The ability of wine yeast to consume fructose

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Introduction

Stuck fermentations have been the subject of numerous studies, and several have determined the factors responsible for this fermentation problem. Research has shown how certain fermentation conditions, such as nutritional deficiencies, high initial levels of sugar, and the presence of inhibiting compounds, can lead to fermentation problems. The results of this type of research are helping winemakers lower the risk of stuck fermentations significantly.

Under oenological conditions, the main sugars fermentable by *Saccharomyces cerevisiae* are glucose and fructose. Both of these hexoses are generally present in musts in equivalent quantities, but the proportions may vary in some musts. *S. cerevisiae* prefers to consume glucose, which explains why, when fermentations become stuck, the remaining sugar is mainly fructose. The frequency of stuck fermentations showing residual fructose raises the question of the ability of yeast to consume this hexose. The kinetics of sugar utilization by *S. cerevisiae* during fermentation is largely driven by sugar transport, and glucose is typically consumed at a faster rate than fructose. In sluggish fermentations, the maximal rate of fermentation is reduced after most of the glucose is consumed, and fermentation can become stuck with a significant concentration of fructose remaining. According to the literature, the level of residual glucose in stuck wines is 10 times lower than the fructose concentration. According to Gafner and Schûtz (1996), it is possible to predict stuck fermentation when the glucose/fructose ratio (GFR) is under 0.1.

During alcoholic fermentation, sugars are consumed mainly during the stationary phase. During this phase, the available nitrogen gradually becomes less available, and since it is an essential nutrient involved in the transport of sugars into the cell via protein synthesis, this partially explains why both the yeast metabolism and the fermentation activity (Salmon, 1996) slow down. The alcohol level also gradually increases, becoming toxic to the yeast cell, and the use of fructose is even more compromised.

At the molecular level, research has confirmed the genes coding for the hexose transporters in yeast. Under oenological conditions, several genes are involved in sugar transport, which is regulated by a large, multi-gene family called HXT. There are 20 HXT genes. Hxt1 and Hxt7 are the main transporters. Hxt2, Hxt6 and Hxt7 are high-affinity carriers, whereas Hxt1 and Hxt3 are low-affinity carriers. Several other Hxt carriers have intermediate affinity. Both the high- and low-affinity carriers have greater affinity for glucose than fructose, which may affect the rate of utilization of those hexoses. Hexose concentrations in the medium will influence the expression of individual HXT genes (Perez et al., 2005; Guillaume et al., 2007). It has been shown that Hxt3 has the highest capacity to support fermentation (Luyten et al., 2002) and very recent studies have also identified that this gene is indeed responsible for the capacity for consuming fructose among certain yeasts (Guillaume et al., 2007). They also showed that a mutation on an allele of the Hxt3 gene was responsible for improving the performance of wine yeast by utilizing fructose during fermentation and in cases of stuck fermentation.

It is now established that variations exist in the capacity of yeast to consume fructose. The objective of this study was to evaluate the fermentation performance of selected yeasts under oenological conditions, paying particular attention to their capacity to consume fructose. A method was developed to measure the "fructophilic index," which would help determine the ability of a particular yeast to consume fructose.

The "fructophilic" character of yeasts

In our experiments, we assessed the yeasts' capacity to utilize fructose, based on measurable phenotypical criteria.

The different commercial yeasts were selected for their capacity to ferment high-sugar musts and for their aptitude for restarting stuck fermentations.

The impacts of several oenological parameters were studied:

- The initial levels of sugars.
- The glucose/fructose ratio (GFR).
- The initial level of yeast-assimilable nitrogen (YAN).
- The temperature of fermentation.

The criteria evaluated for each yeast were:

- **Fermentation activity** – Fermentation kinetics are represented by the speed of fermentation in terms of time or of CO_2 released.
- **The kinetics of glucose and fructose consumption** – In order to evaluate and differentiate the capacity of yeasts vis-à-vis their fructose uptake, the glucose and fructose contents were measured throughout fermentation to evaluate the kinetics of sugar consumption.

