Differences in the glucose and fructose consumption profiles in diverse *Saccharomyces* wine species and their hybrids during grape juice fermentation

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Running title: Sugar consumption during wine fermentation

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Abstract

Yeasts with a high fructose consumption capability are very important for winemakers to solve problems associated with sluggish or stuck fermentations causing undesirable sweetness in wines. In the present study, we analyze the kinetics of glucose and fructose consumption during wine fermentations performed at low (12 ºC) and high (28 ºC) temperatures by twelve different yeast strains belonging to the species *Saccharomyces cerevisiae, S. bayanus* var. *uvarum, S. kudriavzevii* as well as interspecific *Saccharomyces* hybrids. Different mathematical equations (sigmoid, exponential and linear decay functions) were used to fit, by means of linear and nonlinear regressions, the sugar degradation along the fermentative process. Temperature had an important influence on glucose and fructose consumption, and clearly different degradation profiles were observed at 12 and 28 ºC. From the obtained equations, times to consume half and total of the initial glucose and fructose concentrations present in the must were calculated, allowing a quantitative comparison among yeasts in order to select the fastest fermentative yeast according to the fermentation temperature. In general, all yeasts assayed showed a slightly higher preference for glucose than fructose at both temperatures, confirming the glucophilic character of *Saccharomyces* wine yeasts. However, at low temperatures, some *Saccharomyces* yeasts showed a fructophilic character at the beginning of fermentation. This kind of studies can be very useful for the wine industry to select yeast strains with different glucose/fructose preferences.

Keywords: *Saccharomyces*; hybrids; wine yeasts; sugar consumption.

1. Introduction

Glucose and fructose are simple reducing sugars found in many foods. Both mono-saccharides have the same empirical formula ($C_6H_{12}O_6$) but a different structure, which determines considerably their physicochemical properties. Grapes, and consequently musts, usually contain equal amounts of fructose and glucose in a range between 160 and 300 g/L of total sugars (Fleet and Heard, 1993), although recently the climatic change is increasing the proportion of fructose respect to glucose in grapes (Jones et al., 2005). During wine fermentations, both mono-saccharides are co-fermented by yeasts producing diverse compounds such us carbon dioxide, ethanol, glycerol, etc. However, yeasts have a slightly higher preference for glucose than for fructose during wine fermentations, resulting in a difference between the consumption of both sugars along the fermentative process (Fleet, 1998; Berthels et al., 2004). This differential consumption results in a preponderance of fructose during the last phases of fermentation, which must be fermented by yeasts under stress conditions such as nitrogen starvation or high levels of ethanol (Bauer and Pretorius, 2000; Perez et al., 2005). As a consequence, a considerable residual fructose level in fermented musts might be present, with the corresponding risk for microbial spoilage of the finished wine. Moreover, fructose is approximately twice sweeter than glucose, producing undesirable sweetness sensations in dry wines (Boulton et al., 1996). Therefore, wine yeasts with a higher capability of fructose consumption are of interest for the wine industry.

Differences between glucose and fructose fermentation rates may be due either to differential transport across the plasma membrane (Guillaume et al., 2007) or to differences in the hexose phosphorylation occurring inside the cell (Berthels et al.,

47 2008). Both hexose transporters and kinases have different glucose/fructose affinities
48 and preferences. To data, at least 20 *Hxt* genes encoding these transporters have been
49 identified (Wieczorke et al., 1999), and three sugar kinases (Hxk1, Hxk2 and Glk1) are
50 involved in the phosphorylation of fructose and/or glucose (Entian and Barnett, 1992).
51 Berthels et al. (2004) studied the discrepancy between glucose and fructose utilization
52 by seventeen *Saccharomyces cerevisiae* yeasts strains during wine fermentations, as
53 well as the influence of certain environmental variables such as nitrogen
54 supplementation and ethanol addition on this property. Recently, these authors showed
55 that discrepancies in glucose/fructose consumption were related with different
56 hexokinase kinetic properties of the *Saccharomyces* strains (Berthels et al., 2008).
57 However, Guillaume et al. (2007) found and studied a *S. cerevisiae* wine yeast strain
58 with a higher fructose utilization capacity. They concluded that this capability was due
59 to a mutated *Hxt3* allele, providing the first demonstration that the pattern of fructose
60 consumption could be altered by expression of a mutated hexose transporter. But some
61 species of the *Saccharomyces* sensu stricto group exhibit, in addition to facilitated
62 diffusion of glucose and fructose, proton symport systems for both sugars. Specifically,
63 fructose/H^+ symport was found to coexist with the facilitated diffusion system for
64 hexoses in the species *S. bayanus* and *S. pastorianus* (syn. *S. carlsbergensis*) (Rodrigues
65 de Sousa et al., 1995; Gonçalves et al., 2000). Therefore, the use of other
66 *Saccharomyces* species could reduce the levels of residual fructose in wines.

67 To the best of our knowledge, few studies have been addressed to analyze and
68 quantify the glucose and fructose preferences of *Saccharomyces* wine yeasts belonging
69 to other species than *S. cerevisiae* (Berthels et al., 2004). The temperature at which the
70 alcoholic fermentation is conducted affects the yeast growth and duration of

fermentation (Fleet and Heard, 1993). In recent years, there is a preference among winemakers to ferment white and rosé wines at low temperatures (10 to 15 °C) to minimize the loss of aromatic volatiles, and red wines to higher temperatures (18–30 °C) to enhance extraction of anthocyanin pigments (Belloch et al., 2008). However, the effect of low and high temperatures on glucose and fructose consumptions by yeasts is still unknown. The use of mathematical equations focused to study the glucose and fructose degradation during wine fermentations by yeasts could be very useful, providing an objective and valuable information for the wine industry in order to select yeasts with lower sugar degradation times at different temperatures. Moreover, profiles obtained for glucose and fructose utilization could be also used by researches to identify yeast strains with higher fructose preferences.

In the present work, we study the glucose and fructose consumption during 'Tempranillo' must fermentations performed at both low (12 ºC) and high (28 ºC) temperatures by twelve *Saccharomyces* strains. Because most of the previous information on this aspect is only available for *S. cerevisiae* strains, we have included in this study diverse strains of *S. bayanus* var. *uvarum*, *S. kudriavzevii*, *S. cerevisiae* as well as their interspecific hybrids described in previous studies (Bradbury et al., 2006; González et al., 2006; Belloch et al., 2009) in order to obtain a better understanding on yeast sugar preferences in other *Saccharomyces* species. The information obtained could also help to solve problems associated with sluggish and stuck fermentations due to high levels of residual fructose.

2. Material and methods

95 *2.1. Yeast strains and inoculum preparation*

96 A total of twelve yeast strains, three *S. cerevisiae* (*Sc*), three *S. bayanus* var.
97 *uvarum* (*Su*), one *S. kudriavzevii* (*Sk*), one hybrid *Sc x Su*, three hybrids *Sc x Sk,* and one
98 triple hybrid *Sc x Su x Sk*, were used in this work (see Table 1 for abbreviations). The
99 hybrid nature of these strains has been demonstrated in previous studies (Bradbury et
100 al., 2006; González et al., 2006; Belloch et al., 2009). Most of the strains were isolated
101 from wine fermentations in different countries (Spain, France, Italy, Switzerland,
102 Germany, Austria and South Africa) and eight strains are currently commercialized as
103 active dry yeasts, as indicated in Table 1. Single colonies from pure cultures of each
104 species were grown in GPY broth medium (0.5% peptone, 4% glucose, 0.5% yeast
105 extract) at room temperature (25 ± 2 ºC) for 24 h to obtain the different inocula.

106

107 *2.2. Fermentation conditions*

108 Experiments were carried out by triplicate by using 550 mL of 'Tempranillo' red
109 must in sterile 750 mL vessels at two different temperatures (12 and 28 ºC). Therefore,
110 a total of 72 microvinifications (12 yeast strains x 2 temperatures x triplicate) were
111 studied. Previously, the must was clarified by settling for 24 h at 4 °C to separate the
112 clear juice from the sediment in presence of 60 mg/L of sulphur dioxide. After filtration,
113 glucose and fructose were added to reach a total reducing sugar concentration of
114 approximately 228 g/L (112±21 g/L of glucose and 116±17 g/L of fructose). The initial
115 yeast assimilable nitrogen was determined by the formaldehyde index method (Aerny,
116 1996), and then the grape must was made up to 250 mg/L with diammonium sulphate.
117 The medium was also enriched with 0.25 g/L of yeast nutrients (Lallemand Inc,

118 Montréal, Canada). Subsequently, the 'Tempranillo' must was sterilized by adding 1
119 mL/L of dimethyl dicarbonate (Fluka, Switzerland) and inoculated independently with
120 the diverse yeasts to reach an initial population of 6.6 $\log_{10}$ CFU/mL. The initial pH of
121 the medium was 3.5±0.1. Fermentations were followed until the total amount of sugar
122 present in the musts was less than 2 g/L.

