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The effect of addition of sucrose and aeration to grape must on growth and metabolic activity of *Saccharomyces cerevisiae*

by

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Incidence de l'addition de saccharose et de l'aération sur la croissance et l'activité métabolique de *Saccharomyces cerevisiae*

Résumé : La supplémentation du moût de raisin en saccharose a un effet inhibiteur sur la croissance des levures, mais augmente l'activité catabolique des populations. Elle sera réalisée de préférence après la phase initiale de croissance des levures (entre le 2ème et le 4ème jour), en même temps qu'une aération momentanée qui stimule la croissance et la vitesse d'utilisation du sucre.

Key words: must, sugar, oxygen, yeast, growth, metabolism, fermentation.

Introduction

In vinification, alcoholic fermentation is controlled by the sugar content of the grape must. Generally, must sugar concentration is between 200 and 300 g/l and this is necessary to obtain the desired wine quality. However, in some circumstances depending on climatic conditions, this concentration may not occur naturally and it becomes necessary to supplement the must by addition of sucrose.

High concentration of sugars in the must may cause the so-called 'stuck fermentation', where yeast growth and metabolism cease before all the sugar is fermented. This undesirable situation may be overcome by aeration of the must during the early stages of fermentation. An aeration of the grape must, performed during the multiplication phase, stimulates yeast growth and fermentation of the must (RIBÉREAU-GAYON *et al.* 1975). However, the oxygen thus introduced does not lead to the most energetic metabolic pathway; glucose, at high concentration, represses the synthesis of cytochrome oxidases; fermentation is the only catabolic process (DE DECKEN 1966). The Crabtree effect functions rigourously under the conditions of vinification (TRAVERSO-RUEDA 1980). Oxygen acts by allowing the biosynthesis of sterols (KIRSOP 1974; LARUE *et al.* 1980). In this way, the multiplication and survival of yeast are promoted (LARUE *et al.* 1980). On the other hand, sugar, at high concentration, slows the growth rate of yeast (LAFON-LAFOURCADE *et al.* 1979) and increases the duration of fermentation.

This work studies the effect of simultaneous addition of sucrose and aeration of must on growth, viability and metabolic activity of *Saccharomyces cerevisiae.*

Material and methods

Filtration-sterilized commercial grape must (Muscat, Salins du Midi, Montpellier) was used (sugar 170 g/l, pH 3.5). Inoculation was made with 10^5 cells/ml of *S. cerevisiae.* The fermentation took place at 19 °C in non-agitated sterile flasks, sealed with a mer-

