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Effect of tyrosine on tyramine formation during beer fermentation

Maria Izquierdo-Pulido *, Abel Mariné-Font, M. Carmen Vidal-Carou

Departament de Nutrició i Bromatologia-CeRTA, Facultat de Farmàcia, Avg. Joan XXIII, s/n, 08028 Barcelona, Spain

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Abstract

Changes in tyramine, in its precursor amino acid tyrosine, and in *Pediococcus* spp. were followed during 54 beer fermentations. No statistical relationship between tyramine production and wort tyrosine levels was found, either between the amount of tyramine formed or the decrease in tyrosine through beer fermentation. However, tyramine formation significantly correlated with *Pediococcus* spp. contamination ($r = 0.876$; $P < 0.001$, $n = 54$). Higher initial tyrosine levels did not induce greater tyramine formation when a similar degree of contamination by *Pediococcus* spp. occurred, either between tyrosine decrease and tyramine formation. Special attention is required in controlling the presence of lactic acid bacteria to minimise the formation of tyramine. However, the amount of tyrosine does not seem to be a critical factor in the tyramine formation during beer fermentation.

Keywords: Tyramine; Tyrosine; Lactic acid bacteria; *Pediococcus* spp; Beer; Biogenic amines

1. Introduction

Undesirable effects after consumption of food products with high contents of biogenic amines, such as histamine and tyramine, have been reported (Stratton, Hutkins & Tailor, 1991). Histaminic intoxications have not been described for beers; however, the appearance of symptoms of severe hypertensive crisis has been described in patients taking classical monoamine oxidase inhibitors (MAOI) after drinking beer containing relatively high amounts of tyramine (Murray, Walker & Doyle, 1988; Shulman, Tailor, Walker & Gardner, 1997; Tailor, Shulman, Walker, Moss & Gardner, 1994).

Production of beer fulfills the three factors that govern the formation of amines (Rice, Eitenmiller & Koehler, 1976). Free precursor amino acids are available, micro-organisms with amino acid decarboxylating activity can occur, and favourable conditions for the growth of micro-organisms can be found. Several studies have reported the formation of biogenic amines, such as tyramine, putrescine, cadaverine, and histamine during beer fermentation (Cerutti, Finoli & Vecchio, 1989; Izquierdo-Pulido, Font-Fábregas, Carceller-Rosa, Mariné-Font & Vidal-Carou, 1996; Zee, Simard, Vaillancourt & Boudreau, 1981). Thus, high levels of biogenic amines have been related to the presence of contaminant micro-organisms, usually species of lactic acid bacteria (i.e. *Lactobacillus* and *Pediococcus*) (Donhauser, Wagner & Geiger, 1993; Izquierdo-Pulido et al., 1996; Zee et al., 1991) since brewer's yeast is unable to form biogenic amines (Izquierdo-Pulido, Font-Fábregas & Vidal-Carou, 1995; Zee et al., 1991).

Little information is available on the relationship between precursor amino acids and the formation of their corresponding amines, even though amino acids have been described as critical factors for amine formation. Eitenmiller, Koehler and Reagan (1978) and Joosten and Stadhouders (1987) concluded from studies performed on sausages and cheese, respectively, that high levels of tyrosine may induce tyramine formation. In beers, Chen and Van Gheluwe (1979) reported that whereas some uptake of histidine was noticed, histamine wort levels remained practically unchanged.

Previous work carried out in our laboratory (Izquierdo-Pulido et al., 1996) demonstrated that lactic acid bacteria contamination, which was identified as species of *Pediococcus* spp., was the main responsible factor for tyramine formation during beer fermentation. Since no previous data were reported about the relationship between tyramine and its precursor amino acid tyrosine during beer fermentation, the aim of the

* Corresponding author. Tel.: +34-934-03-59-30; fax: +34-934-03-59-31.

E-mail address: izquier@farmacia.far.ub.es (M. Izquierdo-Pulido).

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present study was to find out whether a relationship between tyramine formation and its precursor amino acid tyrosine could be established. Special attention was given to studying the influence of tyrosine when a similar degree of contamination with *Pediococcus* spp. occurred. This is the first time that the actual influence of a precursor amino acid on amine formation during beer fermentation is reported.

2. Material and methods

The beer studied was a lager, Pilsner, and a bottom brewer's yeast (*Saccharomyces cerevisiae* var. *uvarum*) was used for all fermentations. All samples were supplied by a Spanish brewery.