The fructophilic index was based on the calculation of the area between the glucose and fructose consumption curves for the CO_2 released (Figure 1) by the same yeast, and is the criteria selected to evaluate each yeast's capacity to consume fructose and to compare them with each other. We focused on the area located in the last half of the fermentation since it is the critical area where the sugars are mainly consumed. The smaller the area, the closer the fructose consump-

tion kinetics is to the glucose consumption kinetics. We chose this value to represent each yeast and to categorize the oenological yeast strains according to their capacity to utilize fructose. The yeasts whose fructose consumption kinetics are similar to that of glucose are the yeasts that present a fructophilic character and can perform better in high fructose situations.

To validate our ranking system, we included in the study a highly reputable control yeast described as having a strong fructophilic character (Guillaume et al., 2007).

Materials and methods

Oenological yeasts. We utilized several commercially available oenological yeasts and in some cases, yeasts selected for their ability to restart stuck fermentations, such as UVAFERM 43 (YSEO®). Nineteen commercially available yeasts were initially trialled, and four remained, based on their outstanding performance to restart stuck fermentation, in addition to the UVAFERM 43 (YSEO®). They were coded Ref. 1 to Ref. 4.

During microvinification fermentations, 1.1 L of medium in fermenters with a 1.2-litre capacity were inoculated with the yeasts. The inoculation rate is 25 g/hL (corresponding to about 5 x 10^6 cells/mL).

Fermentation environments. In order to compare different, commercially available oenological yeasts, we chose to work in a standard environment: a synthetic medium that mimics the composition of a must (MS300) described by Bely et al. (1991), with some modifications to the initial sugar level (we systematically utilized fructose in a quantity equal to that of the glucose, or in a higher quantity for the experiments where the GFR was <1). Similarly, we varied the total nitrogen concentrations from 100 mg/L to 400 mg/L according to the experiment.

Fermentation. The fermentations were carried out with constant stirring, at 18°C, 24°C or 28°C, in fermenters with a 1.2-litre capacity.

Rate of fermentations

CO_2. The quantity of CO_2 released was determined by the automatic measuring of the loss of weight from each fermenter every 20 minutes. The validity of this technique, developed by the INRA in Montpellier by Jean-Marie Sablayrolles to estimate the sugar and alcohol levels, has been described in numerous papers, including El Haloui et al. (1988) and Sablayrolles et al. (1987).

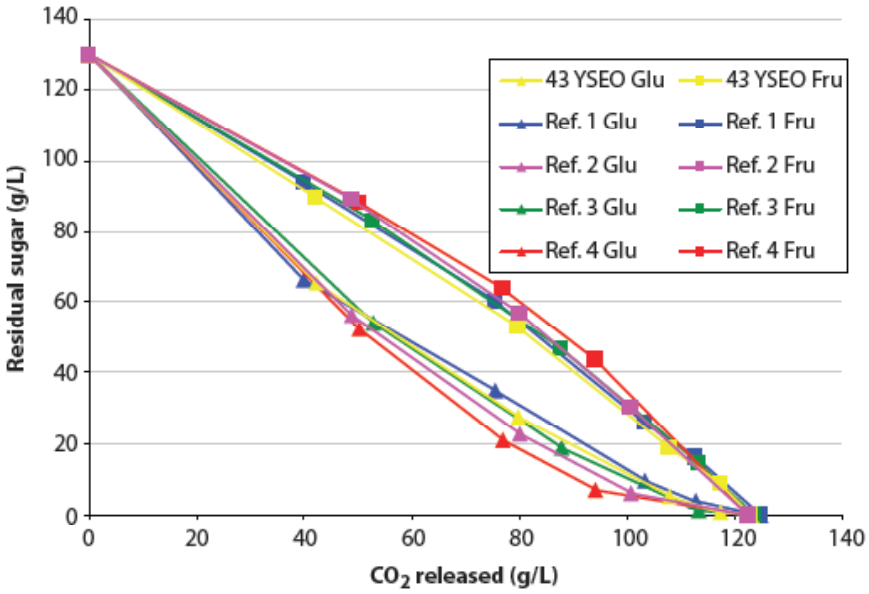

Fig. 1. Evolution of the glucose and the fructose during alcoholic fermentation. Comparison of 5 strains of Saccharomyces cerevisiae. Milieu MS300 Glucose/Fructose (130 g/L of each sugar); 24°C.

