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Prediction of the production kinetics of the main fermentative aromas in winemaking fermentations

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ABSTRACT

A novel dynamic model was developed for predicting the synthesis kinetics of the principal aroma compounds produced by yeasts during winemaking fermentations: isobutanol, isoamyl alcohol, isoamyl acetate, ethyl hexanoate and ethyl octanoate. The parameters of the model were identified from nine fermentations performed at temperatures between 18 and 30 °C and with different initial nitrogen contents, in the range of 70 to 410 mgN/L. The model was validated in six independent experiments with conditions in the same range. Predictions were accurate for these volatile compounds: the mean difference between experimental and estimated values for fermentative aroma synthesis throughout the process was below 10%, for both the fermentations used to build the model and those used for validation. This model is the first to simulate the production kinetics of fermentative aromas and provides new insight into the synthesis of these volatile compounds. It will facilitate the development of innovative strategies for controlling the production of those aromas in winemaking, through management of the principal control factors: assimilable nitrogen content and temperature during the alcoholic fermentation.

1. Introduction

Wine aroma is one of the principal attributes determining consumer preference [1–3]. Most fruity aroma compounds, including esters in particular, are produced by the yeast during alcoholic fermentation. Several studies have assessed the influence of fermentation parameters on the final fermentative aroma content of the wine, focusing mostly on higher alcohols and esters [2]. These final concentrations are influenced principally by the concentration of assimilable nitrogen [2]. The synthesis of higher alcohols is not a monotonic function of initial nitrogen content; production is optimal for an initial assimilable nitrogen content of 200 to 300 mg/L [4–8]. The final concentrations of both acetate and ethyl esters are generally higher for musts with high initial nitrogen contents [3,6,9–11]. Fermentation temperature is the second most important factor influencing the final aroma composition of the wine. Larger amounts of higher alcohols are generally produced at higher temperatures [12,13], but this relationship is not systematic [14] and depends of the compound studied [6]. By contrast, the final liquid concentrations of esters are systematically lower at high temperatures, particularly for ethyl esters [6,7,12,14].

Despite the large number of studies carried out, the available data are highly fragmentary. There is also currently no model mimicking the production kinetics of fermentative aroma compounds during the process of fermentation. The available dynamic models focus on the main reaction of the alcoholic fermentation: the bioconversion of sugars into ethanol and CO_2 [15–22].

In this study, we determined the production kinetics of the main fermentative aromas for various values of initial nitrogen concentration and temperature, using an online monitoring device [23]. We then used the data collected to construct the first dynamic model predicting the synthesis of five fermentative aromas (two higher alcohols, one acetate ester and two ethyl esters) during the process of fermentation.

2. Materials and methods

The data used to build the model were obtained in a previous study [6], in which nine pilot-scale fermentations were performed with musts containing three levels of assimilable nitrogen (70, 230 and 410 mgN/L), at three temperatures (18, 24 and 30 °C), corresponding to the conditions generally used in winemaking. The

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Nomenclature

Symbols and abbreviations

A, B, C	Parameters describing the dependence of S_{trans} on N_0
$D_1 \ldots D_{12}$	Parameters describing the dependence of Y_1 and Y_2 on N_0 and T
k_1	Function involved in the modeling of biomass production
MOMAF	Modeling of the main reaction of alcoholic fermentation
MODAPEC	Modeling of aroma production in enological conditions
M	Total production of fermentative aroma compounds (mg/L)
N	Residual nitrogen concentration (mgN/L)
N_0	Initial assimilable nitrogen concentration (mgN/L)
N_{ST}	Function involved in the modeling of sugar consumption
S	Residual sugar concentration (g/L)
S_{trans}	Amount of consumed sugar corresponding to the transition between the two linear phases of production (g/L)
T	Temperature (°C)
X	Size of the cell population (10^9 cells/L)
X_{max}	Maximum population size during stationary phase (10^9 cells/L)
Y_1	Production yield of fermentative aromas from sugar during the first linear phase of production (mg/g)
Y_2	Production yield of fermentative aromas from sugar during the second linear phase of production (mg/g)
ν_N	Function involved in the modeling of nitrogen consumption
ν_{ST}	Function involved in the modeling of sugar consumption

concentrations of volatile compounds in the headspace of the fermenter were monitored with a specific online GC system [23].

