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Influence of *Saccharomyces cerevisiae* var. *uvarum* on histamine and tyramine formation during beer fermentation

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Data are provided on the effect of brewer's yeast on the formation of histamine and tyramine during beer fermentation in pilot plant. Biogenic amines were determined by spectrofluorometric methods. *Saccharomyces cerevisiae* var. *uvarum*, a bottom yeast, did not produce histamine or tyramine during fermentation. Yeast recycling did not influence biogenic amines formation.

INTRODUCTION

Toxic effects after consumption of food products with high contents of biogenic amines, such as histamine and tyramine, have been reported (Stratton *et al.*, 1991). Among them, the best known are the histaminic intoxications (Taylor, 1985) and the hypertensive crisis due to the interaction between these amines and monoamine-oxidase inhibitor (MAOI) drugs (Lippman & Nash, 1990). Histaminic intoxications have not been described for beers; however, the appearance of symptoms of a severe hypertensive crisis were described in a patient taking a MAOI drug (tranylcypromine) after drinking 250 ml of an Irish beer (Murray *et al.*, 1988). It has been strongly recommended that beers should be included among the prohibited foodstuffs for patients on MAOIs (D'Arcy, 1988).

In addition to toxicological implications, biogenic amines have been proposed as indices of defective food manufacturing processes related with poor sanitary conditions (Cerutti, 1989; Hálasz *et al.*, 1994). Cerutti *et al.* (1989) suggested that it is possible to obtain beers with low amine levels using good quality raw materials and applying suitable technologies. Several authors have concluded that these amines are mainly formed through the decarboxylation of their precursor amino acids during beer fermentation (Narziss *et al.*, 1984*a*,*b*; Cerutti *et al.*, 1989; Izquierdo-Pulido *et al.*, 1991). However, it is still not clear whether amine production is a result of yeast fermentative activity, or whether contaminant microorganisms, such as *Lactobacillus* and *Pediococcus*, are the actual mediators (Zee *et al.*, 1981; Hálasz *et al.*, 1994). Literature reports of yeast effects on biogenic amines formation during brewing are scarce. Chen and Van Gheluwe (1979) and Zee *et al.* (1981) studied the influence of *Saccharomyces uvarum* (a top or ale yeast); however, we are not aware of research done with bottom or lager yeasts. The aim of our work was to study the influence of *Saccharomyces cerevisiae* var. *uvarum*, a bottom fermentative yeast, on the production of amines during beer fermentation. An additional objective was to check whether yeast recycling (a normal brewing practice) played any role in the biogenesis of those amines. The present study was done in a pilot plant using glass fermenters. Working on a small scale allowed us to control different factors, including uniformity of sampling, and to work with pure yeast and sterile wort, avoiding possible microbial contamination.

MATERIALS AND METHODS

Fifteen pilot-scale fermentations were conducted using EBC-style glass fermenters (3 litres capacity, 150 cm high) recommended by the European Brewery Convention (EBC, 1977). Samples were withdrawn using a sterile syringe through a rubber stopper located 50 cm from the top of the fermenter. Glass fermenters were thoroughly cleaned and disinfected every time a new fermentation process started. Inocula of a pure *Saccharomyces cerevisiae* var. *uvarum* culture and sterile worts were used.

Preparation of yeast inoculum and wort

An initial yeast inoculum was propagated in 100 ml of sterile wort in a conical flask in a gyratory shaker at 250 rpm for 48 h at 20°C. Afterwards, the content of the conical flask was poured into another flask with 3

litres of sterile wort. After slow agitation for 3 days at room temperature, a low temperature (4°C) was applied on the flask to provoke yeast flocculation. Yeast was collected and kept at 4°C. Worts were sterilized at 121°C for 15 min, then cooled to 10°C and used immediately for fermentation.

Pilot-scale fermentations

Fermentation was started by adding a yeast inoculum to 3 litres of sterile wort and then the mixture was transferred to a glass fermenter. The yeast inoculum employed was calculated to obtain a final count of 20 million cells per ml of wort. Fermenters were connected to a refrigerated bath at a constant temperature (10°C). The yeast/wort ratio and the temperature were the same as those used in an industrial brewery. Samples were taken every day during the 6 or 7 days that the fermentation lasted. One portion of sample was used for microbial analysis and the other portion was centrifuged to remove yeasts, filtered (0·22 μm filter) and then frozen (–20°C) until amine analysis were done.

