

MODELLING OF THE PRODUCTION OF FERMENTATIVE AROMAS DURING WINEMAKING FERMENTATION

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Abstract

In this study, we determined the production of the main fermentative aromas (acids) during winemaking fermentation. We applied the dynamic model for describing of biomass and ethanol production, total acids, volatile acids and sugar consumption. The parameters of model were identified from fermentation experimental data of synthetic medium.

Key words: winemaking, fermentative aromas, modelling

1. INTRODUCTION

Saccharomyces wine yeast strain, selected as good producers of aroma compounds, when grown on synthetic microbiological medium, have been tested in wine fermentations. Some complex products such as wine, have in his composition more than 1000 volatile molecules and therefore, one can be tempted to think that the final aroma perception is the result of the interactions of several hundred volatiles, which means that we have almost no chance to understand, or predict the aroma of these products. (1)

Four senses are involved in defining the organoleptic quality of wine: sight, smell, taste and touch. The term aroma is normally used to describe the smell of a young, fresh wine; primary aromas originate during fermentation. The aroma and flavour of wine are one of the main characteristics that define the differences among the vast array of wines and wine styles produced throughout the world. They are affected by the innumerable possible variations in wine's production, both in viticulture and in winemaking. (2)

Some volatile aroma compounds arise directly from chemical components of the grapes, many grape-derived compounds are released and/or modified by the action of flavour-active yeast and bacteria, and a further substantial portion of wine flavour substances result from the metabolic activities of these wine microbes. The concentration of aroma compounds at the end of fermentation are also impacted by the losses in the CO₂ released. (3,4,5)

Mouret et al. [9] were studied the impact of the temperature and assimilable nitrogen on synthesis of the principal fermentative aromas by yeasts. Morakul et al [10] elaborated the model to quantify the gas-liquid partitioning of four the most important volatile compounds produced during winemaking fermentation, namely isobutanol, ethylacetate, isoamyl acetate and ethyl hexanoate. The dynamic model kinetic of the aroma compounds during winemaking fermentation was developed in [11]. A stoichiometric model for wine fermentation was constructed to simulate batch cultures of *S. cerevisiae*. Five differential equations describe the evolution of the main metabolites and biomass in the fermentation tank. Malherbe et al. [6] were proposed a dynamic model of alcoholic fermentation in wine making conditions. They studied the effect of assimilable nitrogen and temperature on fermentation kinetics involved during the grape must fermentation but also on the synthesis of flavour markers determining the aromatic profile of wine [7,8,9]. Carrau et al. [3] studied the yeast physiological behavior and metabolite production in response to nitrogen supplementation using chemically defined model medium.

2. MATERIALS AND METHODS

2.1 Yeast strains

In experiment, two yeast strains of *Saccharomyces cerevisiae* were used. *S. cerevisiae* TC-2 and TC-3 are autochthonous cultures isolated from natural sources (vine) and they are part of collection of microorganisms of Faculty of Chemical and Food Technology (Slovak University of Technology, Bratislava, Slovakia). Strains are characterized by high production of esters and are suitable for production of white wines with expressive fruity aroma.

2.2 Preparation of inoculum

Yeast starters for fermentation were prepared from a yeast strain culture grown aerobically for 24 h in a 100 ml of liquid medium (20 g.l⁻¹ glucose, 10 g.l⁻¹ yeast extract (Merck); pH 6.5) in a 500 ml cultivation flask, on an orbital shaker (2 Hz) at 28 °C.

After cultivation, concentration of the yeast biomass was determined by counting in a Bürker chamber. The calculated volume of biomass was withdrawn and centrifuged (10 min, 1 370 ×g). Separated biomass was washed with MilliQ deionized water, centrifuged again and finally added to fermenting medium to achieve the starting concentration of biomass 10⁶ cells.ml⁻¹.

2.3 Experimental fermentation

For experimental fermentation YD medium of following final composition was used: 225 g.l⁻¹ glucose, 8 g.l⁻¹ yeast extract (Merck), pH 3.5. Fermentation took place in 5-liter fermenter: 4 l of medium in fermenter. Before fermentation, 900 g of glucose was dissolved in 3500 ml of MilliQ deionized water and sterilized directly in fermenter. After sterilization, sterile water solution of yeast extract (32 g in 500 ml) was added and pH was adjusted by L-tartaric acid (Sigma Aldrich). Medium was aseptically inoculated, and the main alcoholic fermentation had proceeded for 2 weeks at controlled temperature 17 °C, without stirring.

