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Short communication

Effect of ammonium concentration on alcoholic fermentation kinetics by wine yeasts for high sugar content

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Abstract

Kinetics of alcoholic fermentation by *Saccharomyces cerevisiae* wine strains in a synthetic medium with high sugar content were established for different nitrogen initial content and are presented for four strains. The composition of the medium was close to grape must except that the nitrogen source consisted mainly in ammonium and was varied from 120 to 290 mg N/l assimilable nitrogen. The overall nitrogen consumed was also estimated in order to determine nitrogen requirement variability.

The effect of assimilable nitrogen was in general greater on sugar consumption rates than on growth and three kinds of effect on sugar consumption rates were observed: (i) existence of an optimal initial nitrogen level for a maximal sugar consumption rate (inhibition if excess), (ii) no effect of nitrogen beyond the intermediary level (saturation), (iii) sugar consumption rate proportional to the initial nitrogen level (activation).

In all cases, the amount of consumed nitrogen increased with its initial concentration and so did the fructophilic capacity of the strains. The optimal requirement varied from 0.62 to 0.91 mg N/g of sugars according to different strains. There was no general correlation between the sugar assimilation rates and the nitrogen requirement.

Keywords: Wine yeast; Ammonium; Fermentation; *Saccharomyces*; Assimilable nitrogen

1. Introduction

In wine-making, the problem of adequate nitrogen level in the grape musts for a good achievement of alcoholic fermentation is not yet totally solved. If the yeast suffers from nitrogen deficiency it can lead to stuck or sluggish fermentation (Salmon, 1989; Bisson and Butzke, 2000). On the opposite, if excessive ammonium addition is done, there could be a risk that the wine had modified characteristics for higher alcohols (Beltran et al., 2005), acetic acid (Bely et al., 2003), ethyl carbamate (Ough et al., 1988) or in some conditions hydrogen sulphide (Wang et al., 2003) content. Despite numerous studies carried out on this topic, the results are not always in agreement nor the conclusions very clear. For some authors addition of a nitrogen source to the must increases biomass concentration for *Saccharomyces cerevisiae* and sugars utilization rates (Henschke and Jiranek, 1993; Bely et al., 2003; Beltran et al., 2005). This last effect could be explained by the stimulation of enzymes de novo synthesis during sugar assimilation even during the stationary phase (Mendeis-Ferreira et al., 2004), some of this enzymes being sugar permease (Salmon et al., 1993). For other authors, the use of ammonium salts to increase the nitrogen content of grape must induce a repression of amino acid consumption by the yeasts and could reduce the fermentation efficiency (Beltran et al., 2005). Thomas et al. (1996) even stated that the flux of carbon through the glycolytic pathway was greater under nitrogen limitation. In fact it seems that nitrogen requirement of *Saccharomyces cerevisiae* could depend on the strain (Jiranek et al., 1995) and on the conditions of fermentation (sugar concentration, temperature, presence of oxygen…) (Valero et al., 2003). In most of the studies sugar concentration not greater than 200 g/l were used. For these reasons, we studied the nitrogen requirement using

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ammonium salts for 11 commercial wine strains from different origin in a synthetic grape must with high level of sugars (240 g/l). The results for the four must representative strains are presented in this paper. The kinetics of growth, glucose and fructose assimilation, as well as the total consumed assimilable nitrogen amount was established for various initial assimilable nitrogen levels in the must. We chose a synthetic medium in order to well define and control its nitrogen content mainly consisting of ammonium. Moreover, ammonium salts are an excellent nitrogen source for *S. cerevisiae* (Torija et al., 2003) and are widely used by winemakers to increase nitrogen content in the legal limit of 1000 mg/l in Europe and 950 mg/l in USA (Henschke and Jiranek, 1993) of ammonium phosphate or sulphate (21% of nitrogen).