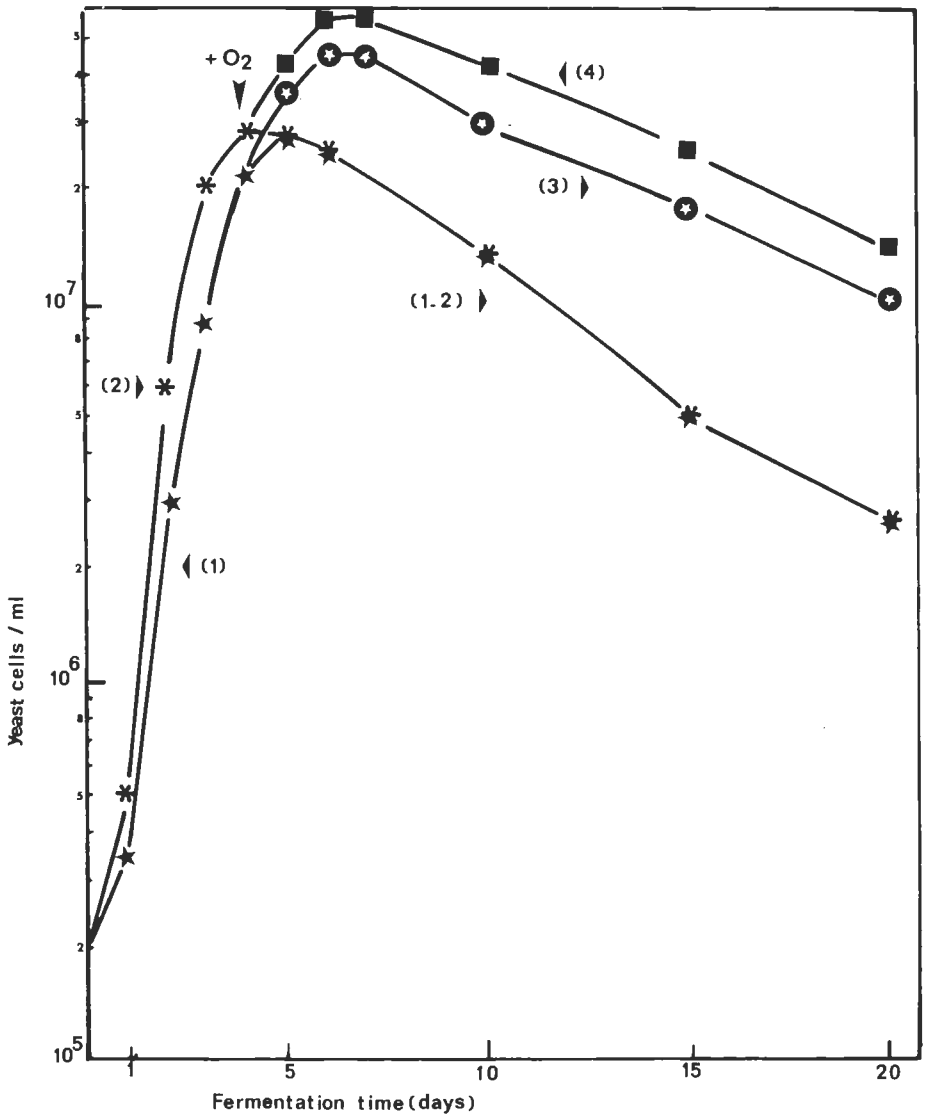

Fig. 1: Effect of supplementation with sucrose and of aeration on the growth of *Saccharomyces cerevisiae* in grape must. — 1: Supplementation with sucrose at day 0, no aeration; 2: supplementation with sucrose at day 4, no aeration; 3: supplementation with sucrose at day 0 and aeration at day 4; 4: supplementation with sucrose at day 4 and aeration at day 4.

Incidence de l'addition de saccharose et de l'aération sur la croissance de *Saccharomyces cerevisiae* dans le moût de raisin. — 1: Addition de saccharose au jour 0, pas d'aération; 2: addition de saccharose au jour 4, pas d'aération; 3: addition de saccharose au jour 0 et aération au jour 4; 4: addition de saccharose au jour 4 et aération au jour 4.

cury fermentation lock, for which anaerobic conditions were assumed. Samples were taken, using a sterile syringe and needle, through an opening in the side of the flask, which was closed by a silicon stopper. Supplementation with sucrose (30 g/l) was made by adding a suitable volume of sterile sucrose solution at 50 %. Aeration was made by bubbling oxygen to saturation into the fermenting medium (8 mg/l) during 10 min.

The following experimental conditions were studied:

Condition (1): Supplementation with sucrose at day 0, no aeration
Condition (2): Supplementation with sucrose at day 4, no aeration
Condition (3): Supplementation at day 0, aeration at day 4
Condition (4): Supplementation with sucrose at day 4, aeration at day 4.

The progress of fermentation was followed by measuring sugar utilization as a function of time (RIBÉREAU-GAYON *et al.* 1982). Yeast cells were counted by plating onto a medium of grape juice agar (LAFON-LAFOURCADE *et al.* 1979).

The expression of viable cells (cells/ml) is given by:

$$P = \frac{\Sigma Ni}{\Sigma Ci} \times \frac{1}{ni}$$

Ni = Number of colonies on the Petri-dishes
Ci = Dilution of the samples
ni = Number of Petri-dishes for each dilution.

The growth rate (μ) from time interval (Δt hours) is given by:

$$\mu\,(h^{-1}) = \frac{\log P_2 - \log P_1}{\Delta t}$$

P_2 = Viable cells at time t_2
P_1 = Viable cells at time t_1
$\Delta t = t_2 - t_1$

The average quantity of sugar consumed per day (mg) per 10^6 cells is:

$$q\,(mg) = \frac{\frac{x_2 - x_1}{\frac{(P_1 + P_2)}{2}} \times 10^6}{\times\, n}$$

x_1 and x_2 = Weight of sugar (mg/ml) consumed at times t_1 and t_2
P_1 and P_2 = Viable populations $\times$ 10^6/ml at times t_1 and t_2
n = Interval between t_1 and t_2 expressed in days.

