Risk Analysis of Tyramine Concentration in Food Production

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Abstract. The contribution is focused on risk analysis in food microbiology. This paper evaluates the effect of selected factors on tyramine production in bacterial strains of *Lactococcus* genus which were assigned as tyramine producers. Tyramine is a biogenic amine sythesized from an amino acid called tyrosine. It can be found in certain foodstuffs (often in cheese), and can cause a pseudo-response in sensitive individuals. The above-mentioned bacteria are commonly used in the biotechnological process of cheese production as starter cultures. The levels of factors were chosen with respect to the conditions which can occur in this technological process. To describe and compare tyramine production in chosen microorganisms, generalized regression models were applied. Tyramine production was modelled by Gompertz curves according to the selected factors (the lactose concentration of 0-1% w/v, NaCl 0-2% w/v and aero/anaerobiosis) for 3 different types of bacterial cultivation. Moreover, estimates of model parameters were calculated and tested; multiple comparisons were discussed as well. The aim of this paper is to find a combination of factors leading to a similar tyramine production level.

Keywords: Gompertz curve, yield factor, multiple comparisons, tyramine production
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INTRODUCTION

Tyramine (TYM) belongs to a group of biogenic amines, which are alkaline compounds formed in foodstuff mainly by microbial decarboxylation. Biogenic amine (BA) precursors are free amino acids (tyrosine is a precursor for tyramine synthesis) provided by proteolytic changes of proteins and/or peptides (see [1]). Contaminating bacteria from family *Enterobacteriaceae* and genera *Pseudomonas* are usually included in groups of microorganisms possessing decarboxylase enzymes. TYM is produced also by some lactic acid bacteria (e.g. strains from genera *Lactococcus*, *Lactobacillus*, *Enterococcus* etc.). Lactic acid bacteria (LAB) also occur in some food as contaminants threatening the quality and safety of the product (e. g. in certain meat products, beer, and wine). Presence of particular decarboxylases is not specific within the species and occurs only among certain number of strains in the species (see [2], [3]).

TYM is an endogenous compound with key functions in metabolism of living organisms. Generally, low concentrations of TYM in food and beverage (basically under 100 mg/kg) do not represent a significant risk for a healthy person. Human intestinal tract has a detoxifying system. However, higher amounts of BAs (generally above 100 mg/kg) may induce undesirable psychoactive and vasoactive effects (hypotension or hypertension, headache, nausea, breathing problems etc.). Moreover, effectiveness of the detoxication system can also be diminished by antihistamines, antidepressants and alcohol. Due to the interference of alcohol with the detoxication metabolism, a lower TYM limit is usually declared for alcoholic beverages: 25-40 mg/L for TYM (see [1], [4]).

Production of biogenic amines (including TYM) by bacteria can be influenced by many external factors (temperature and pH of the environment, aero/anaerobiosis, availability of carbon sources, presence of growth factors, growth phase of the cells, NaCl concentration etc.), which can affect mainly the kinetics of decarboxylase reactions (see [1], [2], [5]). Biogenic amines can also be produced by LAB strains which are commonly used for technological purposes as starter cultures [2]. It would be suitable to describe the kinetics of biogenic amine production under conditions which are similar to those during the technological process of cheesemaking. However, such information occurs very rarely in current specialized literature [2], [5].

PROBLEM DELINEATION AND APPLIED STATISTICAL METHODS

The aim of the work was to model the effect of selected factors (the concentration of lactose in the range of 0-1% w/v, NaCl in the range of 0-2% w/v and an aerobic/anaerobic environment) on TYM production in one bacterial

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strain of *Lactococcus lactis* subsp. *lactis* (001) and two bacterial strains of *Lactococcus lactis* subsp. *cremoris* (002, 003) which are tyramine-positive. These bacteria are used within the biotechnological process of cheese production as starter cultures. Therefore, the levels of factors were chosen with respect to the conditions which can occur within the technological process. To describe and compare TYM production for chosen microorganisms, nonlinear regression models were applied.

TYM production has been modelled by Gompertz curves according to selected factors. Parameterization of Gompertz's curve was carried out considering [2], [5], and it was used in the form of

$$y = \alpha \exp\left\{-\exp\left[\frac{\mu \mathrm{e}}{\alpha}(\lambda - t) + 1\right]\right\},$$

where y is the tyramine content (mg/L); μ is the tyramine production rate (mg tyramine/L h); α is the asymptote defined as maximum tyramine production; λ is the delay period (the time until the tyramine production was detected for the first time (hours)); t is time (hours). Parameter estimates were carried out by nonlinear least squares; standard deviations of parameter estimates were determined, and in the course of statistical analysis, asymptotic normality of observed parameters was assumed (see [6] p. 24 Theorem 2.1). Multiple comparisons of parameters were performed by the Bonferroni method; again, asymptotic normality was assumed (see [7] p. 197 formula (5-27)).