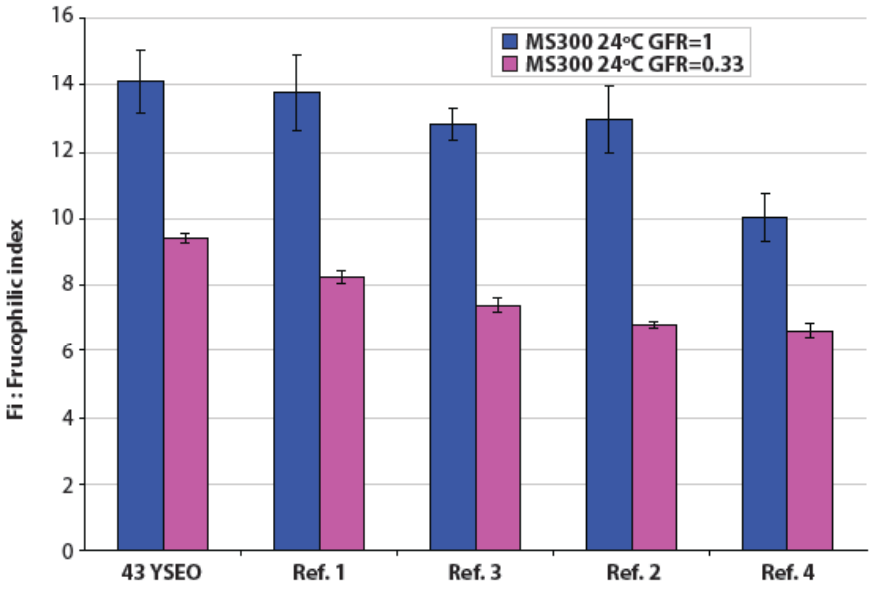

Fig. 2. Impact of the GFR on the fructophilic index for different commercial yeasts.

Rate of CO_2 production (dCO_2/dt). The speed of CO_2 production was calculated by the polynomial smoothing of the 11 last values of CO_2 released. The frequent acquisitions of the release of CO_2 and the precision of the weighing (0.1 g to 0.01 g) allow us to repeatedly calculate the fermentation speed with great precision (Bely et al., 1990).

Glucose and fructose consumption

Samples were taken during fermentation. After centrifugation, the sugars in the supernatant were dosed with the help of the ENZYTEC™ D-Glucose/D-Fructose kits (Scil Diagnostics GmbH, Germany). Different oenological conditions were studied, including the different initial levels of sugars, but only the following oenological conditions were reported:

1. Temperature of fermentation: 24°C. Synthetic medium high in YAN (MS300) and high in sugars, total sugars: 260 g/L, GFR = 1 (glucose = 130 g/L and fructose = 130 g/L)..

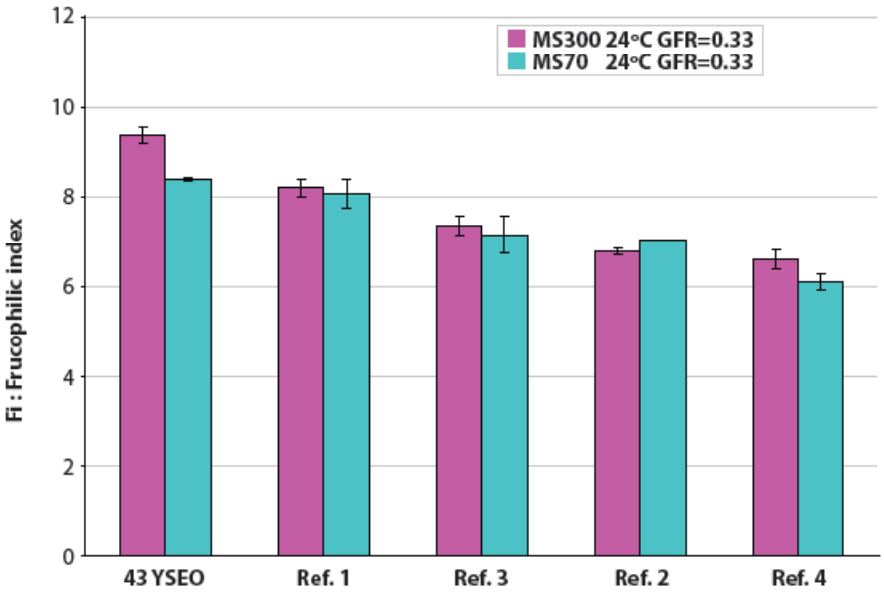

Fig. 3. Ranking of selected yeasts based on the difference in sugar consumption in a medium with the glucose/fructose ratio = 0.33 and with different levels of nitrogen (media deficient in nitrogen or high in nitrogen).

