spermidine et essentiellement la spermine. Quelques bières de spécialité telles que les bières acides et les bières produites avec des cultures mixtes sont caractérisées par une présence constitutive d'amines biogènes due au métabolisme de la microflore typique impliquée. Cette publication fait la démonstration qu'il est possible de réduire le taux d'amines biogènes de façon significative (jusqu'à 95 %) dans les bières de spécialité tout en maintenant le procédé aussi traditionnel que possible. En mélangeant le moût fraîchement refroidit avec une bière âgée contenant des bactéries d'acide lactique qui sont caractérisées par leur absence d'activité de décarboxylase d'acide aminé.

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