123

124 *2.3. Analytical determinations*

125 Samples were taken by triplicate along fermentation and analysed to determine
126 their glucose and fructose concentrations using a chromatographic system, which
127 consisted of a GP40 gradient pump, an ED40 pulsed electrochemical detector, and an
128 AS3500 autosampler system (Dionex Corporation, Sunnyvale, CA, USA). The mobile
129 phase consisted of a mixture of water and 1.0 M sodium hydroxide (52:48, V/V) at a
130 flow rate of 0.4 mL/min. The anion-exchange column used was the CarboPac MA1
131 (Dionex, 4 x 250 mm) with guard (4 x 50 mm). Sugar concentrations were expressed as
132 g/L, and then transformed to percentage (%) of sugar still present in must respect to the
133 initial concentration (100%) for curve-fitting purpose.

134

135 *2.4. Fitting the sugar consumption to diverse mathematical equations*

136 Glucose and fructose utilization by yeasts during fermentation were fitted by
137 means of the three following mathematical equations:

138 1) Linear decay function, used previously by Berthels et al. (2004). Function to
139 fit: $Y=S_0–K*t$. Where Y (dependent variable) is the percentage of glucose or fructose

still present in must, t (independent variable) is the time (days), S_0 is the value of interception in the origin, and K is the kinetic constant (concentration*$days^{-1}$).

2) Exponential decay function, used previously by Arroyo-López et al. (2008). Function to fit: $Y=D+S*e^{-K*t}$. Where Y is the percentage of glucose or fructose still present in must, t is the time (days), D is a specific value when $t \rightarrow \infty$, S is the estimated value of change, and K is the kinetic constant ($days^{-1}$).

3) Sigmoid or altered Gompertz decay function, used previously by Lambert and Pearson (2000). Function to fit: $Y=A+C*e^{-e^{\wedge}(K*(t-M))}$. Where Y is the percentage of glucose or fructose still present in must, t is the time (days), A is the lower asymptote when t tends to infinity ($t \rightarrow \infty$), K is the kinetic constant ($days^{-1}$), C is the distance between the upper and lower asymptote, and M is the time when the inflection point is obtained.

Equations were fitted by means of linear and non-linear regression procedures with Statistica 7.0 software package (StatSoft, Tulsa, OK, USA), minimizing the sum of squares of the difference between experimental data and the fitted model. Fit adequacy was checked by the proportion of variance explained by the model (R^2) respect to experimental data. For each yeast and temperature the three equations were tested, but only the function with the highest R^2 was chosen. Subsequently, the obtained equations were used to calculate the time necessary to consume 50% of the initial sugar concentration present in must (t_{50}), and the time to consume the total of the initial sugar present in must (t_{end}) for glucose and fructose sugars.

3. Results

3.1. Effect of temperature on the sugar degradation profiles

Glucose and fructose consumptions by yeasts were considerably influenced by temperature. At 28 ºC, three different kinetic responses were found as a function of the yeast strains (Figure 1 and Table 2). Several equations (see material and methods) were used to fit sugar degradation profiles, and in all cases the fit was very good with a R^2 between 94.9 and 99.7%. In a first group, formed by the *S. cerevisiae* strains ScT73, FCRY and FRCH, the *S. bayanus* var. *uvarum* BM58 and the *Sc x Sk* hybrid W27, the sugar consumptions during fermentation were explained by means of exponential and linear decay models for glucose and fructose, respectively. Their respective functions are included in Table 2 and a graphic example of this behavior is shown in Figure 1 for yeast ScT73. As can be seen, fructose decreased linearly while glucose decreased exponentially. In a second group, formed by type strains of *S. bayanus* var. *uvarum* (1969) and *S. kudriavzevii* (Sk), the *Sc x Sk* hybrids AMH and VIN7 and the triple hybrid *Sc x Sk x Su* CBS, the glucose and fructose consumptions were both explained by means of exponential decay function (as an example see Figure 1 for yeast CBS). Finally, the *S. bayanus* var. *uvarum* strain 12600 and the *Sc x Su* hybrid S6U formed a third group which showed a linear decay kinetic for glucose and fructose (see Figure 1 for S6U). In all cases, except for the strain AMH, yeasts showed a slightly higher preference for glucose uptake than fructose during the whole fermentation process, as can also be deduced of their respective decay kinetic constants depicted in Table 2. Even though the process started with approximately equal amounts of both sugars, the concomitant but slower utilization of fructose than glucose led to a difference between their respective concentrations. Discrepancies in the utilization of both sugars during

186 fermentation was more obvious for certain yeasts exhibiting an exponential decay
187 kinetics for glucose but linear for fructose, as can be observed for ScT73 in Figure 1.
188 For the remaining yeasts, the differences in the assimilation of both sugars were lower
189 (Figure 1, S6U).

190 Glucose and fructose concentrations rapidly decreased at the beginning of
191 fermentation at 28 ºC, which was indicative of a short lag phase for all yeasts under
192 study. Towards the end of fermentation, the differences between glucose and fructose
193 concentrations decreased considerably. Except for yeast W27, hybrid strains between *Sc*
194 *x Sk* (AMH and VIN7) and the triple hybrid CBS (*Sc* x *Sk* x *Su*), showed sugar
195 degradation patterns similar to those exhibited by *S. kudriavzevii* but clearly different to
196 those of the *S. cerevisiae* parental.

197 Sugar consumption at 12ºC was completely different to those exhibited at 28 ºC.
198 At lower temperature, glucose degradation was very similar for all yeasts but with
199 different kinetic constants. Glucose degradation followed in all cases a sigmoid decay
200 response that was well fitted by means of an altered Gompertz function (R^2 between
201 95.5 and 99.9%). At the beginning of fermentation (0 to 2 days), only a slight decrease
202 in the glucose concentration was observed, and during this period some yeasts (ScT73,
203 BM58 and Sk) consumed fructose even faster than glucose (see Figure 2). With respect
204 to fructose consumption, three different kinetics were observed (Figure 2 and Table 3)
205 showing all of them a good fit with a R^2 between 92.0 and 99.8%. An exponential decay
206 kinetic for fructose was observed for yeasts ScT73 and BM58, while a linear
207 consumption was only reported for yeast Sk (Figure 2, Table 3). However, most strains
208 showed a sigmoid decay response for fructose similar to glucose but with lower kinetic
209 constants, which is indicative that yeasts underwent a greater lag phase at 12 ºC.

210

211 *3.2. Effect of fermentation temperature on sugar degradation times*

212 From a technological point of view, it is very important to obtain the times
213 required to consume half (t_{50}) and total (t_{end}) glucose and fructose initial concentrations
214 present in must. This way, we can predict the extent of the fermentative process
215 depending on the initial sugar concentration of musts. These values can be easily
216 obtained for each yeast and type of sugar with the equations depicted in Tables 2 and 3.

217 At 28 ºC, the yeasts with the lowest t_{50} values for glucose were FCRY and
218 FRCH, with 1.08 and 1.03 days, respectively. The rest of values, except for the hybrid
219 AMH, were included in a very narrow interval (between 1.03 and 2.14 days) (see Table
220 4 for standard deviations). Comparing yeast by yeast, the time to consume 50% of
221 fructose was always higher than the time required to consume 50% of glucose (except
222 for the hybrid strain AMH). This fact is clearly indicative of a slight preference for
223 glucose uptake than fructose at this temperature, and confirms the glucophilic character
224 of the wine *Saccharomyces* strains. However, low values of t_{50} for fructose were also
225 obtained for yeasts FCRY and 1969 (with 1.40 and 1.62 days respectively), indicative
226 of higher fructose rate consumption for both yeasts at the beginning of fermentation.
227 Table 4 shows the differences among yeasts in the time required to consume 50% of
228 glucose and 50% of fructose at 28 ºC respect to their initial concentrations present in the
229 must. The smallest differences are observed for yeasts FCRY and 1969.

230 The fastest consumption of glucose at 28 ºC was observed for yeasts FCRY and
231 12600 (2.90 and 2.88 days respectively). The other strains took between 2.88 – 10.34
232 days to consume the total glucose content. Times to consume fructose were longer than

233 those for glucose, with values between 2.96 and 15.33 days. FCRY was the fastest yeast
234 to consume all fructose present in the must (2.96 days), while yeast 1969, the type strain
235 of *S. bayanus* var. *uvarum*, originally isolated from black currant, was unable to
236 consume the fructose present in the must, leaving a residual fructose concentration of
237 ~19 %. Table 4 shows the differences among yeasts with respect to the time required to
238 consume the total glucose and fructose present in the must at 28 ºC. It can be observed
239 again that FCRY exhibited the smallest differences between t_{end} values for glucose and
240 fructose, which is indicative of similar glucose and fructose consumption rates.

