OPTIMIZATION OF WINE ACTIVE DRY YEAST REHYDRATION:

INFLUENCE OF THE REHYDRATION CONDITIONS ON THE RECOVERING FERMENTATIVE ACTIVITY OF DIFFERENT YEAST STRAINS

Virginie SOUBEYRAND, Anne JULIEN**, and Jean-Marie SABLAYROLLES***

* Unité Mixte de Recherche-Sciences pour l'Oenologie, Laboratoire de Microbiologie et Technologie des Fermentations, 2 Place Viala 34060 Montpellier Cedex 1. France

** Lallemand S.A., 19, Rue des Briquetiers, BT59, 31703 BLAGNAC Cedex

Wine Active Dry Yeast (WADY) must be rehydrated before inoculation into the must. This work studies the effects of five rehydration conditions (temperature, water hardness, sugar concentration, agitation and duration) on the recovering fermentative activity of three strains. The effects of conditions varied by yeast strain, however, they were not as influential as expected.

There is a standard protocol for the rehydrating Wine Active Dry Yeasts (WADY). It consists of adding 10 mL of clean water or sugared water (50g/L of glucose) at 37°C to 1g of ADY. The rehydration is carried out at 37°C, for 30 minutes. However, several years ago, several studies showed that some wine active dried yeast strains do not rehydrate well via this protocol. The objective of this work is to study the effects of different rehydration conditions on the recovering fermentative activity of the yeast cells.

ICV-K1-INRA, CEG and EC1118 were the yeast strains studied. These yeasts, produced by Lallemand (Canada), are widely used in winemaking all over the world. They were selected for this study since their behaviors during rehydration are different (the rehydration of CEG can be more difficult than the other two).

Fermenters were inoculated with 25 $g.hL^{-1}$ active dry yeast (corresponding to about 5.10^6 cells/ml). The media used was the synthetic medium described by Bely et al. (Bely et al., 1990) containing 200 $g.l^{-1}$ sugar and an amino acid composition similar to the nitrogen levels of standard grape must.

Selecting the method for rapid evaluation of the recovering fermentative activity of WADY

Three methods were tested for their accuracy; monitoring the pH, dissolved O_2 and dissolved CO_2. The system selected to evaluate the recovering fermentative ability of the WADY after rehydration is the monitoring of dissolved CO_2 in the fermentative medium 50 minutes after the inoculation. Indeed, this measure is the most rapid and reproducible (*Figure 1*). The monitoring of released CO_2 correlates accurately in only a fraction of the time with the results obtained by the carbon dioxide electrode (*Figure 2*).