Results

In grape must supplemented with sucrose before fermentation (Fig. 1), on day 3 of fermentation the viable yeasts population was 66 % lesser than in must supplemented on day 4 (Fig. 1, curves 1 and 2), but they reached the same maximum value (2.8×10^7 cells/ml). In the decline phase, the evolution of viable populations was similar.

A momentary aeration of must supplemented with sucrose before fermentation caused, 2 d later, an increase of 25 % in the maximum viable population (Fig. 1, curve 3). During the decline phase, the start of which was delayed until day 10, the viable population was three times greater. An aeration and addition of sucrose simulta-

Table 1

Effect of supplementation with sucrose and aeration on the sugar utilisation during the alcoholic fermentation of grape must [1)]

Incidence de l'addition de saccharose et de l'aération sur la dégradation du sucre au cours de la fermentation alcoolique du moût de raisin

Conditions [2)]	Weight of sugar consumed (g/l) at fermentation day						
	2	4	5	8	10	15	20
1	50	57	82	120	130	157	167
2	52	62	95	127	140	157	167
3			95	132	155	180	192
4			102	145	162	195	200

[1)] Initial sugar concentration 170 g/l. Initial viable yeasts level: 10^5 cells/ml; dry yeast: *Saccharomyces cerevisiae*.

[2)] See Fig. 1.

neously on day 4 of fermentation had a comparable effect on the maximum viable population (Fig. 1, curve 4) but the effect of aeration on survival of population was greatest.

Table 1 indicates the fermentative activity of the yeast cells. One observes a more rapid start of fermentation in must supplemented with sucrose on day 4. Nevertheless,

Table 2

Effect of the initial concentration of sugar and of aeration on the catabolic activity of yeasts populations

Incidence de la concentration initiale en sucre et de l'aération sur l'activité catabolique des levures

Conditions[1)]	mg of sugar consumed per 10^6 cells between the days:		
	3—4	6—10	10—15
1	1.8	0.65	0.48
2	1.5	0.76	0.58
3	1.8	0.43	0.22
4	1.5	0.40	0.24

[1)] See Fig. 1.

at the spontaneous arrest of fermentation the same quantity of sugar was consumed, whatever the stage of sucrose addition to the grape must (167 g/l). Momentary aeration of grape must supplemented with sucrose before fermentation permitted an increase of this value of 25 g/l of sugar and a complete fermentation when aeration and addition of sucrose were simultaneously done (Table 1).

On the other hand, during the first days of fermentation between days 3 and 4 (Table 2) a larger amount of sugar was consumed per unit of viable biomass (mg/10^6 cells) at higher initial sugar concentrations (condition 1 and 3). But 2 d after aeration,