241 However, at 12 ºC, the yeast with the lowest t_{50} value for glucose was Sk (4.48
242 days), while the yeast with the highest value was the non-wine strain 1969 (9.40 days).
243 Once again, the time to consume 50% of fructose was higher than the time to consume
244 50% of glucose, but this difference was very small for some yeasts such as ScT73,
245 BM58, Sk and W27. The lowest value of t_{50} for fructose was obtained for yeast Sk with
246 4.88 days, which showed a slight preference for fructose uptake respect to glucose at the
247 beginning of fermentation (see Figure 2, Sk). Table 4 shows the differences among
248 yeasts in the time (t_{50}) required to consume 50% of glucose and 50% of fructose of the
249 initial concentration present in 'Tempranillo' must at 12 ºC. The strain Sk clearly
250 showed the lowest t_{50} values for both sugars.

251 At 12 ºC, the lowest times to consume the total of the initial glucose
252 concentration present in the must were obtained for the yeasts Sk and 12600 (6.78 and
253 7.02 days respectively). The highest value was obtained for yeast 1969 with 14.23 days.
254 Again, t_{end} for fructose were higher than for glucose for all yeasts assayed, with values
255 between 9.93 and 21.41 days. In this case, Sk was the yeast that faster consumed all
256 fructose present in the must, while the yeast ScT73 was the slowest. The differences

among yeasts in the times (t_{end}) to consume at 12 ºC the total glucose and fructose contents of the must are shown in Table 4. Yeast Sk had the lowest values for both sugars.

Finally, Table 5 shows the ratios between t_{50} and t_{end} parameters obtained for glucose and fructose at 12 ºC divided by those obtained at 28 ºC. These ratios are useful to determine the most affected yeast when temperature decreases. For example, yeast ScT73 underwent a 4.38-fold increase in t_{50} for glucose when temperature changed from 28 ºC to 12 ºC and only a 1.83-fold increase for parameter t_{end}. Thus, time to consume the total glucose for yeasts ScT73 increase 1.83-fold when temperature decreased 16 ºC. In this way, for fructose, the increase was 2.16-fold for t_{50} and 3.71-fold for t_{end} (Table 5). In general, the yeast FRCH exhibited a high effect of temperature on its glucose consumption (8.06-fold increase for t_{50} and 5.14-fold increase for t_{end}). However for fructose, the yeasts with greater variations were VIN7 and FCRY (7.49-fold and 6.92-fold increases for t_{50}; 3.48-fold and 4.67-fold increase for t_{end}, respectively). In general, the type strain of *S. kudriavzevii* (Sk) was less affected than other yeasts when temperature decreased from 28 to 12ºC (low values in Table 5).

4. Discussion

This is the first time than sugar degradation times, independently obtained for glucose and fructose, are supplied for diverse *Saccharomyces* species and their hybrids during wine fermentations. These values were obtained by means of mathematical equations which were used to fit the sugar consumption profiles along the fermentative process. This information could be very useful for the wine industry to select the most

appropriate yeasts to ferment at low or high temperatures, of course, complemented with the organoleptic characterization of the yeast strains.

At 28 ºC the fastest fermentative yeast was FCRY, showing the lowest t_{50} and t_{end} values for glucose and fructose. The fructose uptake in this *S. cerevisiae* strain was practically similar than glucose, which is very appreciated by winemakers. FRCY is indicated for the production of young wines. This strain has a short latency time and worked till the complete sugar depletion. At 12 ºC, the yeast with a better sugar consumption performance was the type strain of *S. kudriavzevii* (Sk). This species has never been described in wine fermentation, and the strain used in this work (*S. kudriavzevii* IFO 1802^T) was isolated from decayed leaves in Japan (Naumov et al., 2000). However, this strain has proven to be a quite good fermentative microorganism at different temperatures (14-32 ºC) in 'Macabeo' and 'Tempranillo' grape musts (González et al., 2007).

Temperature is a very important factor influencing sugar consumption. For all yeasts assayed, glucose and fructose increased their t_{50} and t_{end} values when temperature decreased from 28 to 12 ºC (a total of 16 ºC), resulting Sk the least affected yeast by this variation. Similar results were also obtained by González et al. (2007) for yeasts *S. cerevisiae* T73, *S. kudriavzevii* IFO1802^T, and the hybrids *Sc* x *Sk* W27 and W46 in wine fermentations. These authors reported an increase in the time required to consume the total sugar concentrations present in the must when temperature decreased from 32 to 14 ºC. The time increased from approximately 4 to 8 days in experiments carried out with 'Tempranillo' and 'Macabeo' grape musts. But temperature also influenced the kinetic response of glucose and fructose consumption by yeasts, and different profiles were reported at 12 and 28 ºC. At 28 ºC, exponential decays for glucose and linear for

304 fructose were observed for strains ScT73, FCRY, FRCH, BM58 and W27 (three *S.*
305 *cerevisiae* strains, one *S. bayanus* var. *uvarum* and a *Sc x Sk* hybrid, respectively). A
306 similar response was reported by Guillaume et al. (2007) for the *S. cerevisiae*
307 Fermichamp strain, isolated from French wines, during fermentation experiments
308 performed with synthetic must at 28 ºC. Moreover, in the present study, we have also
309 found an exponential-exponential decay profiles for glucose and fructose consumptions
310 for the *S. kudriavzevii* strain (Sk) and its hybrids AMH, VIN7 and CBS. In general, at
311 28 ºC, *S. kudriavzevii* hybrid strains had a response to fructose consumption similar to
312 *S. kudriavzevii* (exponential decay) and different to *S. cerevisiae* (linear decay), the
313 other parental species. The only exception was the hybrid W27, exhibiting a response
314 similar to *S. cerevisiae*. However, Berthels et al. (2004) showed similar sugar
315 degradations (exponential-exponential) for diverse *S. cerevisiae* strains at 20 ºC in
316 'Colombard' grape must. Finally, we do not have any references for the other profile
317 found in this study (linear-linear decay) for yeasts S6U and 12600 (Figure 1, S6U), a
318 hybrid strain between *Sc x Su* and a strain of *S. bayanus* var. *uvarum* isolated from
319 sweet wines, respectively. The presence of an active fructose transport in *S. bayanus*
320 was reported years ago by Rodrigues de Sousa et al. (1995), which could explain the
321 similar uptake for glucose and fructose found for both strains at this temperature. Mateo
322 et al. (2001) studied the evolution of the total sugar content (glucose+fructose) for *S.*
323 *cerevisiae* and *S. bayanus* strains during fermentation of 'Monastrell' must at 21 ºC.
324 These authors reported different kinetics of sugar degradation (exponential and
325 sigmoid), depending on the yeast used as starter. The strain of *S. bayanus* used in that
326 work showed a larger latent stage than *S. cerevisiae*. Therefore, according to our results
327 and previous studies, it is very difficult to reach any conclusions about the presence of
328 species-specific profiles for the sugar degradation.

329 The kinetics of sugar degradation changed considerably at low temperatures (12
330 ºC). At this temperature most yeasts showed for glucose and fructose consumption a
331 sigmoid-decay response, which was fitted by means of an altered Gompertz function. At
332 the beginning of fermentation both sugars decreased slightly, but then, sugar
333 consumption rate increased (Figure 2, VIN7). González et al. (2007) reported this
334 behavior for the evolution of total sugar content in 'Tempranillo' and 'Macabeo' grape
335 musts at 14 ºC. During the first 2-4 days, total sugar only decreased slightly for the four
336 yeasts assayed. However, the linear (Sk) and exponential decay (ScT73, BM58) found
337 in this work for fructose consumption at 12 ºC (Figure 2, ScT73 and Sk), has never been
338 reported.

339 Important differences among *Saccharomyces* strains with respect to their glucose
340 and fructose degradation profiles were noticed in this work, and in most of the cases,
341 fermentation of fructose always lags behind that of glucose. This behaviour was also
342 reported by Berthels et al. (2004) for commercial wine yeast strains. However, in some
343 cases (for yeasts ScT73, BM58 and Sk at 12 ºC, and AMH at 28 ºC) the fructose
344 consumption initiated before than glucose consumption. The search for fructophilic
345 yeasts is a priority for the wine industry, especially now when the concentration of
346 fructose is increasing in musts due to climatic change (Jones et al., 2005). Berthels et al.
347 (2004) mentioned that a possible explanation between glucose and fructose discrepancy
348 during wine fermentations could be a different ethanol sensitivity of the strains, or a
349 differential rate of nitrogen utilisation, which could affect the sugar transport or
350 phosphorylation. These authors showed that fructose utilisation was stimulated to a
351 greater extent by nitrogen supplementation. However, fructose was significantly more
352 inhibited than glucose by high ethanol levels. Finally, they suggested that determination

of the difference between glucose and fructose consumption along the fermentative process should be considered as a standard procedure during strain evaluation and selection.