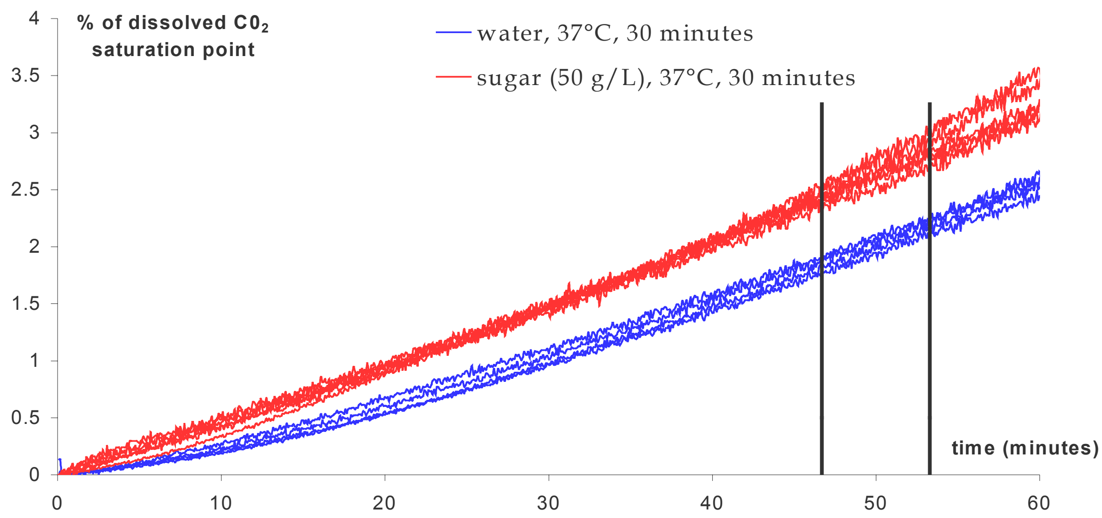

Figure 1 : Monitoring of the dissolved CO_2 in the fermentative medium after rehydration of the ICV-ICV-K1-INRA-INRA yeast in two different mediums (with and without sugar).

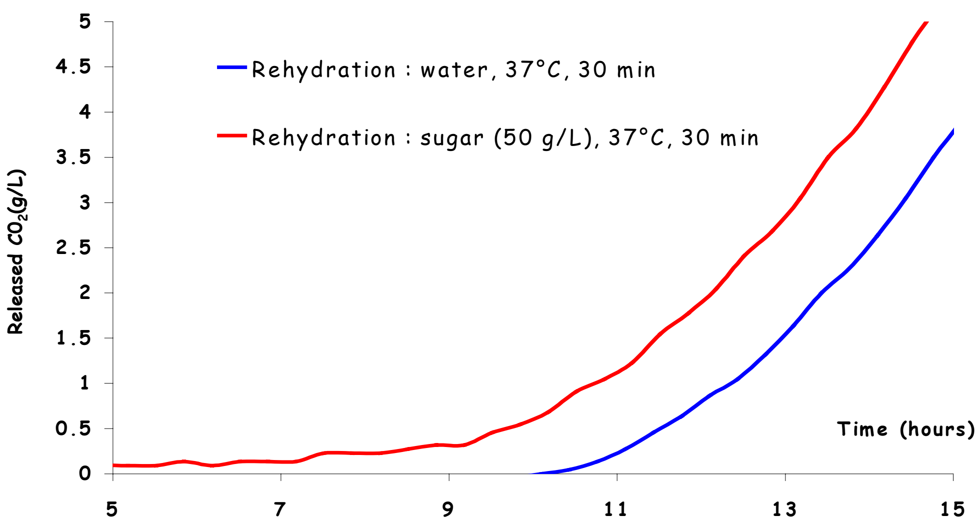

Figure 2: Monitoring of the released CO_2 in the fermentative medium after rehydration of the ICV -K1-INRA yeast in two different mediums (with and without sugar).

Effects of the rehydration conditions on the recovering fermentative activity of WADY (CO_2 dissolved concentration measured 50 minutes after rehydration).

The effects and the interactions of the rehydration conditions are evaluated by two statistical plans. The conditions are temperature, sugar concentration, duration, water hardness, and agitation. They are conditions that winemakers can easily control during rehydration.

For each condition, three levels were tested; ' low', 'medium' and 'high' levels. To reduce the number of trials, two plans were used (Table 1): the first one (PLAN A) addresses the 'low' and 'medium' conditions while the second one (PLAN B) addresses the 'medium' and 'high' conditions.

PLAN A

Condition	Low	Medium
Temperature (°C)	27	35
Sugar concentration (g/L)	0	25
Duration (min)	10	30
Water hardness (° DHT)*	0	12.5
Agitation	No agitation	Continuous

PLAN B

Condition	Medium	High
Temperature (°C)	35	43
Sugar concentration (g/L)	25	50
Duration (min)	30	60
Water hardness (° DHT)*	12.5	25
Agitation	No agitation	Continuous

*: 25°DHT (Total Dissolved Solids) is equivalent to a 300-ppm concentration of the total salts (Ca, Mg).

Table 1: Description of the statistical plans.

These plans do not directly determine the effect of one condition, but instead the combined effect of one condition and of one interaction (or the added effect of two interactions).