2.1. Samples

Fifty-four industrial beer fermentations were followed. Samples were obtained every day during the 6 or 7 days of fermentation. Samples were aseptically drawn, collected in sterile flasks, and immediately sent to the laboratory under refrigeration. One fraction was kept in sterile flasks for immediate microbial analysis, and the other was centrifuged at 4000 rpm for 30 min at 4°C. The supernatant was stored at −20°C for tyrosine and tyramine analyses.

2.2. Tyramine and tyrosine determination

Tyramine determination was done following an HPLC procedure (Izquierdo-Pulido, Vidal-Carou & Mariné-Font, 1993) The method is based on the formation of ion pairs between the biogenic amines and octanesulphonic acid present in the mobile phase. Their separation is carried out through a reverse phase column. A post-column derivatization with *o*-phthalaldehyde is followed by spectrofluorimetric detection. Tyrosine determination was carried out according to the method developed by Rivas, Font and Mariné (1981). The method consists of an extraction of tyrosine from the sample in an ion exchange resin Dowex 50Wx4 (50–100 mesh) with 2N NH_4OH. The eluate was evaporated to dryness and dissolved in 0.2N HCl. Finally, a fluorescent complex between tyrosine and α-nitroso-β-naphtol was formed, and a spectrofluorometric reading at 450 nm/540 nm was made.

2.3. Microbial analysis

Pediococcus spp. were counted on NBB agar plates (Nachweis Medium für bierschädliche Bakterien, Döhler, Darmstadt, Germany) (Back, 1980; Dachs, 1981). One hundred microliters of a sample, or of a dilution in Ringer solution, was spread and incubated at 28°C for up to 14 days under anaerobic conditions. Duplicate plate counts were obtained throughout.

2.4. Statistical analysis

Correlation coefficients were calculated to assess the relationship between tyramine formation, tyrosine contents and *Pediococcus* spp. counts. All analyses were performed with the statistics package SPSS for Windows 6.0.1 (SPSS Inc. Chicago, IL).

3. Results and discussion

The tyrosine contents found in worts fluctuated from 100.0 to 205.0 mg/l. Batalla and Torrent (1990) pointed out that the amino acid composition of the wort depends upon the barley variety and the conditions of malting and mashing steps. Worts studied were obtained from malts of the same barley variety. Therefore, differences in tyrosine contents seem to be related to characteristics of each batch during malting and mashing.

In all the monitored fermentations, initial tyrosine levels decreased as fermentation progressed, but the decreases varied greatly between the distinct fermentations studied. Final levels ranged from 17.8 to 165.5 mg/l. Thus, in some fermentations 20% of the tyrosine of the wort disappeared during the process, while in others, the percentage was about 70%. An explanation of this variability could be that the brewer's yeast takes up amino acids in an orderly manner. At the beginning of fermentation, arginine, aspartic acid, asparagine, glutamic acid, glutamine, lysine, serine, and threonine are used rapidly. The other amino acids, among them tyrosine, are taken up only slowly, or not until later stages (Hardwick, 1995). In those fermentations, tyramine formation fluctuated from less than 1 to 25 mg/l (Table 1). Production of levels of tyramine above 5 mg/l were observed in 59% of the fermentations followed, while small amounts of tyramine were formed in 19% of the processes.

Table 1
Distribution of beer fermentations as a function of the tyramine formation (mg/l) and the levels of tyrosine in wort (mg/l)

	Tyramine formation[a]			
Tyrosine in wort	No formation[b]	1.0–5.0	5.1–15.0	15.1–25.0
100.0–135.0 ($n=22$)	3[c]	4	9	6
136–170.0 ($n=18$)	4	3	7	4
171.0–205.0 ($n=14$)	5	3	4	2
Total ($n=54$)	12	10	20	12

[a] Expressed as final value (found in beer) minus initial value (found in wort).
[b] Less than 1 mg/l.
[c] Number of fermentations.