Conditions of yeast recycling

Five consecutive fermentations were carried out in glass fermenters with the same yeast but recycled for every new fermentation. For the first fermentation, an inoculum of a fresh yeast was used. At the end, yeast was harvested in aseptic conditions, recycled and used for a new process. Yeast recycling was done with phosphoric acid (Hough, 1991). Two hundred μl of 75% phosphoric acid were added to 100 ml of yeast. The yeast was briefly shaken and kept at 4°C at least 30 min before using for another fermentation. Yeast was recycled up to four times, as the cooperating brewery usually did. This process of 5 consecutive fermentations was repeated using a fresh inoculum of the same pure yeast culture. Samples were drawn and manipulated as described above.

Analytical methods

Histamine was determined according to a spectrofluorometric method (Vidal-Carou *et al.*, 1989). Tyramine determination was carried out according to Rivas-Gonzalo *et al.* (1979). Each analysis was always carried out in duplicate.

Microbial analysis

Yeast cell counting was done with a Thoma cell, after diluting samples with distilled water (EBC, 1977). Contaminant flora, such as wild yeasts and lactic acid bacteria, were monitored with WLN (Oxoid, Unipaph Ltd, Hampshire, UK) (Roecken & Schulte, 1986) and NBB (Döhler, Darmstadt, Germany) (Back, 1980; Dachs, 1981) plates, respectively. WLN plates were incubated at 37°C for 3–5 days in aerobic conditions and NBB plates were incubated in anaerobic conditions at 28°C for 10 days.

Statistical analysis

Data were analyzed by one-way ANOVA followed by testing of specific mean differences using Fisher's test. Significant differences were established at $P \leq 0{\cdot}05$. Analyses were performed by means of the SPSS Statistical software package (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Effect of yeast on biogenic amine formation

In a previous work done in the same cooperating brewery, histamine and other amines, such as cadaverine, putrescine, agmatine and spermine, remained constant and even decreased, e.g. spermidine, during beer fermentation (Izquierdo-Pulido *et al.*, 1994). Those amines proceed mainly from raw materials and the mash process. Only tyramine formation was detected (final levels ranging from 9·50 to 30·10 mg/l). Therefore this study focused on tyramine evolution. Histamine evolution was also included because of its important toxicological implications. Furthermore, legal regulations or recommendations of maximum tolerable contents in foods have been only proposed for this amine (Stratton *et al.*, 1991).

No contaminant flora were detected in any of the fermentation samples. We observed that yeast followed a typical bottom-yeast growth-curve in all fermentations. Yeast counts at the beginning of the fermentation were about 20×10^6 cells/ml, increasing up to 40×10^6 to 50×10^6 cells/ml in 4 or 5 days of fermentation. Final yeast counts ranged from 25×10^6 to 15×10^6 cells/ml.

Initial levels of histamine in wort ranged from 0·45 to 0·85 mg/litre and for tyramine from 1·35 to 2·90 mg/litre. Evolution of histamine and tyramine during fermentation is shown in Fig. 1. An ANOVA was carried out to test differences in amine contents among the different days of fermentation. No significant differences ($P > 0{\cdot}05$) were found for both amines, indicating

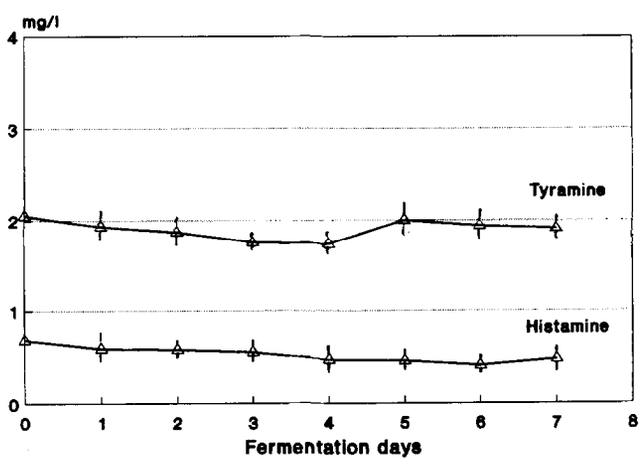

Fig. 1. Evolution of histamine and tyramine contents during beer fermentation using pure yeast and sterile wort. Data are averages (± standard deviations) of 15 processes.