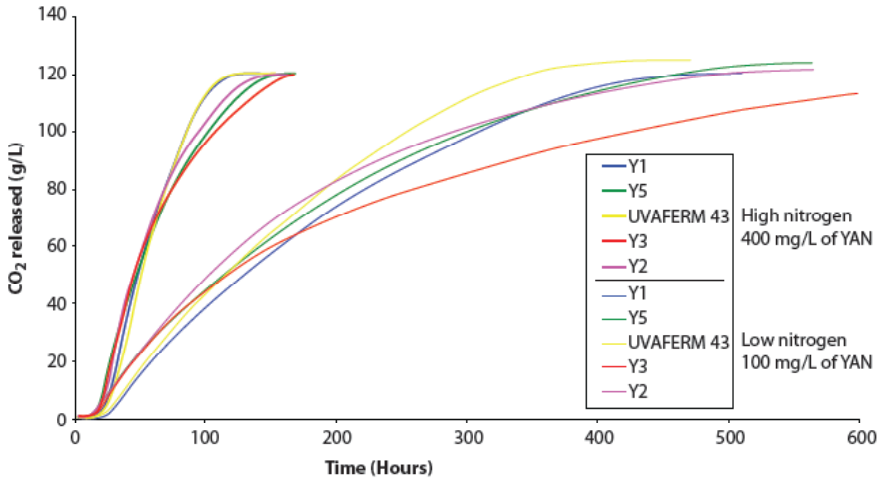

Fig. 4. Comparison of the fermentation behaviour of yeasts in a medium with the glucose/fructose ratio = 0.33 and with different levels of nitrogen (media deficient in nitrogen or high in nitrogen).

2. Temperature of fermentation: 24°C. Synthetic medium high in YAN (MS300), total sugars: 260 g/L, GFR = 0.33 (glucose = 65 g/L and fructose = 195 g/L).

3. Temperature of fermentation: 24°C. Synthetic medium deficient in YAN (MS70), total sugars: 260 g/L, GFR = 0.33 (glucose = 65 g/L and fructose = 195 g/L).

4. Temperature of fermentation: 18°C. Synthetic medium high in YAN (MS300), total sugars: 260 g/L, GFR = 0.33 (glucose = 65 g/L and fructose = 195 g/L).

5. Temperature of fermentation: 28°C. Synthetic medium high in YAN (MS300), total sugars: 260 g/L, GFR = 0.33 (glucose = 65 g/L and fructose = 195 g/L).

Given the number of conditions tested, not all the data on fermentation kinetics and rate of sugar consumption have been reported in this article.

Results

The impact of glucose/fructose ratio

The single variable between oenological conditions 1 and 2 was the GFR: the respective levels of the two hexoses were identical in condition 1 while in condition 2 there were three times more fructose than glucose. Both sugars were monitored during fermentation, and the uptake difference of both sugars was calculated to show the fructophilic index. Figure 2 shows the results of the five yeasts tested in conditions 1 and 2, and regardless of the GFR level (equal to 1 or 0.33), UVAFERM 43 (YSEO®) was the yeast that showed the best ability to consume the fructose. The ranking of the yeasts in terms of their capacity to consume fructose is maintained for both these different glucose/fructose ratios. It also shows that when the GFR is lower than 1, the fructophilic index is also lowered. However, we notice that some yeast is less affected than others. For example, UVAFERM 43 (YSEO®) and Ref. 4 appear to be less affected than the other three, as shown by the level of reduction of the fructophilic index.

The impact of the nitrogen content

When we compared oenological conditions 2 and 3, where the only variable was the initial level of YAN, with a GFR <1, we observed that the UVAFERM 43 (YSEO®) yeast still presents the best performance vis-à-vis fructose consumption (Figure 3), and that the capacity of the yeasts to utilize the fructose is almost maintained, no matter whether YAN was available or there was a nitrogen deficiency (<150 mg/L). Figure 4 shows the impact of nitrogen deficiency on the fermentation activity of yeasts. Fermentation times are about four times longer in the MS70, and there is a notable effect on the maximum speed of fermentation, as in the case of a nitrogen deficiency, the yeast metabolism is slowed significantly. This concurs with the literature (Salmon, 1989, Salmon et al., 1993). Working with a medium deficient in nitrogen is an opportunity to better discern the behaviour of the yeasts, and to demonstrate the variability in the need for nitrogen among yeasts. These findings are completely coherent with a prior study (Julien et al., 2001). These findings also show that the initial levels of nitrogen have a very significant influence on the fermentation activity of yeasts, but do not impact their variable capacity to utilize fructose. In both conditions, UVAFERM 43 (YSEO®) completes the fermentation the earliest with a steady fermentation rate.

