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Levels of biogenic amines as a measure of the quality of the beer fermentation process: Data from Belgian samples

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Abstract

Biogenic amines were determined by HPLC in four beer types. In spontaneously fermented beers (SF beers), the amounts of vasoactive amines reached high levels, namely a mean value (1) above 20 mg/l for tyramine and (2) close to 10 mg/l for histamine. Considering the bacterial origin of these amines, we established a calculation formula for a Beer biogenic amine index (Beer BAI), reflecting the microbiological quality of the fermentation process. Using this formula we determined a mean Beer BAI value lying between 0.84 ± 0.89 (high quality) in low fermented beers and 16.2 ± 13.9 in SF beers. BAI values $\geqslant 10$ (poor hygienic fermentation process) corresponded to beers showing values of vasoactive BA (>10 mg/l) that could cause health troubles in certain types of consumers (people under treatment with monoamine-oxidase medication or genetically more sensitive to food-born BA).

Keywords: Vasoactive amines; Microbiological quality; Biogenic amine index; Beer fermentation type

1. Introduction

Moderate amounts of biogenic amines (about 50 mg/kg food) can be ingested with food without effect on consumer health, as detoxifying enzymes (monoamine and diamine oxidase or MAO and DAO), found in epithelial cells lining the gut, alter them. However, upon intake of high loads of biogenic amines with food, the detoxification system is unable to process them sufficiently. This detoxification deficiency has been associated with some cases of food poisoning (Grotheer & Stockemer, 2001; Shalaby, 1996), expressed as headaches, respiratory distress, heart palpitation, hypertension or hypotension, and several allergy-related disorders. Cases of scombroid fish poisoning episodes are well documented (for review, see Lehane, 2000). Involvement of histamine as the main chemical in this poisoning is supported by the occurrence of symptoms identical to those of intravenous histamine administration or allergic reaction, the efficacy of antihistamine therapy and the presence of increased levels of histamine in plasma of patients. Regardless of the food type, high amounts of biogenic amines have been reported for products resulting from the fermentation process and/or ripening (essentially fish, fermented sausage, cheese and fermented alcohol beverages). The prevailing biogenic amines in fermented and/or ripened foodstuffs are histamine, tyramine, cadaverine and putrescine, while fresh products only show significant amounts of the polyamines spermidine and spermine. The production of BA in foodstuffs is a characteristic of several groups of microorganisms able to decarboxylate amino acids, such as Enterobacteriaceae, *Pseudomonas* spp., Micrococcaceae, Enterococci and lactic acid bacteria (Giraffa, 2002; Silla Santos, 1996; Silva & Gloria, 2002; Straub, Kicherer, Schilcher, & Hammes, 1995). As a result, the determination of biogenic amines has been proposed as an indication of food microbiological quality.

Pioneers in this area, Mietz and Karmas (1977), assessed the quality of fish on the basis of its contents of histamine, cadaverine, putescine, spermidine, and spermine and devised a biogenic amine index (BAI) reflecting the freshness status of the food. Since then, this

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first proposal has been revisited. Thus, (Yamanaka, Shiomi, & Kikuchi, 1987), followed the cadaverine level as a quality index for salmonid fish spoilage. Alternatively, the agmatine amount – alone or in combination with cadaverine – has been proposed as a quality index in common squid (Yamanaka, Shiomi, & Kikuchi, 1989) and hake stored on ice (Ruiz-Capillas & Moral, 2001a, 2001b), while Veciana-Nogues, Marine-Font, and Vidal-Carou (1997) suggested monitoring histamine, tyramine, cadaverine and putrescine in fresh and canned tuna. More recently, putrescine concentration has been proposed as a chemical indicator of carp meat quality (Krizek, Pavlicek, & Vacha, 2002). Besides fish, meat and dairy products also show high amounts of tyramine, histamine, putrescine, cadaverine and tryptamine with spoilage. An index based on the ratio of spermidine/spermine levels was considered appropriate for the evaluation of chicken meat quality (Silva & Gloria, 2002). Rises of biogenic amine levels were also associated with sausage fermentation, as well as with cheese ripening (Bodmer, Imark, & Kneubühl, 1999; Mietz & Karmas, 1977; Okamoto, Sugi, Koizumi, Yanagida, & Udaka, 1997; Silla Santos, 1996).