5. Conclusions

This study shows that glucose and fructose consumption profiles were considerably influenced by temperature, and the kinetics of degradation for both sugars were different depending on the yeast strains used as starter. In general, yeasts had a slightly higher preference for glucose than for fructose at both temperatures (12 and 28 ºC), but with strain- and time-dependent discrepancies. Among all yeasts assayed, FCRY was the best fermentative yeast at 28ºC in 'Tempranillo' must, showing the lowest (and similar) t_{50} and t_{end} values for glucose and fructose. At 12ºC, the best fermentative yeast was Sk for both sugars, although ScT73 and BM58 also showed a slight preference for fructose uptake than glucose at the beginning of fermentation. Results obtained in this work can be very useful for industry and research to select yeasts with different glucose/fructose preferences, and develop further studies on the molecular basis of sugar assimilation differences among yeast species. In this way, new wine strains with interesting industrial applications could be obtained by hybridization or genetic manipulation.

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Table 1. Yeast strains used in the present study and source where were isolated.

Strain[†]	Species/Hybrid	Commercial and non-commercial strains	Source
ScT73	*S. cerevisiae*	Lalvin T.73[††]	Wine (Spain)
FCRY	*S. cerevisiae*	Fermol cryophile[††] AEB	Wine (France)
FRCH	*S. cerevisiae*	Fermol Remis Champagne[††] AEB	Sparkling wine (France)
BM58	*S. bayanus* var.*uvarum*	BM58[††]	Wine (Spain)
1969	*S. bayanus* var. *uvarum*	CECT1969 [T]	Black currant (The Netherlands)
12600	*S. bayanus* var. *uvarum*	CECT12600	Sweet wine (Spain)
Sk	*S. kudriavzevii*	IFO1802 [T]	Decayed leaves (Japan)
S6U	Hybrid of *S. cerevisiae* x *S. bayanus* var. *uvarum*	Lalvin S6U[††]	Wine (Italy)
W27	Hybrid of *S. cerevisiae* x *S. kudriavzevii*	Lalvin W27[††]	Wine (Switzerland)
AMH	Hybrid of *S. cerevisiae* x *S. kudriavzevii*	Assmanhausen[††]	Wine (Germany)
VIN7	Hybrid of *S. cerevisiae* x *S. kudriavzevii*	VIN7[††]	Wine (South Africa)
CBS	Hybrid of *S. cerevisiae* x *S. bayanus* x *S. kudriavzevii*	CBS 2834	Wine (Switzerland)

[†] Yeast reference used in the present work.

[††] Yeast strains currently commercialized as active dry yeasts.

[T] Type strain.

Table 2. Type of model and equations used to predict sugar consumption in 'Tempranillo' must at 28 ºC according to yeast strains.

Yeast	Sugar	Type of function used	Equation†
ScT73	Glucose	Exponential decay	$Y=-13.77(\pm9.16)+117.09(\pm11.18)*e^{(-0.437(\pm0.114)*t))}$
	Fructose	Linear decay	$Y= 103.57(\pm5.03) – 17.91(\pm1.11)*t$
BM58	Glucose	Exponential decay	$Y=-43.92(\pm6.95)+150.66(\pm6.78)*e^{(-0.272(\pm0.027)*t))}$
	Fructose	Linear decay	$Y= 100.73(\pm0.64) – 16.95(\pm0.43)*t$
Sk	Glucose	Exponential decay	$Y=-14.32(\pm14.46)+120.66(\pm14.89)*e^{(-0.395(\pm0.094)*t))}$
	Fructose	Exponential decay	$Y=-24.74(\pm14.60)+132.60(\pm10.71)*e^{(-0.199(\pm0.014)*t))}$
W27	Glucose	Exponential decay	$Y=-23.93(\pm0.95)+126.92(\pm1.45)*e^{(-0.385(\pm0.002)*t))}$
	Fructose	Linear decay	$Y= 98.56(\pm2.82) – 18.60(\pm1.58)*t$
S6U	Glucose	Linear decay	$Y= 101.03(\pm2.21) – 23.79(\pm2.97)*t$
	Fructose	Linear decay	$Y= 100.35(\pm7.43) – 19.17(\pm2.96)*t$
AMH	Glucose	Exponential decay	$Y=-32.94(\pm1.64)+132.23(\pm3.42)*e^{(-0.134(\pm0.004)*t))}$
	Fructose	Exponential decay	$Y=3.10(\pm6.24)+82.97(\pm6.85)*e^{(-0.223(\pm0.035)*t))}$
FCRY	Glucose	Exponential decay	$Y=-49.44(\pm12.93)+151.65(\pm13.93)*e^{(-0.393(\pm0.070)*t))}$
	Fructose	Linear decay	$Y= 94.73(\pm2.80) – 31.86(\pm0.69)*t$
FRCH	Glucose	Exponential decay	$Y=-12.40(\pm1.78)+112.54(\pm1.81)*e^{(-0.575(\pm0.032)*t))}$
	Fructose	Linear decay	$Y= 97.33(\pm2.23) – 24.45(\pm0.31)*t$
1969	Glucose	Exponential decay	$Y=-9.88(\pm4.94)+115.81(\pm7.81)*e^{(-0.499(\pm0.088)*t))}$
	Fructose	Exponential decay	$Y=10.00(\pm10.61)+91.32(\pm13.01)*e^{(-0.567(\pm0.285)*t))}$
12600	Glucose	Linear decay	$Y= 102.90(\pm1.69) – 35.72(\pm0.58)*t$
	Fructose	Linear decay	$Y= 94.20(\pm1.56) – 25.79(\pm0.09)*t$
VIN7	Glucose	Exponential decay	$Y=-12.21(\pm0.43)+115.64(\pm1.36)*e^{(-0.490(\pm0.084)*t))}$
	Fructose	Exponential decay	$Y=-27.71(\pm3.01)+129.77(\pm0.70)*e^{(-0.297(\pm0.072)*t))}$
CBS	Glucose	Exponential decay	$Y=-6.41(\pm2.08)+111.13(\pm5.13)*e^{(-0.448(\pm0.032)*t))}$
	Fructose	Exponential decay	$Y=-24.83(\pm13.47)+125.33(\pm12.33)*e^{(-0.225(\pm0.079)*t))}$

†Y= % sugar present in must (dependent variable); t= time (units, days) (independent variable). Standard deviation obtained from triplicate experiments in parentheses.

Table 3. Type of model and equations used to predict sugar degradation in 'Tempranillo' must according to yeast strains at 12 ºC.