The absolute measurement error is assessed by repeating the same measurement often allowing the determination of a "threshold effect".

The effects of the interactions are low compared to the effects of the condition. Therefore, if a sum has a significant effect, it is assumed it is associated to the condition (and not to the interaction of conditions.) To evaluate the level of significance, the following ratio is calculated for each effect:

R = Value of the effect / Value of the threshold effect.

Three brackets are defined:

- **$|R| < 1$**, no significant effect ("0");
- **$1 < |R| < 2$**, significant effect ("+/-");
- **$2 < |R|$**, very significant effect ("++/--").

Yeast Strain Condition	ICV-K1-INRA	EC1118	CEG
Temperature	+	+ +	+
Sugar concentration	+	+ +	++
Duration	+	+	0
Water hardness	0	0	- -
Agitation	0	+	-

Table 2: Effects of the rehydration conditions on the recovering fermentative activity of the 3 yeast strains for PLAN A.

Yeast Strain Condition	ICV-K1-INRA	EC1118	CEG
Temperature	-	0	- -
Sugar concentration	+	0	+
Duration	0	+ +	0
Water hardness	- -	0	0
Agitation	0	0	0

Table 3: Effects of the rehydration conditions on the recovering fermentative activity of the 3 yeast strains for PLAN B.

The raw data (not reported) show that the standard conditions (37°C, 30 minutes, with or without 50 g/L of glucose) represent the optimal conditions for all three strains.

Generally speaking, the effects of the rehydration conditions are less important than expected, in particular, for the ICV-K1-INRA strain. However CEG and EC1118 strains appear more sensitive to the conditions of rehydration.

For ICV-K1-INRA and CEG agitation and rehydration duration have a weak effect. For EC1118 the increase of the duration from 30 to 60 minutes has a weak positive effect under PLAN B (*Table 3*).

Similarly, the hardness has generally a weak effect. A negative effect can be noted occasionally between 0 to 12.5° for CEG (*Table 2*), between 12.5 and 25° for ICV-K1-INRA (*Table 3*).

For all the tested yeast strains, the increase of temperature from 27°C to 35°C has a positive effect especially for EC1118. The increase of temperature from 35 to 43°C (*Table 3*) has either a negative effect (ICV-K1-INRA and CEG), or no effect (EC1118).

Sugar concentration increase is positive with all the yeast strains, in particular between 0 and 25 g/L (*Table 2*). This effect is more marked with the CEG and the EC1118 strains than with the ICV-K1-INRA strain. However other studies (data not presented) show that the presence of sugar in the medium of rehydration causes significant drops in pH having a potentially negative effect on the viability of certain strains. In other words, it is difficult to conclude the benefit of adding sugar during rehydration.

EC1118 behavior is different from the two other strains:

- The positive effect of the temperature increase between 27 to 35°C is far more marked (*Table 2*),
- The temperature increase from 35 to 43°C has no influence (*Table 3*), while it has a negative effect with the ICV-K1-INRA and the CEG yeast strains,
- The increase of duration from 30 to 60 minutes has a weak positive effect (*Table 3*), whereas it has no effect with the two other yeasts.

Conclusions

Generally speaking, two practical conclusions for the rehydration protocol of the WADY are revealed by this study:

- Firstly, the rehydration conditions given by the yeast producers are quite optimal (water with or without 50 g/L of sugar, 37°C, 30 minutes, low water hardness, gentle agitation) and proper adaptation.

-Secondly, the rehydration conditions applied in this study did not significantly affect the three yeasts, at least in term of CO_2 release in the first hours.

This work will be continued with other yeasts. In addition, future studies will also track the effect of rehydration conditions on the complete alcoholic fermentation.

References

Bely M., Sablayrolles J.M., Barre P.: Description of alcoholic fermentation kinetics: its variability and significance, in: American Journal of Enology and Viticulture, 41, (4), 319-323 (1990).