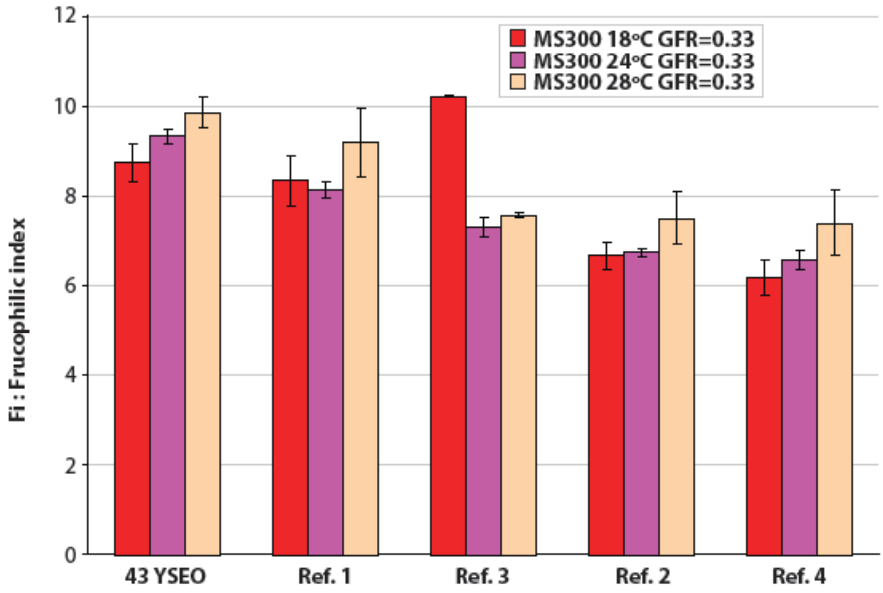

Fig. 4. Ranking of selected yeasts based on the difference in sugar consumption in a medium with the glucose/fructose ratio = 0.33 at different temperatures.

The impact of temperature

We studied the impact of the temperature on the yeasts' capacity to uptake fructose (Figure 5). Results indicated that this capacity increased with the temperature whatever the yeast, except for one specific yeast (Ref. 3). In this case, we see the fructophilic index increased significantly when fermentation was carried at 18°C, compared to fermentation at a higher temperature, but also compared to the other yeasts. This yeast (Ref. 3) is well known for being well adapted to fermenting at low temperature and this could explain its behaviour.

Except for this particular situation, the ranking among the selected yeasts remains the same, with the better fructophilic index for the UVAFERM 43 (YSEO®), whatever the temperature.

The fact that the yeasts' capacity to uptake the fructose is lower at low temperature can be explained by the slower yeast metabolism when the fermentation temperature decreases.

Conclusion

The UVAFERM 43 (YSEO®) yeast consistently showed the smallest area between the glucose and fructose consumption curves during the last half of the fermentation, and therefore has the highest fructophilic index, which means this yeast has the best fructose uptake capacity, whatever the GFR, the nitrogen or temperature levels. This behaviour, although reported only on five yeasts in this paper, was tested on 19 other selected yeasts with the same results.

The selected yeasts differed in their capacity to consume fructose, and that is an indicator of performance in potentially problematic must, where the GFR is lower and/or the must conditions are difficult. The fructophilic index measured as the area difference between glucose and fructose consumption can be a tool used to evaluate the fructophilic capacity of wine yeasts, and to characterize this phenotype and avoid stuck fermentations.

The study of the characterization of the UVAFERM 43 (YSEO®) continues with an in-depth investigation on its ability to restart stuck fermentations and to develop reliable protocols for such situations.

Need more information?

In case you may need more information on the product(s) and details discussed in the above-mentioned article, please contact Piet Loubser, the area manager for Lallemand in South Africa, tel (021) 913-7555, fax (021) 913-5550 or ploubser@lallemand.com.

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