Only the additional experiments used for model validation are described below.

2.1. Fermentation

2.1.1. Yeast strain and culture media

Fermentations were carried out with the commercial *Saccharomyces cerevisiae* strain Lalvin EC 1118® (Lallemand SA, Montreal, Canada). Fermentation tanks were inoculated with 200 mg/L active dry yeast rehydrated by incubation with 50 g/L glucose for 30 min at 35 °C.

We used different synthetic media (SMn, with 'n' indicating the assimilable nitrogen content) mimicking grape musts [24]. The concentrations of sugars, salts, vitamins, trace elements and anaerobic factors and the proportions of the various nitrogen sources were identical to those used by Mouret et al. [6]. Yeast assimilable nitrogen (YAN) concentration ranged from 150 to 340 mgN/L (SM150, SM230 and SM340).

2.1.2. Tanks and fermentation control

Fermentations were run at pilot scale, in 10-L stainless steel tanks, at 21, 24 and 26 °C. The amount of CO_2 released was measured automatically with a gas mass flow meter, for online measurement of the rate of CO_2 production (dCO_2/dt).

The reproducibility of the experiments was assessed by performing the fermentation in SM230 at 24 °C in triplicate. For all the volatile compounds assessed, the coefficient of variation for the gas concentration between the triplicates was low throughout the process of fermentation, with mean values of 7%, 9%, 6%, 6% and 6% for isobutanol, isoamyl alcohol, isoamyl acetate, ethyl hexanoate and ethyl octanoate, respectively. Based on these low coefficients of variation, the other fermentation trials were each performed only once.

2.2. Analysis of higher alcohols and esters

2.2.1. Online monitoring

The concentrations of volatile compounds in the headspace of the fermenters were measured hourly, with an online GC device [23]. Five fermentative aromas from three chemical families were considered: two higher alcohols (isobutanol, isoamyl alcohol), one acetate ester (isoamyl acetate) and two ethyl esters (ethyl hexanoate, ethyl octanoate). The concentrations of these compounds in the liquid phase were calculated with previously determined gas/liquid partition coefficients [25,26].

2.2.2. Volatile compound balances during fermentation

Gas-liquid balances were calculated for the five volatile compounds considered [6,23]. For each molecule, losses in the gas, accumulation in the liquid and total production (M) were determined throughout the fermentation process.

Total production was calculated by adding the amount remaining in the liquid phase to the amount lost in the gas phase [25]. This total production corresponds to the total amount of the compound produced by the yeast and is therefore related to yeast metabolism.

2.3. Modeling

The mathematical model (presented in Section 3) was implemented in Matlab 7 (The Matworks Inc., Natick, MA). The parameters were identified by nonlinear regression analysis, with Statistic Toolbox in Matlab. Full details on the parameter identification procedure are given in Section 3.1.4.

3. Results and discussion

3.1. Model construction

In a previous study, we highlighted specific relationships between sugar consumption and the total production of volatile compounds, depending on the fermentation conditions [6]. We showed that, for any fermentation, there are two successive linear phases of aroma compound production from sugar [6], as illustrated in Fig. 1a and b. We therefore began by modeling changes in the production yields of these compounds (aroma compound vs. sugar) from the initial nitrogen concentration and temperature values. We then integrated these yields into a previously developed model predicting the kinetics of sugar consumption during the fermentation process (called MOMAF, for "modeling of the main reaction of alcoholic fermentation", [19]). We thus obtained a dynamic model predicting the production kinetics of volatile compounds throughout the alcoholic fermentation from the initial nitrogen concentration and temperature values (called MODAPEC, for "modeling of aroma production in enological conditions"). The structure of these models and their interactions are presented in Fig. 2.

3.1.1. Transition between the two production yields

The first step was to quantify and predict the sugar consumption corresponding to the transition between the two linear phases for the five compounds.