Alcoholic beverages constitute another category of fermented products that sometimes bear substantial quantities of biogenic amines, as shown for beers (Dumont, De Geeter, & Hyughebaert, 1992; Fernandes, Judas, Oliveira, Ferreira, & Ferreira, 2001; Izquierdo-Pulido, Albala-Hurtado, Marine-Font, & Vidal-Carou, 1996a, 1996c; Kalac, Hlavata, & Krizeck, 1997), wines (Caruso et al., 2002; Csomos, Heberger, & Simon-Sarkadi, 2002a; Csomos & Simon-Sarkadi, 2002b; Gasarasi et al., 2003; Hajos, Sass-Kiss, Szerdahelyi, & Bardocz, 2000; Kalac & Krizeck, 2003; Kanny et al., 2001; Leitao, Teixeira, Crespo, & San Romao, 2000; Torrea & Ancin, 2002) and sparkling wines (Del-Campo, Lavado, Duenas, & Irastorza, 2000). The description of a tyramine-dependent hypertension crisis following the consumption of a beer by a patient under treatment with monoamine oxidase inhibitor (Tailor, Shulman, Walker, Moss, & Gardner, 1994), singled out high-risk consumers regarding BA-enriched beverages. Besides secondary effects of medicine, other factors contributing to a high sensitivity toward ingested amines include insufficient DAO-activity caused by genetic predisposition, gastrointestinal disease, or inhibition of amine-metabolising activity by other amines (such as the known inhibitory effect of putrescine and cadaverine on histamine oxidase) and alcohol (for review, see Stratton, Hutkins, & Taylor, 1991).

Biogenic amines have already been reported to occur in a few samples of Belgian beer (Dumont et al., 1992), as well as in a large sampling of European beers, including several Belgian types (Izquierdo-Pulido et al., 1996a, 1996c). Though results of those studies were in agreement regarding the frequency of amine types among beer samples, they reached somewhat different conclusions about the level of health threatening amines (mainly histamine and tyramine) in Belgian samples. These first investigations also suggested that BA content could be related to the brewing process and consequently the BAI value, defined according to Mietz and Karmas (1977), could largely fluctuate from one fermentation type to another. Surprisingly, compared to BAI values obtained for solid foodstuffs, all BAI values reported by Dumont et al. (1992) for Belgian beers were high (between 3.97 and 16.1), suggesting that none of the analysed samples were of good microbiological quality (i.e. corresponded to a Mietz and Karmas BAI value <1.0). The present study has been undertaken with the aim of reconsidering the suitability of the Mietz and Karmas BAI index for beers. To this end, we determined the BA content profile of a wide range of beer samples of four fermentation categories.

2. Materials and methods

2.1. Sampling

Beer samples ($n = 297$) were purchased from local stores. They covered four different brewing processes, namely low or bottom fermentation (LF, 18 samples), top fermentation (TF, 36 samples), top fermentation, followed by a secondary fermentation into bottle (TF + BSF, 184 samples), and spontaneous fermentation (SF, 42 samples). Most of measurements were made between June 1997 and January 2001. As large amounts of biogenic amines were seen in beers produced by spontaneous fermentation, this category of products was more systematically investigated, in order to identify a link between this brewing process and a high concentration of amines. Consequently, though they only account for about 2.3% of the Belgian consumption, beers produced by spontaneous fermentation account for 15% of the total samples analysed in this study. Similarly, samples of top fermented beers, with (TF + BSF) or without (TF) a secondary fermentation after bottling, are well represented in our data (65% of all samples, against 24% of the Belgian consumption), because the present work attempted to cover (as much as possible) the large variety of products comprised in these beer categories. As this study is aimed at defining and discussing the suitability of a biogenic amine index (BAI) that would fit with beer samples of any origin, the trademark of analysed samples has voluntarily not been mentioned.

