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Research Note

Histamine and Tyramine in Beers. Changes During Brewing of a Spanish Beer

ABSTRACT

A preliminary study of the evolution of histamine and tyramine during a brewing process was carried out. A clear formation of tyramine was observed during the main fermentation (from 2·5 to 40·5 mg/litre), but histamine formation was negligible in this same period. Good correlations ($p < 0{\cdot}001$) were found between formation of tyramine and alcoholic content, and between this amine and apparent extract during the main fermentation. In addition, a statistical comparison was done of the biogenic amine contents in 64 samples corresponding to the four types of beer established by the Spanish law on the basis of their extract of original wort and their alcoholic content. No significant statistical differences were found between the histamine and tyramine contents in the four types of beer ($p = 0{\cdot}05$).

INTRODUCTION

Histamine and tyramine can be formed in foods by microbial decarboxylation of their precursor amino acids (histidine and tyrosine). High amounts of these amines in foods can cause direct toxic effects such as 'histaminic intoxications' (Taylor, 1985) and food-induced migraines (Crook, 1981), and also indirect toxic effects such as interaction with monoamine-oxidase inhibiting (MAOI) drugs (Ponto *et al.*, 1977; Murray *et al.*, 1988). Zee *et al.* (1981) reported that these substances in beers are a toxicological problem affecting beer consumers in Canada. Moreover, relationships between amine contents and hygienic defects during food manufacturing, and their possible

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role as 'indicators' of poor-quality raw materials have also been reported (Rice & Koehler, 1976; Ramantanis *et al.*, 1984). Biogenic amines in beers could be related to yeast activity and/or to microbial contaminations (*Lactobacillus*, *Pediococcus*,...) during brewing and/or storage of beers (Steiner & Laezlinger, 1978; Chen & Van Gheluwe, 1979; Zee *et al.*, 1981; Cerutti *et al.*, 1985). At the moment, no contents or relationships have been conclusively reported to establish whether a brewing process has been correct based on the final amine contents in beer, but Thalaçker (1982) considers histamine as an 'undesirable' component of beers, and Cerutti *et al.* (1985) indicate that it would be necessary to include biogenic amine determinations in the quality control programme of breweries.

In this work, data are provided regarding histamine and tyramine evolution during a real brewing process of a Spanish beer. A survey was done to determine the stage of brewing when these amines were produced, and to establish relationships with apparent extract and alcoholic content. Likewise, histamine and tyramine levels in 35 beers, 20 special beers, five special extra beers and four nonalcoholic beers were compared.

MATERIALS AND METHODS

Samples

Samples corresponding to various stages of brewing were supplied by a Spanish brewery. They were passed through a sterilizing filter (0·22 μm) and later stored at -18°C until analysed. Ten samples were collected: the first sample was wort, four samples corresponded to main fermentation, four samples corresponded to secondary fermentation, and the last one was final product: filtered, pasteurized, bottled and ready for sale.

Sixty-four samples of beers all acquired in the Spanish market, were studied. Samples were stored at -18°C just prior to carrying out the analysis.

Analytical methods

Histamine was determined according to the spectrofluorometric method of Vidal-Carou *et al.* (1989). This method consists of the following steps: (a) alkalinization of the sample and extraction with *n*-butanol; (b) histamine transference to 0·1 M HCl; (c) formation of the fluorescent complex with *o*-phthalaldehyde; (d) spectrofluorometric reading at 350 nm emission and 430 nm excitation. Its reliability for beer was tested in terms of precision (CV = 7·65%, for a content of $0{\cdot}85 \pm 0{\cdot}065$ mg/litre, $n = 8$), and recovery ($93{\cdot}40\% \pm 2{\cdot}75$, $n = 24$, after addition of 5, 10 and 15 μg of histamine).

Tyramine was determined according to the spectrofluorometric method proposed by Rivas-Gonzalo *et al.* (1979). This method consists of the following steps: (a) alkalinization of the sample and mixing with grain sand and anhydrous sodium sulphate; (b) extraction with ethyl acetate in a glass column; (c) tyramine transference to 0·2M HCl; (d) formation of the fluorescent complex with α-nitroso-β-naphthol; (e) spectrofluorometric reading at 450 nm emission and 540 nm excitation. Its reliability was also verified for precision (CV = 4·65%, for a content of $16{\cdot}50 \pm 0{\cdot}75$ mg/litre, $n = 8$), and recovery (94·45% $\pm$ 2·60, $n = 24$, after addition of 10, 20 and 30 μg of tyramine).

Alcoholic content, extract of original wort (or also 'original gravity', the percentage of extract (w/w) including sugars, corresponding to wort) and apparent extract (the specific gravity or density of beer expressed in % w/w) were determined automatically using a Technicon Beer Analyzer (Technicon International Division, 1979). The alcohol is determined thermometrically in one channel of the apparatus and the beer density is determined in another channel of this system by a Paar density meter. The extract of original wort is calculated automatically from the alcoholic content and density by a system computer.