2.4 Sampling

At regular intervals (every 24 h), samples of fermenting medium were taken and analysed. Before sampling, content of fermenter was shortly stirred to homogenize the yeast biomass. After determination of biomass concentration samples were centrifuged and analysed in terms of the other analytical parameters.

2.5 Analytical methods

Concentrations of reducing sugars have been analysed by Schoorl method concentration of alcohol and extract pycnometrically (OIV-MA-AS312-01A, OIV-MA-AS2-03B). Total volatile acids (expressed as acetic acid) have been separated from the sample by steam distillation and then as well as total acids (expressed as the tartaric acid) determined by acid-base titration with 0.1 mol.l⁻¹ KOH (OIV-MA-AS313-01, OIV-MA-AS313-02). Yeast assimilable nitrogen was determined by formol titration technique.

2.6 Mathematical model

A simplified model for the kinetic description of sugar consumption and ethanol, total acids and lactic acid production was established.

Growth of yeasts is expressed by Gompertz equation

$$\log(N) = A + C \exp^{-\exp^{-B(t-M)}} \quad (1)$$

where $\log(N)$ is logarithm in time t , A is logarithm of initial amount of microorganism ($A = \log N_0$), C number growth cycles (approximately $\log N_{\max} - \log N_0$), M is time when growth rate is maximal, B is growth rate at time M .

Growth rate of yeasts is expressed by Monod equation

$$\mu = \mu_{\max} \frac{c_s}{K_s + c_s} \quad (2)$$

resp. including product inhibition

$$\mu = \mu_{MAX} \frac{c_S}{(K_S + c_S)(1 - \frac{c_P}{K_P})} \quad (3)$$

Substrate consumption(S), production of biomass (X) and main metabolic products (P) is expressed:

$$S: \frac{dc_S}{dt} = r_S = -Y_{S/X} r_X \quad (4)$$

$$X: \frac{dc_X}{dt} = r_X = \mu c_X \quad (5)$$

$$P: \frac{dc_P}{dt} = r_P = Y_{P/X} r_X \quad (6)$$

Initial conditions:

$$t = 0 \quad c_S = c_{S0} \quad c_X = c_{X0} \quad c_P = c_{P0} \quad (7)$$

3. RESULTS AND DISCUSSION

Experimental data have been simulated by solving the system of Eqs (1-6) with initial condition (Eq.7). A serious model of wine fermentation must describe the key fermentation components (e.g. sugar, biomass, ethanol) as well as aroma compounds.

The curve for total production of biomass is depicted in Fig.1. Our model adequately describes experimental data. Comparison of experimental data of ethanol production and the substrate consumption is presented in Fig.2. Kinetics parameters of model are summarized in Table 1.

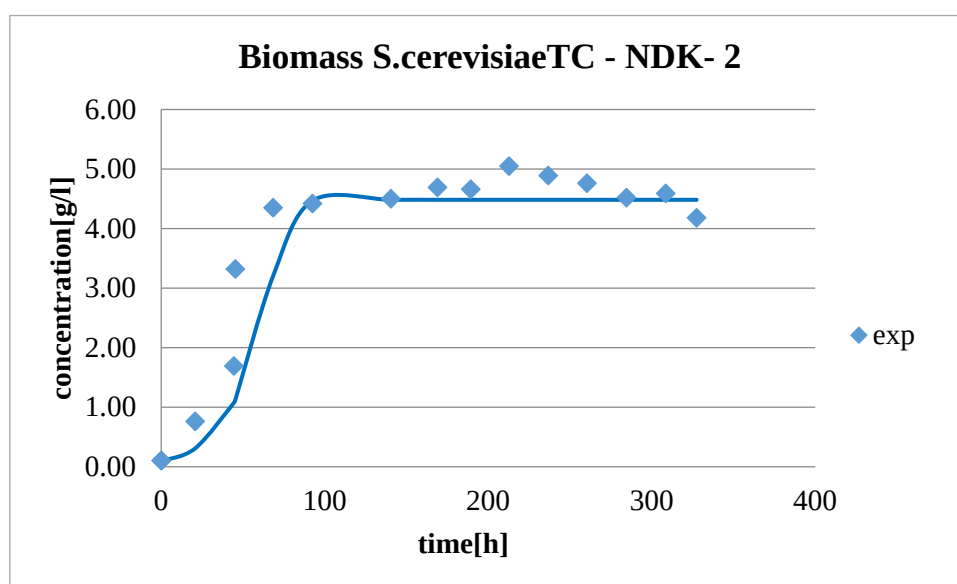


Fig. 1. Biomass production (solid line - calculated, points - experimental data)