2.2. Analytical method

Biogenic amines were determined using high-performance liquid chromatography (HPLC), based on

ion-paired chromatographic partition on a C18 reversed phase column, involving a post-column derivatization with the fluorescent *o*-phthalaldehyde (OPA). This technique corresponds to the official method used in the USA to determine histamine content in food products (Williams, 1984) and has also been routinely used to assess biogenic amine amounts in Spanish foodstuffs (Hernandez-Jover, Izquierdo-Pulido, Veciana-Nogues, & Vidalcarou, 1996). The method was linear for each amine between 0.50 and 200 mg/l. The reproducibility of the analytical performance (column efficiency and detector accuracy) was assessed through the measurement repeatability of an internal standard added to all samples (1,6 diaminohexane or 1,6 HD). Nine different biogenic amines were simultaneously determined in each sample, namely (1) tyramine (TYR), (2) putrescine (PUT), (3) histamine (HIS), (4) cadaverine (CAD), (5) agmatine (AGM), (6) β-phenylethylamine (PHE), (7) spermidine (SPD), (8) tryptamine (TRY) and (9) spermine (SPM). Amine concentrations were expressed as mg/l of beer.

The precision of the method was assessed by repeated measurements of each amine (spiked into samples of beers at 5 mg/l for PUT, CAD and HIS, at 10 mg/l for TYR, AGM, PHE, SPD and TRY and at 20 mg/l for SPM). The coefficients of variation were, respectively, 2.98%, 5.38%, 3.87%, 6.19%, 4.17%, 5.11%, 9.81%, 4.40% and 6.97% for TYR, PUT, CAD, HIS, AGM, PHE, SPD, TRY and SPM. Standard curves were prepared separately in the range of 10–200 μg/ml, for each amine. These concentration ranges were chosen in accordance with the expected values of each amine in beer samples. The correlation coefficient of each curve was above or equal to 0.99, indicating a linear relationship between amine concentration and detector response. Given that assay sensitivity can be defined as the smallest detectable concentration yielding a signal-to-noise ratio of 3:1, the minimal concentration limits were, respectively, 35, 20, 35, 20, 60, 45, 50, 60 and 100 μg/ml for TYR, PUT, CAD, HIS, AGM, PHE, SPD, TRY and SPM. These detection limits appeared to be below the mean beer concentrations.

For each analysis, 50 ml beer samples to which 1,6 diaminohexane (10 mg/l) was added as internal standard, were degassed under vacuum. After centrifugation (2000*g*, for 10 min), samples were filtered through a nylon acrodisc syringe filter (diameter: 26 mm, porosity 0.45 μm; Gelman) before injection (75 μl) into the chromatographic system consisting of a Merck-Itachi separation module equipped with 1 LiChroCART 125-4 HPLC-cartridge (RP-18e, 5 μm, Merck) heated at 40 °C. The mobile phase, driven at a flow rate of 1.0 ml/min, was a mix of an aqueous A solvent (0.1 M sodium acetate, 0.01 M octane sulfonate, pH 5.2) and of an organic B solvent (acetonitrile: solvent A; 34:66; v:v), showing a gradual change from 20% solvent B at the injection time to 80% solvent B after 50 min. Each elution was followed by a equilibration period (10 min) in the initial condition for the solvent (20% solvent B). The post-column derivatization was performed using *o*-phthalaldehyde (OPA Merck, 0.2 g in 5 ml of 100% methanol) dissolved in a 3.1% H_3BO_3 solution containing 2.6% KOH, 1% Brij-35 (Sigma) and 0.3% 2-mercaptoethanol. The resulting fluorescent amines were detected with a fluorescence spectrophotometer (Merck-Itachi F1000) at room temperature.

2.3. Statistical analysis

Means of paired data were compared using the *T*-test. Multiple mean comparisons (using Scheffé's contrasts) were only performed after assessing the homogeneity of variances using the Bartlett test and obtaining a significant result with ANOVA. Asterisks indicate statistical significance between groups of values ($^{*} = P < 0.05$ and $^{**} = P < 0.01$).

3. Results and discussion

3.1. BA occurrence in beer sample categories

Beer samples analysed in the present study ranged into four fermentation types (LF: low fermentation; TF: top fermentation; TF + BSF: top fermentation followed by a second fermentation into bottle and SF: spontaneous fermentation). The HPLC determination method used in this study allowed us to simultaneously detect nine amines in each sample. Contrary to what was reported by Izquierdo-Pulido et al. (1996a, 1996c), SPM and SPD were detected in none of the analysed samples. By contrast, PUT and AGM presented a fairly constant concentration in all samples (Fig. 1). Considering the inability of yeasts used in the low fermentation process to produce BA (Izquierdo-Pulido, Font-Fabregas, & Vidal-Carou, 1995, 1996a), together with the stability of the AGM/PUT ratio within LF and TF beers (i.e. ± 1.45), one could consider that both amines are natural components brought to the beverage by raw material, as reported earlier (Izquierdo-Pulido et al., 1996a; Kalac & Krizeck, 2003).