Yeast	Sugar	Type of function used	Equation[†]
ScT73	Glucose	Sigmoid decay	$Y=3.91(\pm1.50)+107.31(\pm2.58)*e^{-e^{\wedge}(0.365(\pm0.062)*(t-6.71(\pm0.91)))}$
	Fructose	Exponential decay	$Y=-20.14(\pm2.05)+121.15(\pm5.90)*e^{(-0.084(\pm0.007)*t))}$
BM58	Glucose	Sigmoid decay	$Y=1.84(\pm0.21)+103.94(\pm3.64)*e^{-e^{\wedge}(0.486(\pm0.105)*(t-6.27(\pm0.07)))}$
	Fructose	Exponential decay	$Y=-18.44(\pm6.94)+119.37(\pm8.32)*e^{(-0.095(\pm0.014)*t))}$
Sk	Glucose	Sigmoid decay	$Y=0.83(\pm0.06)+106.43(\pm4.49)*e^{-e^{\wedge}(0.551(\pm0.072)*(t-4.95(\pm0.48)))}$
	Fructose	Linear decay	$Y= 98.49(\pm4.36) – 9.94(\pm0.36)*t$
W27	Glucose	Sigmoid decay	$Y=2.90(\pm1.89)+97.13(\pm1.89)*e^{-e^{\wedge}(1.900(\pm0.028)*(t-6.71(\pm0.14)))}$
	Fructose	Sigmoid decay	$Y=-0.83(\pm4.78)+134.2(\pm38.6)*e^{-e^{\wedge}(0.317(\pm0.266)*(t-6.93(\pm0.81)))}$
S6U	Glucose	Sigmoid decay	$Y=4.70(\pm0.47)+99.54(\pm0.12)*e^{-e^{\wedge}(0.585(\pm0.052)*(t-6.92(\pm0.08)))}$
	Fructose	Sigmoid decay	$Y=0.19(\pm2.10)+107.33(\pm0.50)*e^{-e^{\wedge}(0.334(\pm0.079)*(t-9.08(\pm0.31)))}$
AMH	Glucose	Sigmoid decay	$Y=4.16(\pm0.93)+112.02(\pm0.48)*e^{-e^{\wedge}(0.414(\pm0.054)*(t-7.98(\pm1.08)))}$
	Fructose	Sigmoid decay	$Y=1.25(\pm4.33)+119.7(\pm13.2)*e^{-e^{\wedge}(0.189(\pm0.048)*(t-11.05(\pm0.40)))}$
FCRY	Glucose	Sigmoid decay	$Y=3.67(\pm0.61)+117.67(\pm2.19)*e^{-e^{\wedge}(0.555(\pm0.062)*(t-7.46(\pm0.38)))}$
	Fructose	Sigmoid decay	$Y=1.14(\pm1.87)+104.8(\pm3.3)*e^{-e^{\wedge}(0.310(\pm0.045)*(t-10.60(\pm0.05)))}$
FRCH	Glucose	Sigmoid decay	$Y=2.03(\pm0.30)+111.92(\pm4.96)*e^{-e^{\wedge}(0.552(\pm0.103)*(t-8.61(\pm0.27)))}$
	Fructose	Sigmoid decay	$Y=-5.60(\pm8.66)+114.3(\pm1.5)*e^{-e^{\wedge}(0.270(\pm0.004)*(t-11.12(\pm0.98)))}$
1969	Glucose	Sigmoid decay	$Y=4.48(\pm1.35)+114.15(\pm4.05)*e^{-e^{\wedge}(0.224(\pm0.009)*(t-9.78(\pm0.85)))}$
	Fructose	Sigmoid decay	$Y=18.47(\pm8.13)+84.1(\pm11.5)*e^{-e^{\wedge}(0.159(\pm0.116)*(t-7.62(\pm6.32)))}$
12600	Glucose	Sigmoid decay	$Y=5.45(\pm0.03)+100.58(\pm4.85)*e^{-e^{\wedge}(0.604(\pm0.006)*(t-6.13(\pm0.20)))}$
	Fructose	Sigmoid decay	$Y=-1.82(\pm2.69)+177.4(\pm72.0)*e^{-e^{\wedge}(0.133(\pm0.055)*(t-5.90(\pm4.06)))}$
VIN7	Glucose	Sigmoid decay	$Y=3.12(\pm0.89)+115.43(\pm7.06)*e^{-e^{\wedge}(0.265(\pm0.044)*(t-9.37(\pm0.68)))}$
	Fructose	Sigmoid decay	$Y=1.33(\pm0.84)+106.1(\pm1.2)*e^{-e^{\wedge}(0.233(\pm0.003)*(t-14.26(\pm0.60)))}$
CBS	Glucose	Sigmoid decay	$Y=2.84(\pm0.00)+113.47(\pm3.10)*e^{-e^{\wedge}(0.303(\pm0.036)*(t-7.93(\pm0.02)))}$
	Fructose	Sigmoid decay	$Y=2.97(\pm1.52)+112.7(\pm1.6)*e^{-e^{\wedge}(0.195(\pm0.002)*(t-13.29(\pm0.16)))}$

[†]Y= % sugar present in must (dependent variable); t= time (units, days) (independent variable). Standard deviation obtained from triplicate experiments in parentheses.

Table 4. Times (days) to consume half (t_{50}) and total (t_{end}) of the initial concentration of glucose and fructose present in 'Tempranillo' must according to temperature and yeast strains.

Yeast	**Glucose 28 ºC**		**Fructose 28 ºC**		**Glucose 12 ºC**		**Fructose 12 ºC**	
	t_{50}	t_{end}	t_{50}	t_{end}	t_{50}	t_{end}	t_{50}	t_{end}
ScT73	1.42 (0.26)	5.17 (0.08)	2.98 (0.09)	5.77 (0.07)	6.23 (0.81)	9.48 (1.39)	6.44 (0.34)	21.41 (2.34)
BM58	1.74 (0.07)	4.54 (0.03)	3.00 (0.05)	5.85 (0.24)	5.72 (0.03)	8.37 (0.52)	5.90 (0.53)	19.99 (0.39)
Sk	1.62 (0.12)	6.11 (1.44)	2.91 (0.35)	8.82 (2.10)	4.48 (0.45)	6.78 (0.24)	4.88 (0.61)	9.93 (0.77)
W27	1.39 (0.00)	4.32 (0.03)	2.61 (0.07)	5.30 (0.30)	6.43 (0.16)	7.54 (0.16)	6.72 (0.51)	11.79 (3.27)
S6U	2.14 (0.19)	4.26 (0.45)	2.62 (0.02)	5.26 (0.43)	6.69 (0.36)	7.82 (1.01)	8.24 (0.64)	11.86 (0.83)
AMH	3.47 (0.17)	10.34 (0.21)	1.97 (1.04)	15.33 (8.31)	7.69 (1.15)	10.40 (0.72)	10.47 (1.42)	16.51 (0.00)
FCRY	1.08 (0.09)	2.90 (0.09)	1.40 (0.05)	2.96 (0.02)	7.33 (0.31)	9.27 (0.59)	9.69 (0.50)	13.85 (0.41)
FRCH	1.03 (0.02)	3.84 (0.00)	1.93 (0.11)	3.98 (0.14)	8.31 (0.22)	10.45 (0.07)	9.87 (0.09)	14.82 (1.04)
1969	1.34 (0.21)	5.06 (0.01)	1.62 (0.60)	*	9.40 (0.81)	14.23 (1.04)	10.99 (2.00)	16.19 (0.05)
12600	1.47 (0.02)	2.88 (0.00)	1.71 (0.07)	3.65 (0.07)	5.97 (0.03)	7.02 (1.27)	7.64 (0.99)	14.09 (0.64)
VIN7	1.28 (0.21)	4.65 (0.84)	1.76 (0.31)	5.32 (0.94)	8.98 (0.57)	13.19 (0.04)	13.19 (0.62)	18.54 (0.66)
CBS	1.51 (0.13)	6.42 (0.17)	2.40 (0.48)	7.63 (0.60)	7.49 (0.07)	11.25 (0.43)	12.60 (0.43)	18.40 (0.21)

*Fructose was not completely consumed at 28 ºC. Standard deviation obtained from triplicate experiments in parentheses.

Table 5. Ratios between t_{50} and t_{end} parameters obtained for glucose and fructose consumption at 12 ºC divided by those obtained at 28 ºC.

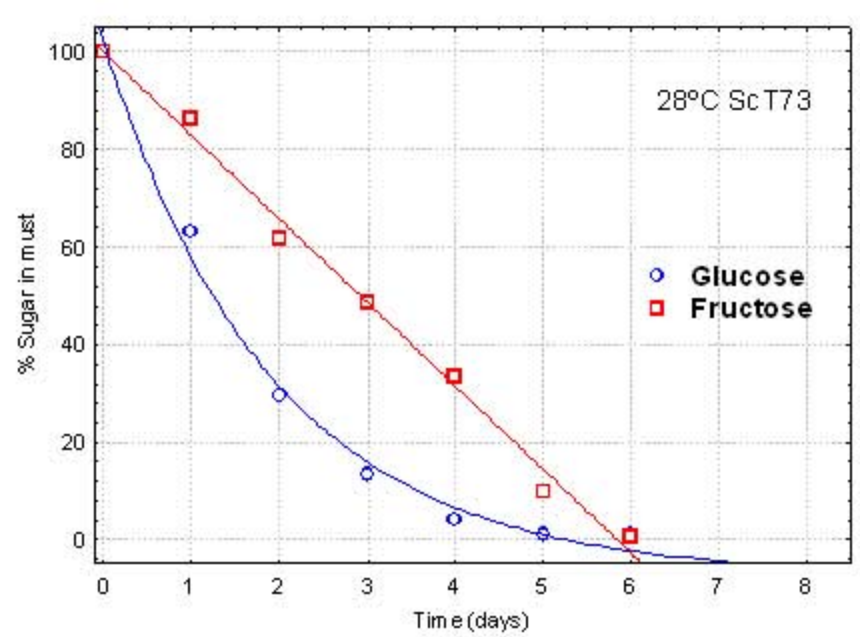
Yeast	Glucose		Fructose	
	t_{50} 12ºC/t_{50} 28ºC	**t_{end} 12ºC/t_{end} 28ºC**	**t_{50} 12ºC/t_{50} 28ºC**	**t_{end} 12ºC/t_{end} 28ºC**
ScT73	4.38	1.83	2.16	3.71
BM58	3.28	1.84	1.96	3.41
Sk	2.76	1.10	1.67	1.12
W27	4.62	1.74	2.57	2.22
S6U	3.12	1.83	3.14	2.25
AMH	2.21	1.01	5.31	1.07
FCRY	6.78	3.19	6.92	4.67
FRCH	8.06	5.14	5.11	3.72
1969	7.01	2.81	6.78	*
12600	4.06	2.43	4.46	3.86
VIN7	7.01	2.83	7.49	3.48
CBS	4.96	1.75	5.25	2.41