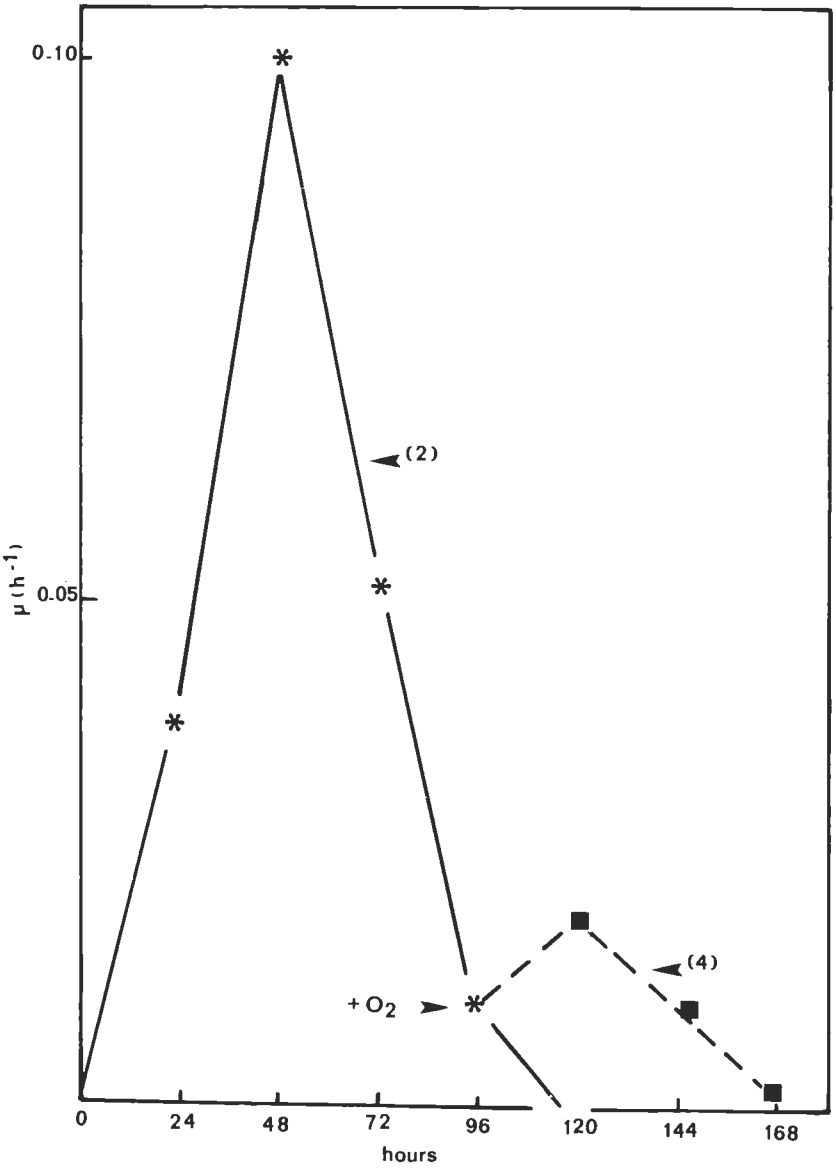

Fig. 2: Effect of supplementation with sucrose and aeration on growth rate of *Saccharomyces cerevisiae* in grape must. — 2: Supplementation with sucrose at day 4, no aeration; 4: supplementation with sucrose at day 4 and aeration at day 4.

Incidence de l'addition de saccharose et de l'aération sur le taux de croissance de *Saccharomyces cerevisiae* dans le moût de raisin. — 2: Addition de saccharose au jour 4, pas d'aération; 4: addition de saccharose au jour 4 et aération au jour 4.

between days 6 and 10, these amounts were lower in the aerated musts (conditions 3 and 4) than in the respective controls (condition 1 and 2).

In the non-aerated musts, the maximum growth rate (μ) of yeast population occurred at day 2 (48 h) of fermentation, the highest value was reached in grape must of low sugar concentration (0.1 h^{-1} instead of 0.09). Then it decreased rapidly and became negative from day 5 (120 h). In the supplemented and simultaneously aerated must (Fig. 2), 24 h after the aeration the growth rate was two times greater and a positive growth rate was maintained for 2 d longer; the start of the declining phase was retarded.

Discussion

The stimulation of the fermentation of grape must results from the sum of three main factors: the total yeast growth, the survival capacity of the population, the catabolic activity of the biomass.

These experiments show:

(i) When the initial sugar content is reduced from 200 to 170 g/l, the growth rate increases, but the catabolic activity per unit of biomass is slightly diminished (Table 2). The net result is an activation at the beginning of the fermentation.

(ii) An aeration during the end of the multiplication phase of the growth cycle causes a strong activation of cellular multiplication and favours the survival of the decline population (Fig. 1), but the catabolic activity per unit of viable biomass decreases (Table 2). The net result is an important stimulation of the fermentation (Table 1).

(iii) The simultaneous aeration and addition of sucrose at the end of the multiplication phase (day 4) mask the growth inhibition by the substrate and avoid its unfavourable effects on the speed of the fermentation (Table 1).

These observations are likely to have an immediate enological application. In the case where an enrichment of the must in sucrose is deemed necessary (chaptalisation), it should be practised in conjunction with an aeration between the days 2 and 4, depending on the rate of fermentation. This process, bringing together the activation due to a moderate initial sugar concentration and that due to a simultaneous addition of sucrose and oxygen, assures an optimal stimulation of the fermentation of the grape must. It allows to avoid certain fermentation difficulties.

Summary

Supplementation of grape must with sucrose has an inhibitory effect on yeast growth, but increases the catabolic activity of yeast populations. It is best done after the initial phase of yeast growth (between days 2 and 4), simultaneously to a short period of aeration which stimulates yeast growth and rate of sugar utilisation.

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