It is, however, noteworthy that putrescine concentration of SF beers (14.0 ± 10.4 mg/l, leading to a AGM/PUT ratio mean value of 0.33) was significantly higher than that of other fermentation types. In this case, one part of the PUT amount could stem from the decarboxylating activity of contaminant bacteria during brewing. In agreement with this hypothesis, SF beers also exhibited the highest levels of other amines of bacterial origin, namely CAD, HIS and TYR (Fig. 2). The vasoactive amine TYR (hypertensive) is the major BA in all these beer samples with a mean concentration

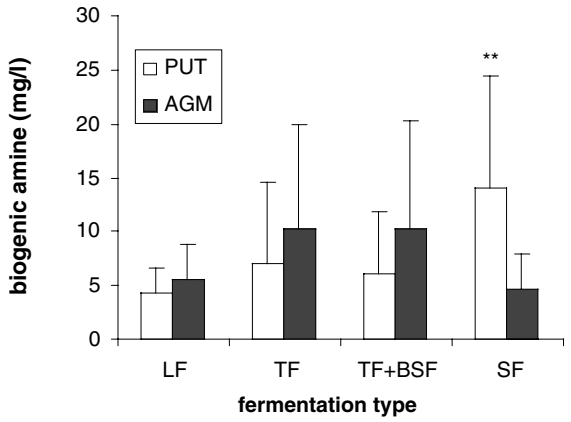

Fig. 1. Distribution of putrescine (PUT) and agmatine (AGM) in beer samples ($n = 280$) from four fermentation types (LF: low fermentation, $n = 18$; TF: top fermentation $n = 36$; TF + BSF: top fermentation followed by a refermentation in bottle $n = 184$; SF: spontaneous fermentation, $n = 42$). Results are expressed as mean values ± standard errors for each fermentation category. Note the significantly higher amount of PUT in SF beers, likely resulting from decarboxylating activity of putrefactive bacteria during the fermentation process.

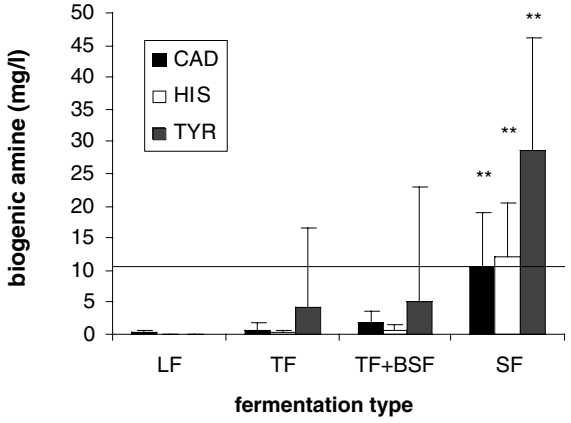

Fig. 2. Distribution of cadaverine (CAD), histamine (HIS) and tyramine (TYR), three typically putrefactive amines, in beer samples ($n = 280$) from four fermentation types (LF: low fermentation, $n = 18$; TF: top fermentation $n = 36$; TF + BSF: top fermentation followed by a secondary fermentation in bottle $n = 184$; SF: spontaneous fermentation, $n = 42$). Results are expressed as mean values ± standard errors for each fermentation category. Note the high amount of each amine (> to the upper level generally considered as safe for the consumer, i.e. 10 mg/l) in spontaneously fermented beers.

value (28.7 ± 17.3 mg/l), largely exceeding the upper limit generally considered to be safe for consumers (i.e. 10 mg/l). Histamine, another vasoactive amine (hypotensive), is also found in high concentration (11.9 ± 8.61 mg/l) in SF beers. Interestingly, not all SF breweries were shown to produce beverages with alarming levels of histamine and tyramine (Fig. 3), an observation that underlined the crucial role of local manufacturing practices in the quality of the final product. It is also interesting to note that, regardless of the final amount of