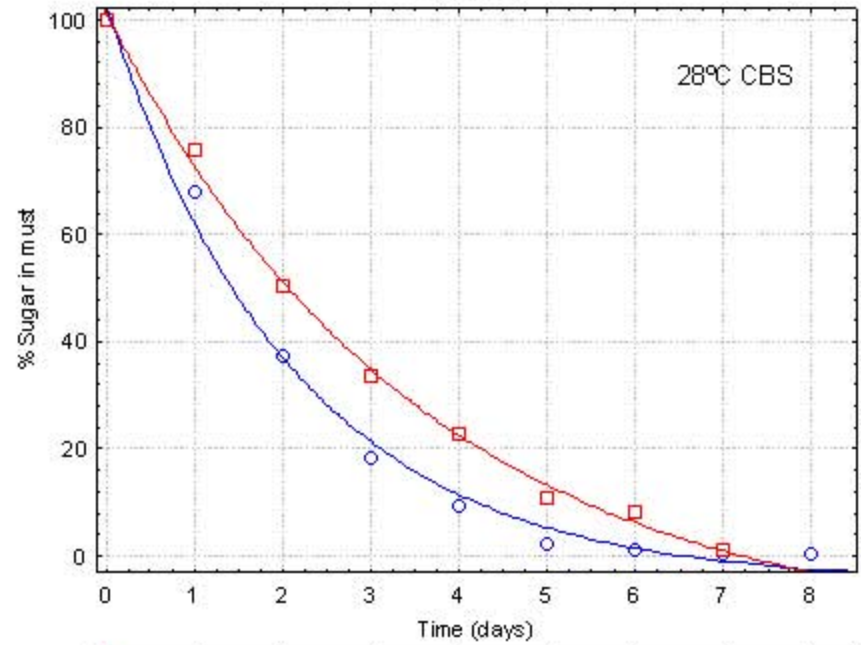
*Fructose was not completely consumed at 28 ºC.

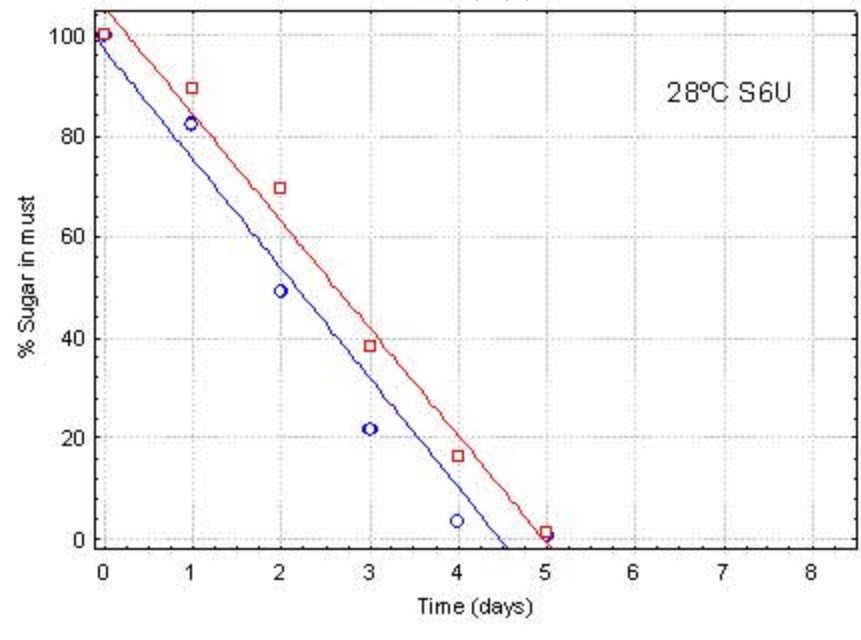
Figure legends

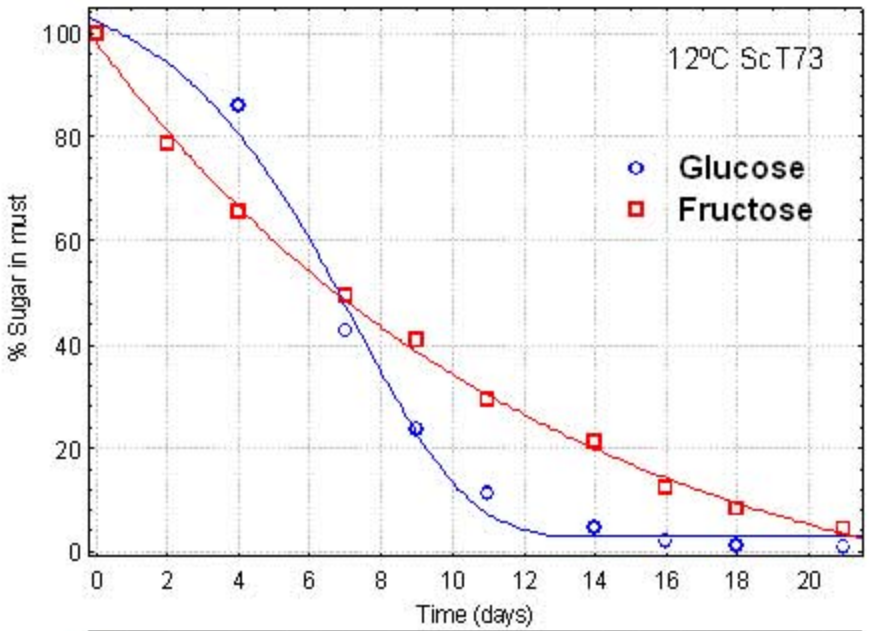
Figure 1. Different glucose (circles) and fructose (squares) consumption profiles observed at 28 ºC for yeasts *S. cerevisiae* T73 (ScT73), the triple hybrid *S. cerevisiae* x *S. bayanus* x *S. kudriavzevii* CBS 2834, and the hybrid *S. cerevisiae* x *S. bayanus* S6U. The other yeasts under study showed similar profiles to these depicted as examples.

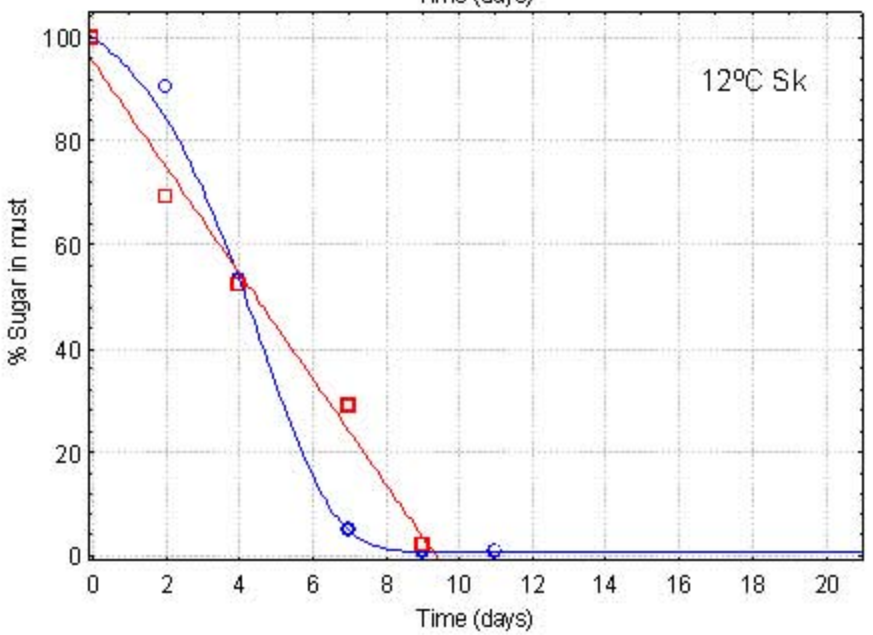
Figure 2. Different glucose (circles) and fructose (squares) consumption profiles detected at 12 ºC for yeasts *S. cerevisiae* T73 (ScT73), *S. kudriavzevii* (Sk), and the hybrid *S. cerevisiae* x *S. kudriavzevii* VIN7. The other yeasts under study showed similar profiles to these depicted as examples.