Furthermore, the yield factor $\beta_y = Y_{TYR/CFU}$ (where the colony-forming unit (CFU) is an estimate of viable bacterial numbers) was modelled using linear regression:

$$y_t = y_0 + \beta_y (N_t - N_0) \cdot 1000,$$

where y_t and y_0 (mg/L) are the concentrations of tyramine at time t and 0, respectively; N_t and N_0 are total numbers of CFU/mL in time t and 0, respectively; and β_y is a constant yield factor for tyramine formation. A test of equality between regression coefficients was transformed into testing of general linear hypothesis (see [7] p. 302); for test statistics, see [7] p. 303 formula (7-17).

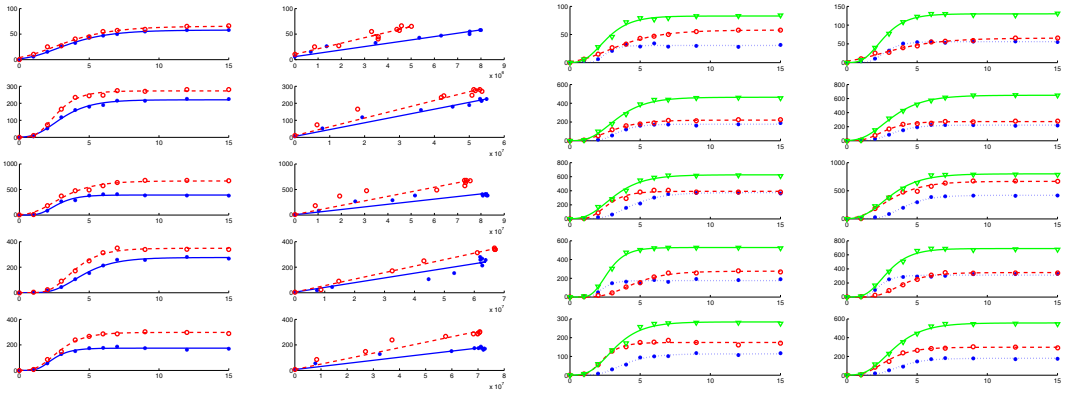
Numerical calculations were obtained using the MATLAB 7.13. software.

STATISTICAL ANALYSIS

In order to assess the influence of four factors, i.e. environment (aerobic AE, anaerobic AN), lactose concentration LC (0%; 0.25%; 0.5%; 0.75%; 1%) w/v, concentration of NaCl (0%, 1%, 2%) w/v and lactic acid bacteria (001, 002, 003) on the tyramine content y (mg/L), 10 observations were made for every variable y (the tyramine content) depending on time (0-15 days), and for various combinations of individual factors. On that account, a total volume of 2x5x3x3=90 sets were available. Temporal progress of the y variable was modelled for every possible combination of the four above-mentioned factors using the Gompertz curve. Consequently, the progress of tyramine y_t was modelled using the β_y yield factor depending on the absolute growth rate (CFU/L day).

While calculating estimates, the main attention was paid to the correct choice of initial estimates. Furthermore, variability of obtained estimates was considered. However, the number of observations in each group was not large (10 observations for each group of four studied factors), variability of estimates did not differ from usual limits. As an example, we present estimates of parameters α, μ, λ and their standard deviations for Gompertz curve from figure 2 – 1. column and 2. row (bacteria 001, AE environment, NaCl concentration 1%, LC 0.5%), where the monitored points demonstrated greater fluctuation around the Gompertz curve. The values of obtained estimates and their standard deviations are 392.07 ± 10.18 for the parameter α, 155.13 ± 24.24 for the parameter μ and 1.40 ± 0.21 for the parameter λ.

The impact of environment on the tyramine content y in a sample of lactic acid bacteria 001 with 1% NaCl concentration and various lactose concentrations can be seen in the left graph in Figure 1. The figure illustrates the environment factor impact on individual curves well. It shows that for given values of other factors, the y variable (tyramine content) is always higher in anaerobic environment. Similarly, the β_y yield factor is always higher in AE environment than in AN environment. Both curves were compared by testing of equality of their parameters. It was proved that the environment factor is statistically significant at a significance level of 5%. To be more precise, the hypothesis of α parameters equality, which describes maximum tyramine production, was rejected in AE and AN environment for all possible combinations of the remaining factors. Hypotheses about equality of μ parameters and equality of λ parameters were rejected by statistical tests approximately in 50% of all cases. Tests on β_y yield factor equality in AE and AN environment were not rejected only in case of the following combinations: bacteria 001,