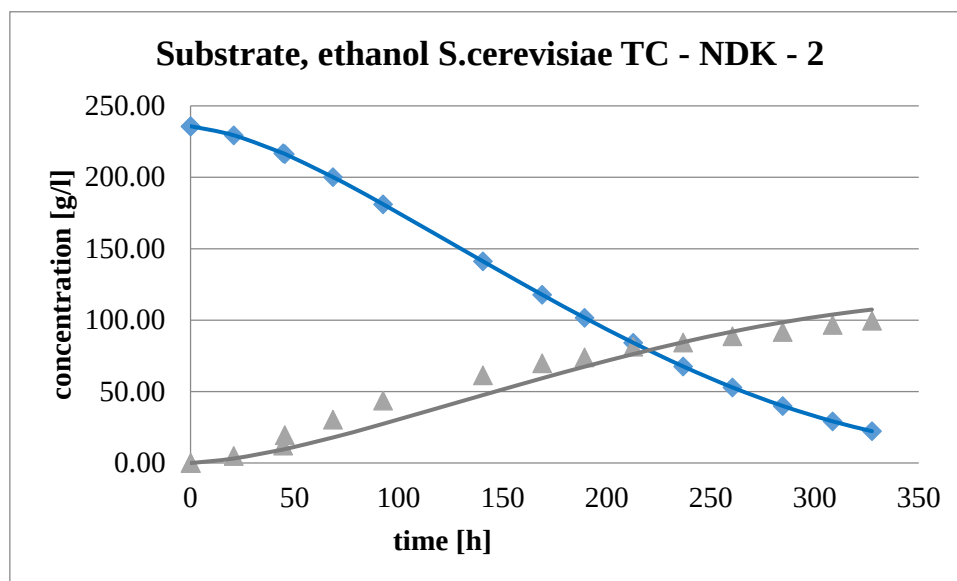


Fig. 2. Substrate consumption and ethanol production (solid lines - calculated, squares - experimental data of substrate, triangles - experimental data of ethanol)

Table 1. Kinetics parameters of model

μ_{MAX}, h^{-1}	$K_S, g/l$	$K_P, g/l$	$Y_{X/S}$	$Y_{P/X}$
0,070	68,099	10,411	0,027	18,543

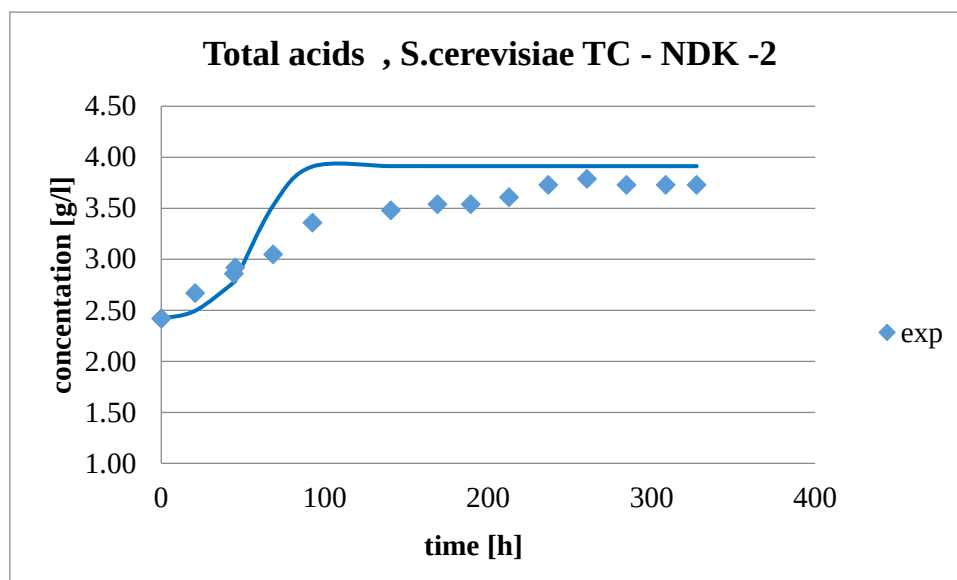


Fig. 3. Total acids production versus time

The acidity of grape juice and wine has a direct impact on its sensory quality and physical, biochemical and microbial stability. The main features of wine acidity include the types and concentrations of the acids.