2. Materials and methods

2.1. Yeast strains

Eleven commercial *Saccharomyces cerevisiae* wine strains provided by Lamothe-Abiet (Bordeaux, France), Lallemand Inc. (Montréal Canada) and Anchor Yeast (Cape Town, South Africa) were used in this study. The results are shown for four of these strains: A, B, C and D. They were maintained on agar slants (peptone 10 g/l, glucose 5 g/l, yeast extract 10 g/l, agar 20 g/l) at 4 °C.

2.2. Fermentations conditions

Prior to inoculation, yeasts were propagated for 15 h at 30 °C in a synthetic medium containing (g/l): glucose, 50; yeast extract, 1; $(NH_4)_2SO_4$, 2; $MgSO_4$, 0.4; KH_2PO_4, 5. Before inoculation the yeast cells were washed with sterile water.

The volume of inoculum was calculated in order to get 3 millions of cells at the beginning of the fermentations in a medium containing (g/l): glucose, 120; fructose, 120; yeast extract, 0.75, citric acid, 0.3; malic acid, 4; tartaric acid, 4; $MgSO_4$, 0.4; KH_2PO_4, 5; sodium oleate, 0.005. The yeast extract (L21, Oxoid) was a vitamins source and at the same time provided 40 mg/l assimilable nitrogen mostly consisting in amino acids. For each strain three levels of total yeast assimilable nitrogen (YAN) were performed by varying the concentration of $(NH_4)_2SO_4$ in order to get 120, 190, and 290 mg N/l (Table 1). The pH was adjusted at 3.3 with NaOH (5 M). Fermentations were carried at 18 °C, in Erlenmeyer flasks of 500 ml containing at the beginning 450 ml. They were shaken only 2 min before taking sample once a day. For one strain all fermentations were inoculated with the same inoculum and carried out at the same time. Fermentations were stopped when sugars were exhausted or their concentration remained constant.

2.3. Yeast biomass concentration and viability

Cells were counted under microscope (× 400) using a Thoma hematocymeter. The experimental error (coefficient variation) was always inferior to 10% (Lange et al., 1993). Yeast viability was assessed by methylene blue staining (Bonora and Mares, 1982).

Table 1
Variable composition in nitrogen of the synthetic medium

Assimilable nitrogen initial level YAN (mg/l)	$(NH_4)_2SO_4$ (g/l)	Assimilable nitrogen from ammonium (mg/l)
120	0.38	80
190	0.714	150
290	1.2	250

2.4. Sugars analysis

Glucose and fructose concentrations were determined by enzymatic analysis using Boeringher test reference EZS862 (R-Biopharm, France) with an experimental error less than 4%.

2.5. Nitrogen content analysis

Ammoniacal nitrogen was determined by enzymatic analysis using Boeringher test reference E1112732 (R-Biopharm, France) and expressed as mg N/l. Total assimilable nitrogen was determined by the Sorensen method (Zoecklein et al., 1995). The coefficient of variation was at maximum 5%.

2.6. Calculation of specific rates

Specific rates for sugars assimilation, ammonium assimilation and growth were calculated in the same way. First, experimental data for kinetics (sugars, ammonium and growth versus time) were adjusted to a mathematical model using Microsoft® Excel™ with stepwise cubic spline function. Secondly, this mathematical model was derivated and then divided by the model fitting growth (expressed in millions of cells per liter) in order to obtain the specific rates versus time (expressed in g or mg N/millions of cells/day).

3. Results and discussion

In Enology, the YAN is usually evaluated by the Sorensen or formol titration method (Zoecklein et al., 1995) and consists in ammonia and alpha-amino nitrogen. According to grape variety and maturity, climate, and fertilization of vineyard, grape must content in YAN mostly ranges from 50 to 500 mg N/l (Bely et al., 2003), with an average value of 120–140 mg N/l. This average value is usually considered as non-limiting to achieve alcoholic fermentation (Butzke, 1990). Taking into account that the initial concentration of sugars in the medium was high (240 g/l) we varied the YAN from 120 to 290 mg/l with an intermediary value of 190 mg N/l to evaluate the requirement of the wine yeasts coming from various