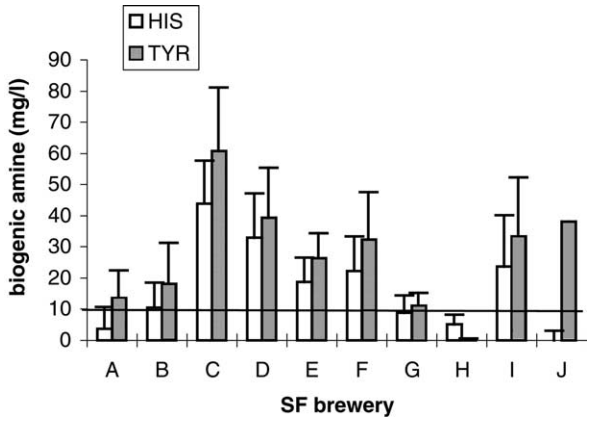

Fig. 3. Comparison of vasoactive amines (HIS and TYR) in samples of SF beers from 10 different breweries (A–J). Results are expressed as mean values ± standard error for each brewery (n was respectively, = 4, 3, 3, 6, 5, 9, 5, 3, 5, and 3 for breweries A–J).

both vasoactive amines, the TYR/HIS ratio was constant (1.51) between breweries (with the exception of breweries H and J), suggesting the intervention of a common decarboxylating micro-organism in each brewery.

Besides SF beers, top fermentation samples (TF and TF + BSF) showed significant amount of tyramine that could in some cases exceed the 10 mg/l threshold value mentioned above. To better characterize the BA content of those few samples, we separately considered beers that exhibited high level (i.e. above 10 mg/l) of either histamine or tyramine (21 samples out of 220 of TF and TF + BSF samples taken together). Among those selected TF samples, beers ranged into two groups (Fig. 4): The first, presenting the typical acid value (pH

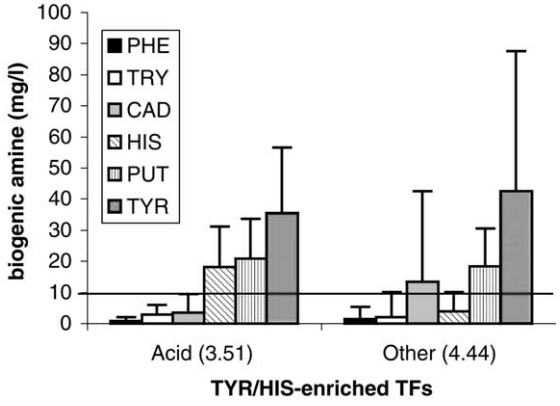

Fig. 4. Distribution of β-phenylethylamine (PHE), tryptamine (TRY), cadaverine (CAD), putrescine (PUT), histamine (HIS) and tyramine (TYR), in selected TFs (i.e. either SF or SF + BSF samples showing amounts of HIS or TYR > 10 mg/l). Results are expressed as mean values ± standard errors ($n = 5$ for acidic TF, $n = 16$ for other TF). Note these samples are, compared to the total number of analysed samples (280), the only ones to exhibit detectable levels in PHE and TRY.

3.51 ± 0.27; "acid TFs") of SF beers (pH 3.62 ± 0.23) was also characterized by a TYR/HIS ratio (1.95) close to that of SF beer (1.51), while the second group presented less acid TFs (pH 4.44) showing a reversed proportion of cadaverine and histamine and consequently a different TYR/HIS ratio (10.9). This suggested that BA did not originate from the same contamination in both cases. Finally, it is also important to note that these samples were the only ones to present low amounts of two other health-threatening amines, namely, β-phenylethylamine (PHE, 0.85–1.45 mg/l) and tryptamine (TRY, 2.96–2.03 mg/l).