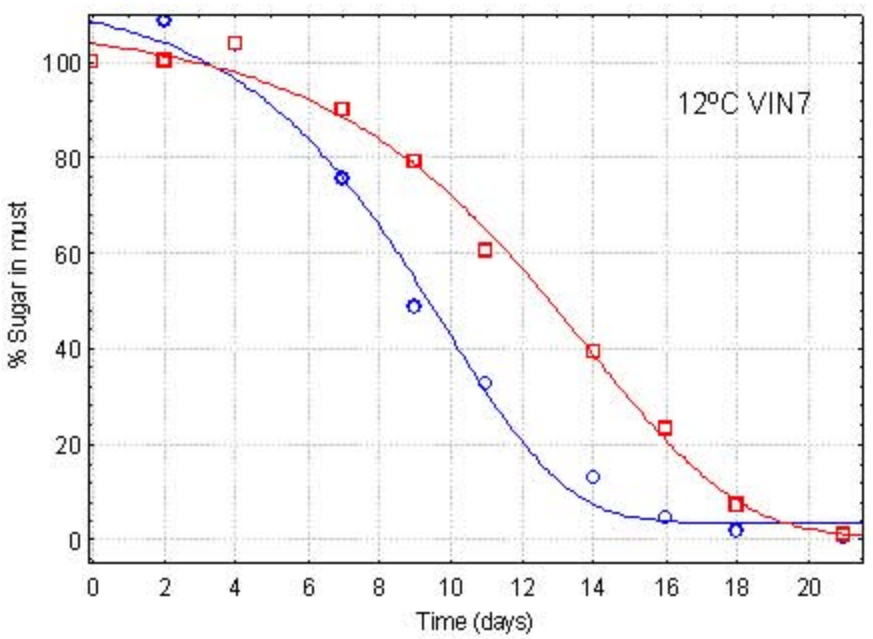

28°C ScT73
Glucose
Fructose
% Sugar in must
Time (days)

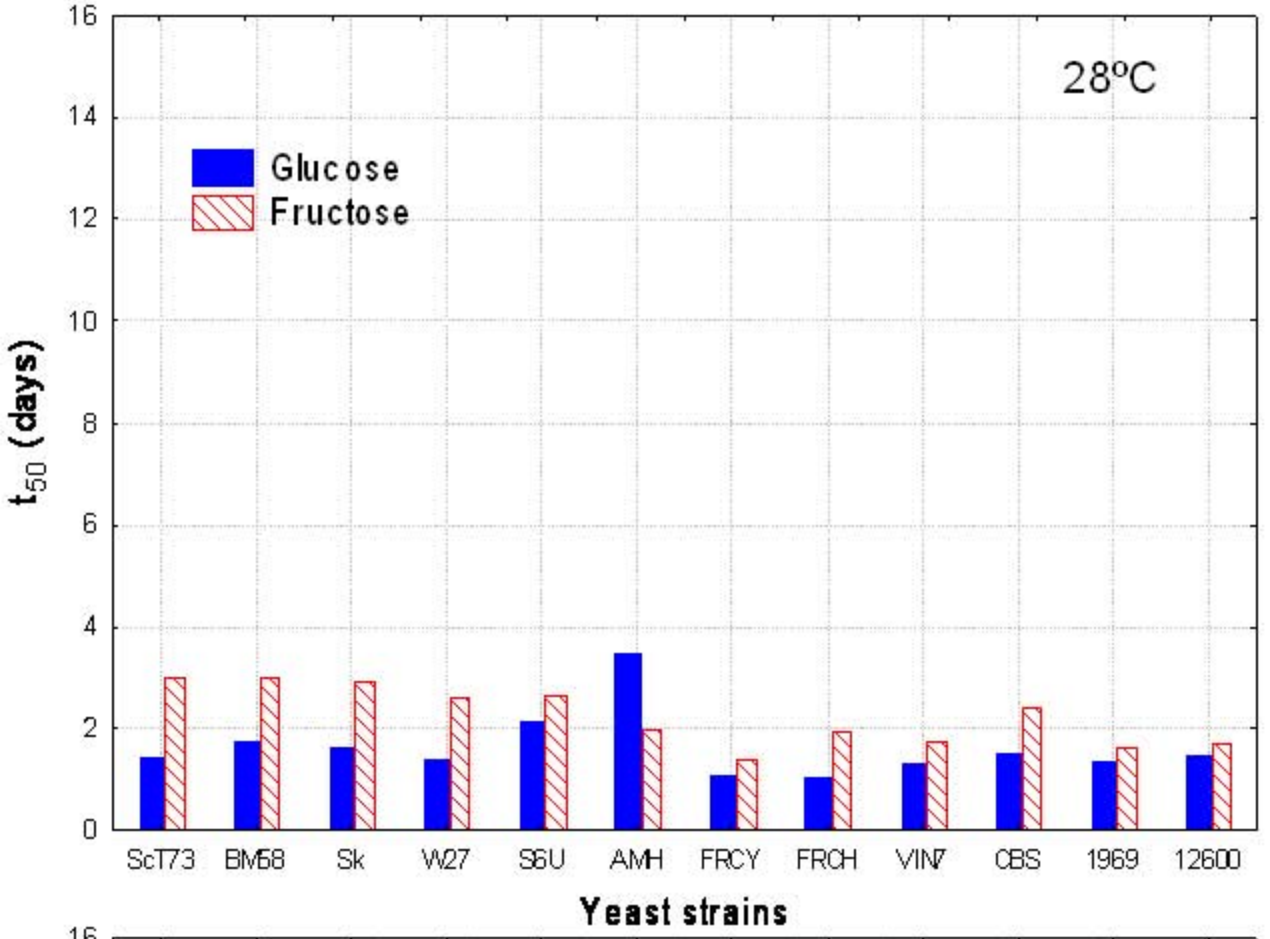

28°C CBS
% Sugar in must
Time (days)

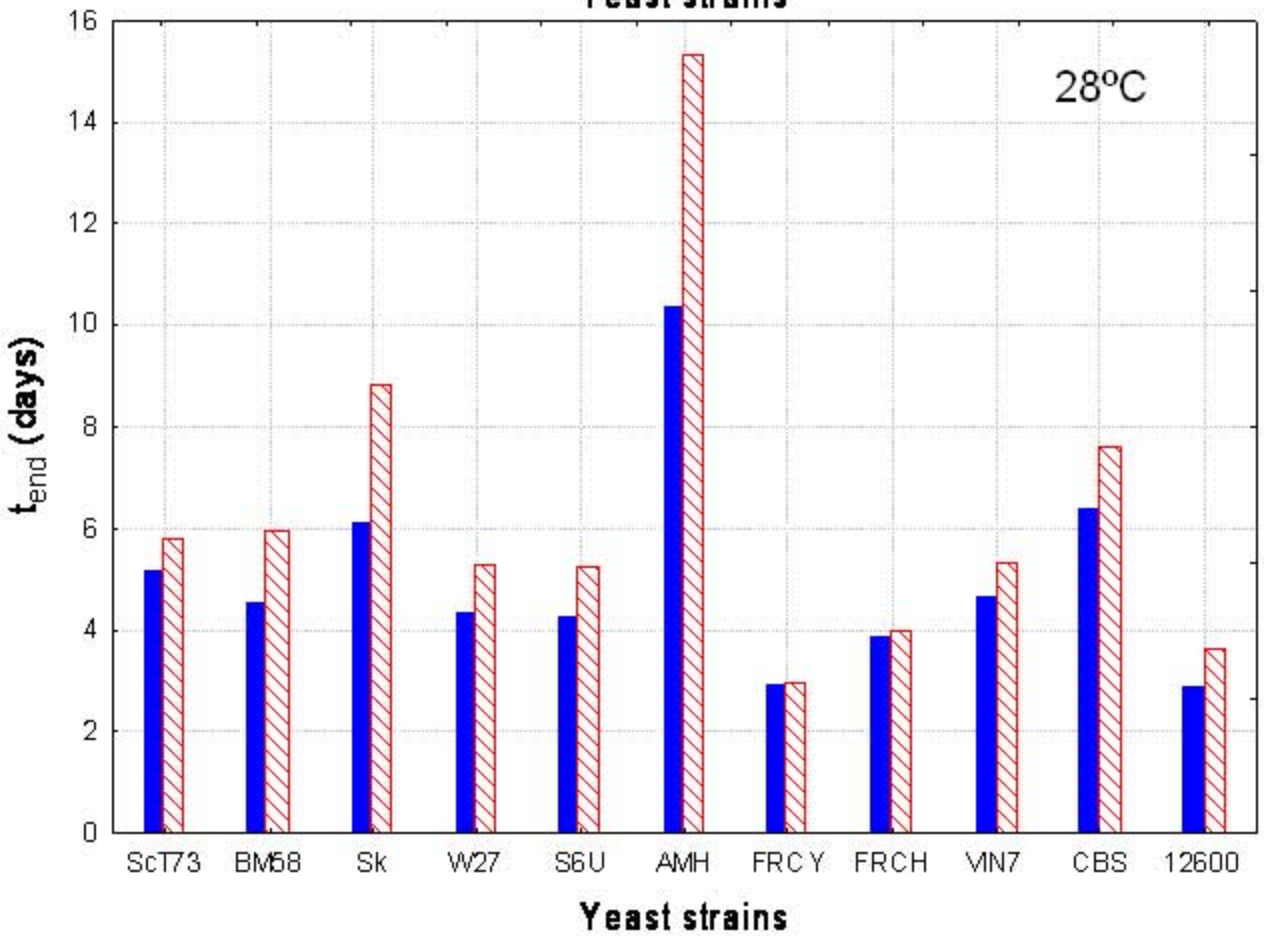

28°C S6U
% Sugar in must
Time (days)