Left graph Right graph

FIGURE 1. Impact of AE (blue) and AN (red) environment (the left graph), and impact of NaCl (0% blue, 1% red, 2% green) (the right graph) on tyramine production. Left graph: bacteria 001, NaCl concentration 1%; Gompertz curves (first column), yield factor (second column); in rows, the CL gradually rises from 0% to 0.25%; 0.5%; 0.75%; 1%. Right graph: bacteria 001; AE environment is in the first column, AN in the second; in rows, the LC rises from 0% to 0.25%; 0.5%; 0.75%; 1%

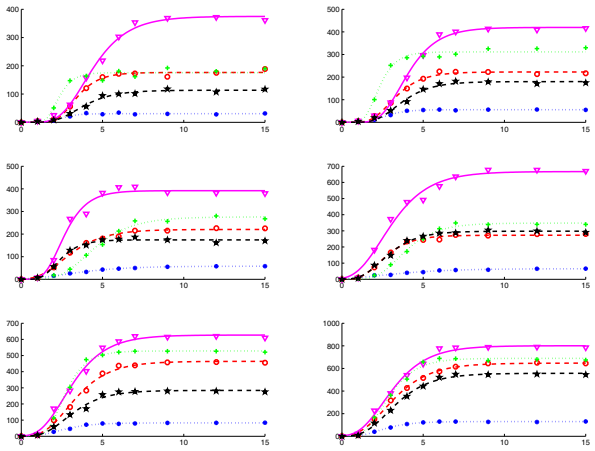


FIGURE 2. Impact of lactose concentracion(0% blue, 0.25% red, 0.5% magneta, 0.75% green, 1% black) on tyramine production for bacteria 001. AE environment is in the first column, AN in the second; in rows the NaCl concentration gradually rises from 0% to 1%; 2%

NaCl concentration 0%, LC $= 0.25\%, 0.5\%$; bacteria 002, NaCl concentration 1% and LC $= 0.75\%$; and bacteria 002, NaCl concentration 2% and LC $= 1\%$. For bacteria 003, the hypothesis about yield factor β_y equality was rejected for NaCl concentration 0%, 2% and for all LC levels; contrariwise, with NaCl concentration of 1%, and LC $= 0.25\%; 0.5\%; 0.75\%; 1\%$, equality of β_y yield factor between AE and AN environment was not rejected.

The impact of NaCl concentration on tyramine production is well illustrated in the right graph in Figure 1. Tests on α parameters (maximum tyramine production) equality for NaCl concentration 0%, 1%, and 2% with given combinations of other factor values (bacteria, CL, environment) shows statistically significant differences at a significance level of 5% in all tested combinations. Values of α parameter (maximum tyramine production) are the highest when NaCl concentration is 2% and the lowest when NaCl concentration is 0%. A similar situation occurred during comparison of yield factor β_y. It reaches its highest values when NaCl concentration is 2%. With NaCl concentration 0% yield factor β_y values were lower or the same as with NaCl concentration 1%.

The impact of LC parameter (lactose concentration) is demonstrated in Figure 2. α parameters equality for various

types of LC factor values (each time five α parameters were compared based on the LC level) in given combinations of other variable values were largely rejected. Values of α parameters were the highest when LC = 0.5%, and the lowest when LC = 0%. In case of yield factor β_y comparison, results were different. Values of β_y parameters were the highest when LC = 0%, and the lowest when LC = 1%. These differences are statistically significant at the level of significance of 5%.

DISCUSSION

Higher production of TYM was observed in the environment with a higher concentration of NaCl in all strains tested. Pereira [8] suggested that Na^+ ions that are involved in regulation of intracellular pH play an essential role in the tyrosine decarboxylation pathway. Na^+ ions are important in sodium/proton antiport system, as they are exchanged with H^+ ions that are removed out of cells.

When lactose was not added to the medium, a lower growth of bacterial cells and a lower production of TYM (in all NaCl concentrations tested) were observed. A cultivation medium without lactose is less suitable for the tested lactococci, which need complex media for their growth. The highest amount of TYM was observed in the environment with 0.5% (w/v) lactose. A further increase in lactose concentration, with the same NaCl concentration, led to a decreasing production of TYM. According to Molenaar [9], decarboxylation of amino acid may serve as a source of metabolic energy. Thus, in sub-optimal cultivation conditions, represented by medium without saccharide source of energy, i.e. lactose, the cells can obtain necessary energy also from decarboxylation processes. Therefore, the amount of TYM produced by a cell as a final product of this metabolic pathway increases. When lactose was added to cultivation broth, the yield factor ($Y_{TYR/CFU}$) decreased, probably because the cell could obtain energy more effectively from saccharides.

The last factor observed was an aerobic/anaerobic environment. In all strains tested under the given conditions, higher production of tyramine was determined under anaerobic conditions.

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