While experimental evidence has clearly established that there is no relationship between commercial yeast (*Saccharomyces cerevisae var. uvarum*) counts and tyramine formation (Izquierdo-Pulido et al., 1995, 1996b), there is a correlation between the production of this vasoactive amine and the presence of lactic acid bacteria (*Pediococcus* spp.) during beer fermentation, so that tyramine levels in beer could be considered as a reliable indicator of the contamination degree by *Pediococcus* spp. during beer fermentation (Izquierdo-Pulido, Font-Fabregas, Carceller-Rosa, Marine-Font, & Vidal-Carou, 1996b, 1997). In agreement with the probable microbial origin of tyramine, histamine, cadaverine and some of the putrescine in SF beers (and a few TF beers), decarboxylating micro-organisms have been reported during the four steps of the spontaneous fermentation process (or lambic fermentation). More precisely, enteric bacteria predominate during the first stage of lambic fermentation, while lactic and acid bacteria occur during the third fermentation step (for review, see De Keersmaecker, 1996). No such bacteriological studies have been performed for Belgian BA-enriched TFs beers. However, on the basis of either their low pH value or their SF-like BA distribution profile, it could be postulated that the fermentation process leading to these beers has been done under poor hygienic conditions.

Considering this aspect, our results have then been exploited to define a "Beer biogenic amine index" or Beer BAI that would allow assessment of the quality of the production process.

3.2. Defining a biogenic amine index for beers

We firstly used the Mietz and Karmas index (M&K BAI) initially developed to qualify the freshness and/or the bacteriological quality of solid foodstuffs (Mietz & Karmas, 1977). Using this BAI calculation (Table 1), most beer samples presented a BAI value corresponding to a moderate contamination of the brewing process by decarboxylating bacteria (M&K BAI between 1 and 10) while only 15 beer samples out of 280 appeared to be contaminant-free (M&K BAI <1, Table 1). In accordance with their high BA content, SF beers also exhibited the highest M&K BAI, which indicated a heavy contamination of the brewing process by decarboxyalting micro-organisms. Surprisingly, though the yeasts used to produce LF beers cannot synthesize BA, the calculated M&K BAI value indicated that just one LF beer sample seemed to come from a bacteria-free brewing. Consequently, the BAI, defined according to Mietz and Karmas, needs to be adapted to really reflect the quality of beer manufacturing regarding the accidental contamination by biogenic amine-producing bacteria.

Table 1
Beer samples distribution according the fermentation type (four types comprising 280 samples) and the BAI value category

BAI value	Fermentation category			
	LF	TF	TF + BSF	SF
M&K BAI[a]				
0–1	1	0	14	0
1–10	17	25	137	4
>10	0	9	33	38
Beer BAI[b]				
0–1	15	24	133	0
1–10	3	12	42	17
>10	0	0	9	25

Three indices have been considered, namely (1) the BAI defined according to Mietz and Karmas or M&K BAI, (2) the M&K BAI corrected for tyramine or M&K BAI + TYR and (3) the BAI proposed for beers from results of this study or Beer BAI.

[a] (CAD + PUT + HIS)/(1 + (SPD + SPM)).

[b] (CAD + HIS + TYR + PUT + PHE + TRY)/(1 + AGM).

Indeed, the M&K BAI formula does not take tyramine into account, which is a serious default considering the predominance of this vasoactive amine in beers. It is also important to note that the M&K BAI formula corresponds to the ratio of amines of bacterial origin to natural amines found in uncontaminated products. In the case of fish, meat and vegetables, the natural amines are essentially spermine (SPM) and spermidine (SPD). While these two amines were not detected in beer samples, our results suggested that we should rather consider PUT and AGM as the naturally occurring amines in beers (Fig. 1). However, contaminant bacteria likely produce only part of the PUT amount found in these beers. Furthermore, owing to the known potentiator effect of PUT on the vasoactive action of HIS (for review, see Shalaby, 1996), one cannot totally exclude this amine from the bulk of health-threatening BA in the formula. Consequently, the Beer BAI calculation formula we propose here consisted of the ratio of BA of bacterial origin (i.e. TYR, PUT, CAD, HIS, PHE and TRY) to the natural BA found in the malt (AGM):

$$\frac{[\mathrm{CAD}] + [\mathrm{HIS}] + [\mathrm{TYR}] + [\mathrm{PUT}] + [\mathrm{PHE}] + [\mathrm{TRY}]}{1 + [\mathrm{AGM}]},$$

where each BA concentration is expressed in mg/l.