Table 2
Tyramine formation (mg/l), levels of tyrosine (mg/l) in wort, and tyrosine decrease (mg/l) during beer fermentation as a function of the degree of contamination by *Pediococcus* spp. (CFU/ml)

				Coefficents of correlations and degree of significnace[c]	
Pediococcus spp.	Tyramine formation[a]	Tyrosine in wort	Tyrosine decrease[b]	Tyramine/tyrosine in wort	Tyramine/decrease tyrosine
4×10^3–1×10^4 ($n=12$)	3.5 (0.9)[c]	155.8 (22.2)	71.9 (18.5)	$R=-0.485$ ($P>0.05$)	$R=0.358$ ($P>0.05$)
1×10^4–1×10^5 ($n=21$)	9.2(4.1)	146.7(26.2)	60.2(14.3)	$R=-0.375$ ($P>0.05$)	$R=0.375$ ($P>0.05$)
1×10^5–1×10^6 ($n=10$)	20.4 (3.7)	138.3 (27.3)	70.1 (23.4)	$R=-0.596$ ($P>0.05$)	$R=0.457$ ($P>0.05$)

[a] Expressed as final value found in beer minus initial value found in wort.
[b] Expressed as initial value found in wort minus final value found in beer.
[c] Mean and standard deviation.

To study relationships between tyramine formation and its precursor amino acid, correlation coefficients were calculated between levels of tyrosine in wort and tyramine formation, and also between tyramine formation and tyrosine decrease during beer fermentation. No statistically significant relationship was found between tyramine production and tyrosine decrease ($r=0.096$, $P>0.05$, $n=54$). In other words, a higher tyramine production was not related to a higher decrease in tyrosine. Although the correlation coefficient between tyramine formation and the levels of tyrosine in wort was statistically significant ($r=-0.410$; $P<0.05$; $n=54$), the coefficient of determination value (r^2) was low (16.8%). Therefore, a definitive relationship cannot be established between tyrosine (in wort) and tyramine. The sign of this correlation was negative, which could imply that lower levels of tyrosine are associated with higher tyramine formation. No previous explanation has been found for this observation, since the few studies done on the relationship between precursor amino acid and amine production have shown the opposite trend. Thus, Joosten and Stadhouders (1987) reported that amino acid decarboxylases are inducible enzymes by high levels of substrate.

From previous studies (Izquierdo-Pulido et al., 1996) in the same brewery, contamination by *Pediococcus* spp., mainly *Pediococcus damnosus*, was critical affecting the tyramine formation during fermentation. Therefore, microbial analyses were also carried out in this study to identify the possible role of tyrosine when contamination with *Pediococcus* spp. is present. First, we found that the relationship between *Pediococcus* spp. and tyramine formation yielded a high correlation coefficient ($r=0.876$, $P<0.001$, $n=54$) with a r^2 of 77%. Therefore, lower or higher tyramine formation could be explained by the degree of contamination with *Pediococcus* spp. during fermentation. Thus, no tyramine formation was detected in fermentations showing *Pediococcus* spp. counts lower than 4×10^3 colony forming units (CFU)/ml. When *Pediococcus* spp. counts ranged from 4×10^3 CFU/ml to 1×10^4 CFU/ml, a slight tyramine formation occurred (Table 2), while higher tyramine levels (20.4 ± 3.7 mg/l) were reached in those fermentations with higher *Pediococcus* spp. counts.

To study whether higher tyrosine levels in wort and higher tyrosine decreases were related to the production of tyramine when a similar degree of contamination with *Pediococcus* spp. occurred, correlation coefficients were calculated between these variables for each level of contamination. None of these coefficients were significant, as shown in Table 2. Consequently, higher levels of tyrosine in wort may not induce higher tyramine formation during fermentation. Likewise, a higher decrease in tyrosine is not related to higher tyramine production for a similar degree of contamination. Finally, tyrosine levels between 17.8 and 165.5 mg/l were found in all the beers (final products), which suggests that sufficient amounts of tyrosine were present in wort to meet yeast requirements and, eventually, to be substrate for decarboxylase enzymes to produce tyramine. Therefore, our results suggest that it is difficult to establish a direct relationship between tyramine and tyrosine. In wines, Buteau, Duitschaever and Ashton (1984) pointed out that the metabolic pathways for the biogenesis of amines are very intricate and more than one compound could give rise to the same end-product. Likewise, a single compound could be the source of many end-products.

In conclusion, we would like to emphasize that to minimise the presence of tyramine in this type of beer, special attention is required in controlling the presence of lactic acid bacteria contamination, in our case *Pediococcus* spp., regardless of the amount of free tyrosine present in the wort.

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