12ºC ScT73
Glucose
Fructose
% Sugar in must
Time (days)


12ºC Sk
% Sugar in must
Time (days)


12ºC VIN7
% Sugar in must
Time (days)


28ºC
Glucose
Fructose
t_{50} (days)
0
2
4
6
8
10
12
14
16
ScT73
BM68
Sk
W27
S6U
AMH
FRCY
FRCH
VIN7
CBS
1969
12600
Yeast strains


28ºC
t_{end} (days)
0
2
4
6
8
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12
14
16
ScT73
BM68
Sk
W27
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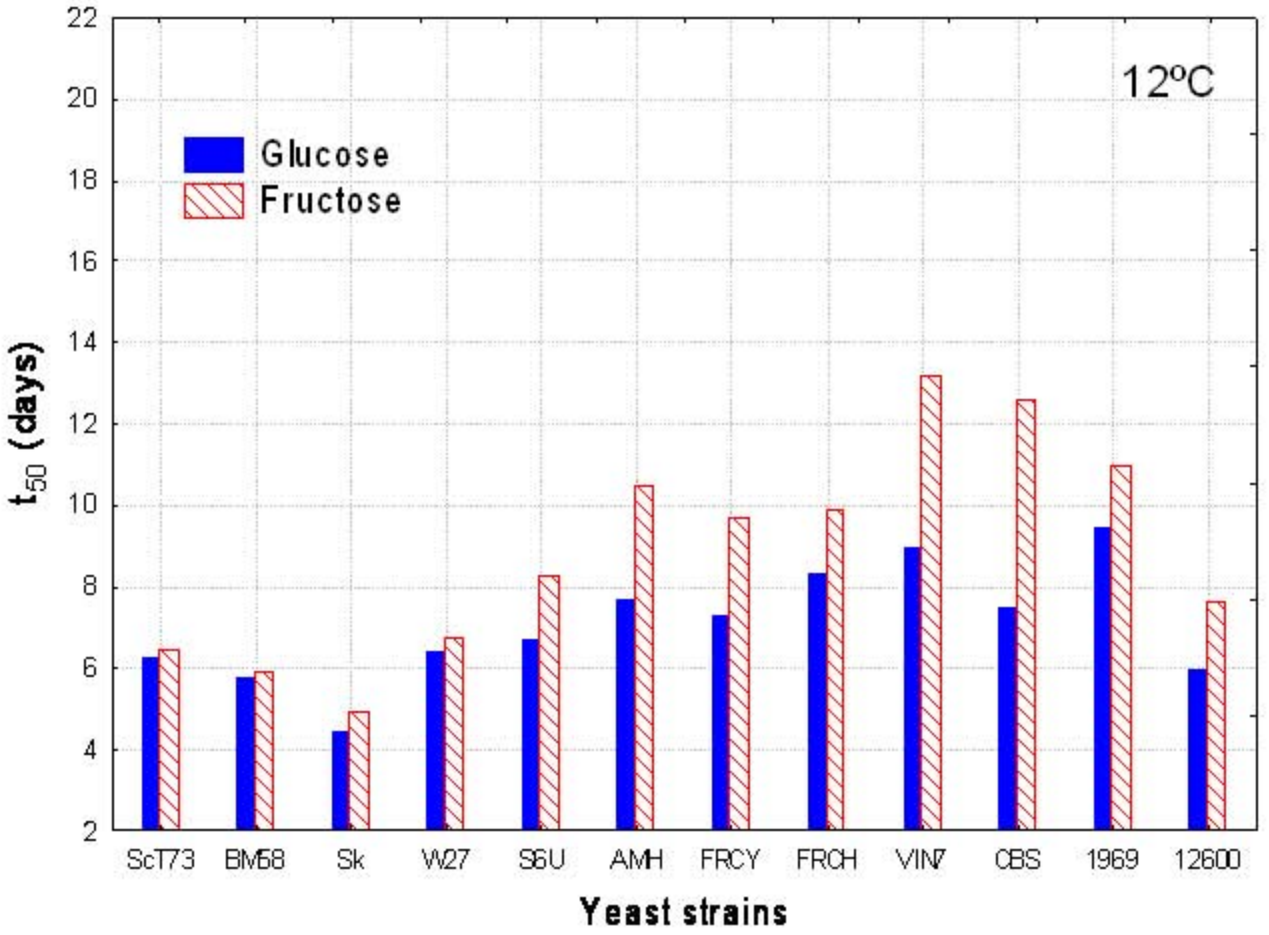
AMH
FRCY
FRCH
VIN7
CBS
12600
Yeast strains


12ºC
Glucose
Fructose
t_{50} (days)
22
20
18
16
14
12
10
8
6
4
2
ScT73
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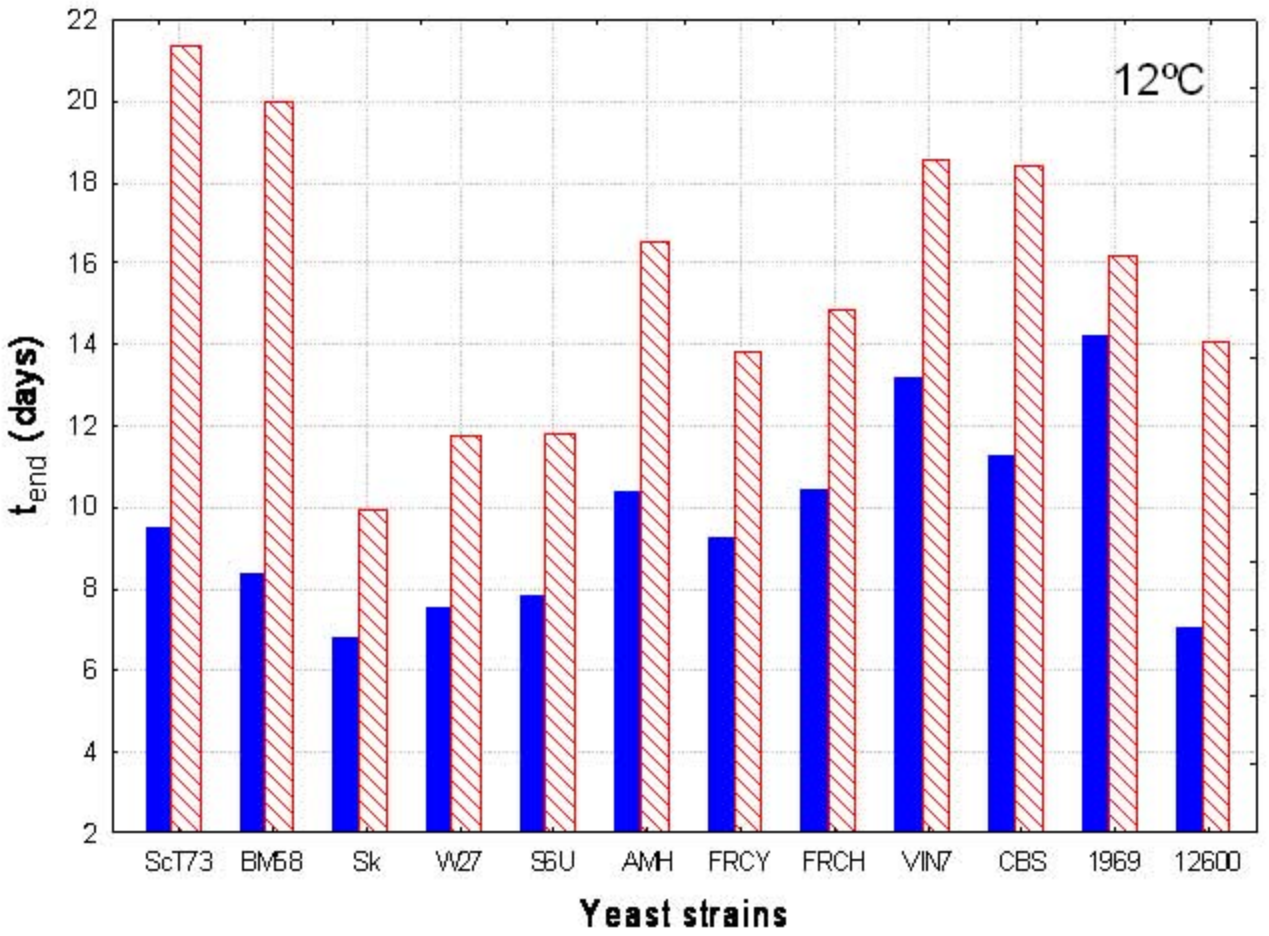
AMH
FRCY
FRCH
VIN7
CBS
1969
12600
Yeast strains


12ºC
t_{end} (days)
22
20
18
16
14
12
10
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2
ScT73
BM68
Sk
W27
S6U
AMH
FRCY
FRCH
VIN7
CBS
1969
12600
Yeast strains