Total acids are one of the most important compounds in wine, contribute a great deal to wine aroma and wine taste. In our model system, the rate of production of total acids could be calculated with adequate accuracy. (see Fig. 3)

We measured the kinetic production of Lactic acid. The presence of this acid is not very popular in wine. Our model has ability to describe the concentration profile of this acid during the fermentation of wine. (see Fig. 4)

The effect of nitrogen limitation during batch fermentation is depicted in Fig. 5. The model was successful to describe experimental data.

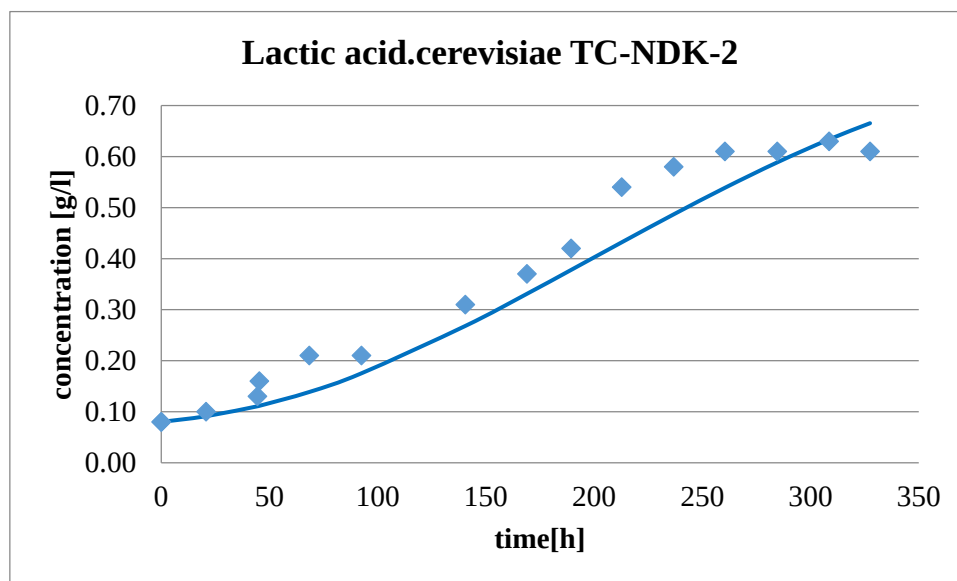


Fig. 4. Lactic acid production versus time

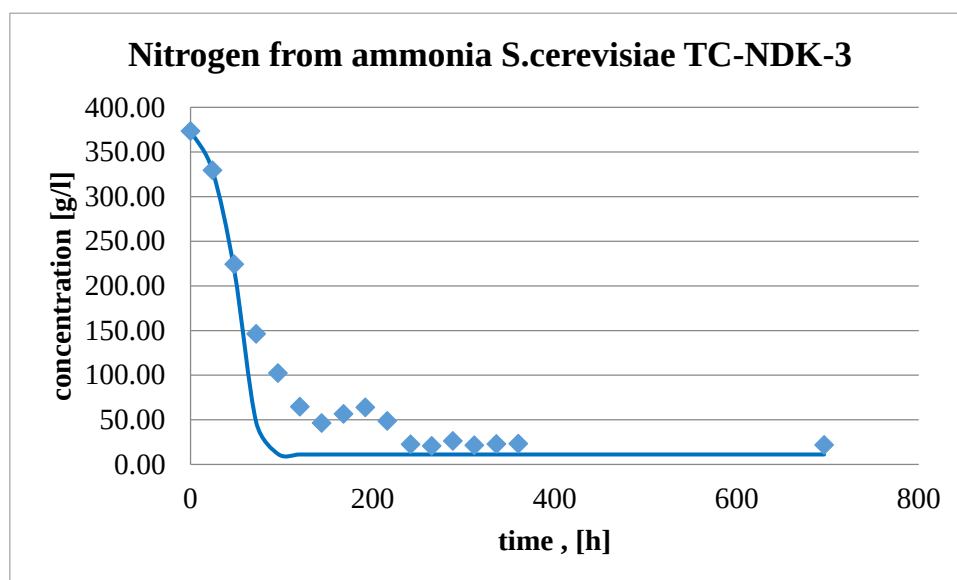


Fig. 5. Nitrogen from ammonia up take versus time

The acidity of grape juice and wine has a direct impact on its sensory quality and physical, biochemical and microbial stability. The main features of wine acidity include the types and concentrations of the acids.

4. CONCLUSIONS

This study developed a simple mathematical model of wine fermentation, which is able to describe substrate and nitrogen consumption, the total acids, biomass and ethanol production. The model fit to experimental data in satisfactory accuracy.

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