Table 1. Evolution of histamine and tyramine contents[a] (mg/litre) during five consecutive fermentations carried out with the same yeast but recycled for every new process

Days	0[b]		I		II		III		IV	
	HIS[c]	TYR[d]	HIS	TYR	HIS	TYR	HIS	TYR	HIS	TYR
0	0·82 ± 0·11	2·17 ± 0·39	0·82 ± 0·03	2·40 ± 0·14	0·70 ± 0·14	2·42 ± 0·32	0·80 ± 0·05	2·27 ± 0·25	0·57 ± 0·11	2·21 ± 0·03
1	0·55 ± 0·07	2·07 ± 0·67	0·55 ± 0·14	2·40 ± 0·07	0·62 ± 0·18	2·70 ± 0·21	0·61 ± 0·12	2·31 ± 0·20	0·45 ± 0·07	1·92 ± 0·25
2	0·57 ± 0·11	2·10 ± 0·42	0·60 ± 0·14	2·07 ± 0·11	0·60 ± 0·13	2·45 ± 0·18	0·35 ± 0·07	2·02 ± 0·13	0·40 ± 0·10	1·90 ± 0·15
3	0·51 ± 0·13	2·12 ± 0·87	0·56 ± 0·08	2·17 ± 0·20	0·40 ± 0·08	2·14 ± 0·16	0·40 ± 0·21	1·85 ± 0·42	0·45 ± 0·11	2·00 ± 0·12
4	0·55 ± 0·09	2·00 ± 0·56	0·40 ± 0·11	2·34 ± 0·32	0·45 ± 0·07	2·40 ± 0·28	0·30 ± 0·07	1·72 ± 0·18	0·35 ± 0·07	1·80 ± 0·21
5	0·42 ± 0·18	1·85 ± 0·78	0·47 ± 0·14	2·40 ± 0·21	0·35 ± 0·07	2·05 ± 0·07	0·42 ± 0·18	1·62 ± 0·39	0·45 ± 0·08	2·50 ± 0·30
6	0·40 ± 0·20	2·17 ± 0·39	0·42 ± 0·18	2·35 ± 0·21	0·45 ± 0·21	2·00 ± 0·64	0·45 ± 0·14	1·65 ± 0·14	0·40 ± 0·11	2·35 ± 0·23

[a]Mean ± SD are averages from two processes. Each amine determination was carried out in duplicate. [b]Recycling time; [c]Histamine; [d]Tyramine.

that no amine formation or reduction occurred during fermentation. Narziss *et al.* (1984a) previously observed that histamine contents decreased during fermentation. Although a slight decrease in histamine contents was observed, this may have been due to unavoidable variations in the analytical method rather than an actual decrease. It would seem that the 'normal' amounts of histamine and tyramine in beer are probably inherited from brewing raw materials.

Our results are consistent with the hypothesis that biogenic amine production is more related to contaminant microorganism activity than to yeast activity. Zee *et al.* (1981), after following amine evolution during small-plant fermentations with *Saccharomyces uvarum*, pointed out that biogenic amines were not formed by yeast. It appears that amines in beers are related, to some extent, to the hygienic conditions of yeast inoculum.

Effect of yeast recycling on biogenic amine formation

No literature has been found about the effect of yeast recycling on amine formation. Our objective was to check if old yeast (yeast used for several fermentations) showed ability to form biogenic amines that was not shown by new cultures of yeast.

No contaminant flora were detected in any fermentation process. Yeast followed a typical growth-curve in all fermentations. Evolution of histamine and tyramine contents is summarized in Table 1. Initial levels of histamine in wort ranged from 0·50 to 0·70 mg/litre and for tyramine from 1·90 to 2·90 mg/litre. No amine formation was detected in any fermentation. No higher or lower final amine contents ($P > 0·50$) were found in the fermentations with 'old yeast' (after being recycled 3 or 4 times) than in those carried out with 'new yeast'. Final histamine levels always ranged between 0·30 and 0·65 mg/litre and final tyramine levels between 1·55 and 2·60 mg/litre regardless of the 'age' of the yeast. From our data, yeast recycling had no influence on biogenic amine evolution.

Haikara (1986) pointed out that as yeast becomes 'older' the number of microorganisms, either gram positive or negative, increases considerably. Phosphoric acid treatment for recycling seemed to be a proper method to control contamination of yeast, at least on a small scale (Hough, 1991), since contaminant microorganisms were not detected.

Current research is underway to study amine production capability of common contaminant microorganisms encountered in breweries. In addition, the influence of precursor amino acid levels on biogenic amine formation is also being studied.

CONCLUSIONS

On the basis of our results, *Saccharomyces cerevisiae* var. *uvarum* alone has no ability to produce biogenic amines during beer fermentation. On the other hand, yeast recycling for several fermentations does not influence biogenic amine contents during fermentation.

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