RESULTS AND DISCUSSION

A preliminary study of the evolution of biogenic amines during the brewing process was carried out. Histamine, tyramine, alcoholic content and apparent extract were determined in samples corresponding to different stages of a brewery process. The evolution of the two amines was very different during this process (Fig. 1). Initial levels of histamine and tyramine found in the wort were 0·6 mg/litre and 2·5 mg/litre, respectively. Histamine

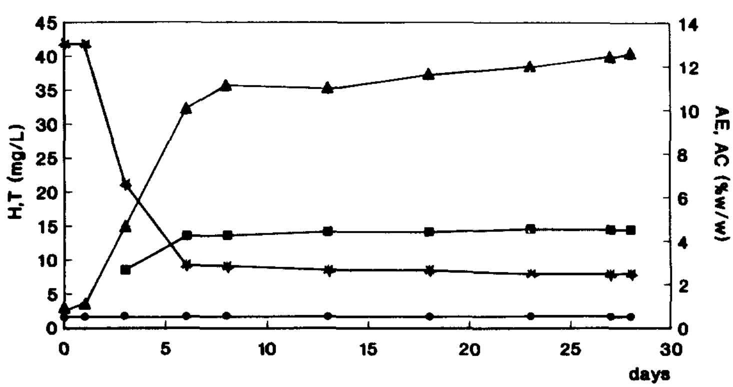

Fig. 1. Changes of (●) histamine (H), (▲) tyramine (T), (★) apparent extract (AE), and (■) alcoholic content (AC), during a real brewing process of a Spanish beer.

and tyramine in wort may derive from the raw materials (Chen & Van Gheluwe, 1979; Narziss *et al.*, 1984*a*,*b*; Wackerbauer & Toussaint, 1984). Formation of histamine was negligible throughout the entire process, from 0·6 to 0·8 mg/litre. On the other hand, a clear formation of tyramine was observed, from 2·5 mg/litre in the wort to 40·0 mg/litre in the final product. The greatest formation of tyramine was in the first 5 days of manufacturing, coinciding with the greatest intensity of alcohol production. A significant statistical correlation ($p < 0{\cdot}001$) between tyramine and alcoholic contents ($y = 0{\cdot}13x + 0{\cdot}13$; $r = 0{\cdot}9784$) was found during the main fermentation. A significant statistical correlation ($p < 0{\cdot}001$) was also found between the decrease in apparent extract and tyramine ($y = 0{\cdot}31x + 14{\cdot}30$; $r = 0{\cdot}9771$). Both correlations indicated that tyramine formation was associated with the fermentation in the studied elaboration process, but do not necessarily imply that yeasts responsible of alcoholic fermentation are also producers of tyramine, since it is possible that other microorganisms can be involved.

A much more gradual increase in tyramine was observed during secondary fermentation, from 35·7 to 40·5 mg/litre. The final filtration had practically no influence on the level of either amine (last two data points on the graph of Fig. 1).

Table 1 shows the ranges of histamine and tyramine contents, the mean values and the standard deviations in the four types of beers established by the Spanish law on the basis of their extract of original wort and their alcoholic content (Ministerio de la Presidencia de Gobierno, 1984). The ranges we obtained and the Spanish legal limits for these analytical parameters are also included. Tyramine ranged from 1·55 to 30·60 mg/litre whereas histamine contents were always lower (in all samples less than 5 mg/litre). Other authors have also found levels of tyramine greater than of histamine (Zee *et al.*, 1981; Ramantanis *et al.*, 1984; Wackerbauer & Toussaint, 1984). By analysis of variance (Table 1), it was verified that there were no significant statistical differences ($p = 0{\cdot}05$) between the concentrations of the amines in the four types of beer considered. Thus the alcohol–tyramine correlation found during the main fermentation was not observed in finished beers from the market. Chen and Van Gheluwe (1978) reported a relationship between histamine content and extract of original wort but this relationship was not observed in samples we studied.

At present, reasons for the different biogenic amine contents in beers do not appear to be very clear. This work shows a great variability in tyramine content in beers from the market and also a clear production of this amine during the main fermentation of a Spanish beer. On the basis of these results, it will be necessary to check the influence of raw material quality and/or possible microbial contaminations in further studies.

TABLE 1
Ranges of Histamine, Tyramine, Alcoholic Content and Extract of Original Wort in the Four Types of Beers established by Spanish Law

Type of beer	*Number of samples*	*Alcoholic content (% v/v)*	*Extract original wort (% w/w)*	*Histamine (mg/litre)*	*Tyramine (mg/litre)*
Beer	35	4·1–5·6	11·1–12·6	0·80 ± 1·80[b]	4·66 ± 5·22[b]
		(4·5–5·5)[a]	(11–13)[a]	(0·35–1·80)[c]	(1·55–30·60)[c]
Special beer	20	5·5–6·5	12·9–15·1	1·17 ± 1·35	7·68 ± 7·72
		(5·5–6·5)	(13–15)	(0·30–4·90)	(1·90–24·75)
Special extra beer	5	6·6–7·9	15·2–17·3	1·49 ± 0·38	4·23 ± 2·14
		(>6·5)	(>15)	(0·90–1·75)	(2·60–7·35)
Non-alcoholic beer	4	0·1–1·0	5·7–6·4	0·38 ± 0·09	3·48 ± 1·17
		(<1·0)	(—)	(0·30–0·50)	(2·10–4·95)
Analysis of variance:	DF = 3, 61; $p = 0{\cdot}05$;	$Ft = 2{\cdot}76$	F(histamine): 2·25 F(tyramine): 1·26		

[a] Spanish law ranges.
[b] Mean and standard deviation.
[c] Range.

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