Using this formula we probably overestimate BAI values in LF beers, as well as in most of TF beers, since the totality of their PUT content is likely introduced by the raw material. Nevertheless, the calculated Beer BAI value for LF beers (0.84 ± 0.89) corresponded to high quality products according to criteria defined by Mietz and Karmas (1977), which is in agreement with the known uncontaminated LF brewing process.

It is important to note that, when applying the Beer BAI formula proposed in the present study to all analysed samples, it appeared (Table 1) that most of LF and TF beers (including TF and TF + BSF) fell in the category of superior quality products. Among those samples, the few presented separately in Fig. 4 (because they showed alarming amounts of either histamine or tyramine) were characterized as having significantly different Beer BAI values. Indeed, the acid group of BA-enriched TFs (pH 3.51, Fig. 4) exhibited a mean Beer BAI value of 6.66 ± 4.70, while the other group of BA-enriched TF (pH 4.44, Fig. 4) presented a Beer BAI mean value (11.2 ± 26.5) reflecting a poor hygienic quality of the fermentation process. These TF samples were also the only ones to exhibit substantial amounts of two other health-perturbing amines, TRY and PHE, a reason to include these amines in the Beer BAI calculation formula.

Similarly to what has been done with the M&K BAI, one could consider that beers showing a Beer BAI value, (i) lower than 1.0 have been produced by a non-contaminated fermentation process (high microbiologic quality), (ii) between 1.0 and 10.0, have been produced by fermentation procedures that could be moderately contaminated by decarboxylating bacteria (intermediate level of microbiological quality) and (iii) higher than 10.0, result from a fermentation procedure that was highly contaminated by amine-producing bacteria (poor microbiological quality). The mean Beer BAI value of SF beers (16.2 ± 13.9, Fig. 5) largely exceeded the threshold value of intermediate quality ($\leqslant 10.0$). As most of these beers contained high amounts of CAD (10 mg/l as mean value), HIS (10 mg/l as mean value) and TYR (28 mg/l as mean value; Fig. 2), their mean BAI value is a good indicator of contamination levels (by enteric and lactic acid bacteria), leading to the accumulation of health threatening amounts of vasoactive amines. Therefore a Beer BAI above 10.0 shows possible amine-related health troubles following the ingestion of BA-enriched drink by high risk consumers (i.e. people presenting a deficiency of their amine detoxification system as a result of either a treatment with a medication inhibiting the amine-oxidases or a genetically acquired inefficiency of this system). In this regard, establishment of a food intolerance data bank, like the one proposed in Germany (Diel et al., 1997), should help high risk consumers to select foodstuffs that are suited for their physiopathological state.

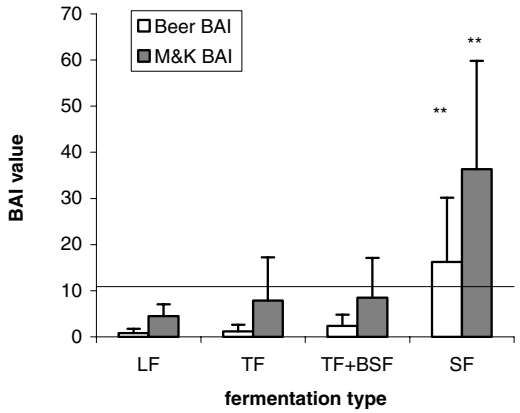

Fig. 5. Comparison of Mietz and Karmas BAI (M&KBAI) and beer biogenic amine index (Beer BAI) values in beer samples ($n = 280$) from four fermentation types (LF: low fermentation, $n = 18$; TF: top fermentation $n = 36$; TF + BSF: top fermentation followed by a refermentation in bottle $n = 184$; SF: spontaneous fermentation, $n = 42$). Results are expressed as mean values ± standard errors for each fermentation category.

Alternatively, the fermentation procedure could be improved, to give an amine-depleted finished product. For instance, it seems that it could be possible to reduce the BA level in spontaneously fermented beers by up to 95% by assembling the freshly cooled wort with old beer containing lactic acid bacteria which have lost their decarboxylating activity (Gasari et al., 2003). Another attractive possibility is the use of fermenting microorganisms that would also exhibit an amine-oxidase activity, as suggested by recent investigations of Gardini, Martuscelli, Crudele, Paparella, and Suzzi (2002) on the role of selected strains of fermentation starters on the BA accumulation in sausages.

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