Title: A comparative study of the wine fermentation performance of *Saccharomyces paradoxus* under different nitrogen concentrations and glucose/fructose ratios

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Running title: *S. paradoxus* wine fermentation performance

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Abstract

Aims: The main goal of the present study is to determine the effects of different nitrogen concentrations and glucose/fructose ratios on the fermentation performance of *Saccharomyces paradoxus*, a non-conventional species for wine making.

Methods and Results: Ethanol yield, residual sugar concentration, as well as glycerol and acetic acid production were determined for diverse wine fermentations conducted by *S. paradoxus*. Experiments were also carried out with a commercial *S. cerevisiae* wine strain used as control. The values obtained were compared to test significant differences by means of a factorial ANOVA analysis and the Scheffé test. Our results show that *S. paradoxus* strain was able to complete the fermentation even in the non-optimal conditions of low nitrogen content and high fructose concentration. In addition, the *S. paradoxus* strain showed significant higher glycerol synthesis and lower acetic acid production than *S. cerevisiae* in media enriched with nitrogen, as well as a lower, but not significant, ethanol yield.

Conclusions: The response of *S. paradoxus* was different with respect to the commercial *S. cerevisiae* strain, especially to glycerol and acetic acid synthesis.

Significance and Impact of the Study: The presented study has an important implication for the implementation of *S. paradoxus* strains as new wine yeast starters exhibiting interesting enological properties.

Keywords: Wine fermentation; *Saccharomyces paradoxus; Saccharomyces cerevisiae*; nitrogen content; fructose; glycerol.

Introduction

Grape must is usually fermented by *Saccharomyces cerevisiae* strains, being the main responsible of the quality and flavour of the final product (Pretorius 2000). Although *S. cerevisiae* is the predominant species, *S. bayanus* var. *uvarum* has been described as adapted to low-temperature fermentations during winemaking (Naumov *et al.* 2000). Recently, Majdak *et al*. (2002) and Orlić *et al*. (2007) reported the possibility to use *S. paradoxus* strains as starters in fermentation because of their excellent contribution to the aroma of the wines. *S. paradoxus* is a widespread species usually present in natural habitats (plants, insects, soils, etc) (Sweeney *et al*. 2004), but also in man-manipulated environments, such as 'pulque', a Mexican traditional fermented beverage made with *Agave* sap (originally described as *S. carbajali*; Ruiz 1938), and from Croatian vineyards (Redžepović *et al*. 2002). It is worth noting that these *S. paradoxus* strains isolated from fermentative environments exhibit physiological properties of biotechnological interest (Redžepović *et al*. 2003; Belloch *et al*. 2008).

The nutritional requirements for *Saccharomyces* species to produce wines with desirable organoleptic characteristics are relative high, and many factors have been found to influence their growth and their metabolic capabilities, including sugar content, temperature, aeration and nitrogen availability (Gardner *et al*. 1993; Bisson 1999; D'Amato *et al*. 2006).

Sugar content is one of the most important factors during wine fermentation. Grape must usually contain very similar amounts of glucose and fructose (Fleet and Heard 1993), but in some ecological conditions and grape varieties, the proportion may differ. As a consequence of the climatic change, fructose concentration in grapes is increasing respect to glucose, affecting the global wine quality (Jones *et al*. 2005). Although glucose and fructose are co-consumed by yeasts during wine fermentation,

53 *Saccharomyces* strains have a preference for glucose, which is usually consumed faster,
54 resulting in a reduction of the glucose/fructose ratio, and the preponderance of fructose
55 towards the end of fermentation (Fleet 1998; Berthels *et al*. 2004). During this phase of
56 fermentation, when nitrogen sources are consumed and ethanol concentrations are high,
57 some strains have difficulties to ferment the remaining fructose, resulting in slugged and
58 stuck fermentations (Bauer and Pretorius 2000).

59 Assimilable nitrogen content is another important factor that directly affects the
60 course of fermentation. Nitrogen deficiency may also lead to delayed or stuck
61 fermentations caused by low biomass yield (Bisson 1999; Varela *et al*. 2004). Nitrogen
62 is an important macronutrient that plays a major role in many of the functions and
63 processes carried out by yeasts. The intrinsic importance of nitrogen content on both
64 yeast growth and its metabolism is well known by winemakers. A minimal
65 concentration of 140 mg l^{-1} is often quoted as necessary for the fermentation of a must
66 with moderate sugar content (200 g l^{-1}) (Bell and Henscke 2005). Moreover, the
67 concentration of assimilable nitrogen also influences the formation of volatile and non-
68 volatile compounds that are important for the organoleptic quality of the wine (Bell and
69 Henscke 2005; Hernández-Orte *et al.* 2006; Vilanova *et al.* 2007).

70 In recent years, there has been an increasing demand for wines with high
71 glycerol levels and reduced ethanol content. Glycerol is the major and the most
72 important non-volatile compound produced by yeasts in wines, and significantly
73 contributes to the wine quality by providing slight sweetness and fullness. It is
74 considered as the third major compound produced during wine fermentation after
75 ethanol and carbon dioxide. The amount of glycerol formed during fermentation by *S.*
76 *cerevisiae* is around one tenth of the amount of ethanol produced, and its concentrations
77 in wine varying between 1 and 10 g l^{-1} (Ough *et al*. 1972), although normal

78 concentrations are in the range 4-9 g l^{-1}. Due to the favorable impact on wine quality,
79 glycerol production is one of the desirable features in wine yeast selection. Glycerol
80 production by yeast is affected by many growth and environmental factors (Gardner *et*
81 *al.* 1993; Remize *et al*. 2000). This metabolite is synthesized by yeasts in response to a
82 hyperosmotic medium.
83 Most fermentation requirements have been studied for *S. cerevisiae* but not for
84 other *Saccharomyces* species. The aim of the presented study is to determine the effect
85 of different concentrations of assimilable nitrogen and glucose/fructose ratios on the
86 fermentation performance and synthesis of ethanol, glycerol and volatile acidity (the
87 major compounds of wine fermentation) by *S. paradoxus* in a wine model system.
88
89 **Materials and methods**
90
91 **Yeast strains and inocula preparation**
92 Two yeast, a commercial *S. cerevisiae* wine strain (SOY51) and a *S. paradoxus* strain
93 (SOY54) isolated from Croatian vineyards, were used in the present study. Yeast
94 cultures were maintained on YEPG medium slopes (yeast extract 10 g l^{-1};
95 bacteriological peptone 10 g l^{-1}; glucose 20 g l^{-1}; agar 20 g l^{-1}) at 4^{o}C and transferred
96 monthly to fresh medium until fermentation experiments were carried out.
97 Starter cultures were prepared according to Wang *et al*. (2003) with slight
98 modifications. Briefly, one colony was transferred into 10 mL of a basal medium of 6.7
99 g $l^{-1}$of Yeast Nitrogen Base (Difco^{TM}, Becton and Dickinson Company, Sparks, USA)
100 adjusted to pH 3·2 and supplemented with 50 g l^{-1} of glucose, and incubated at 30^{o}C
101 overnight. Subsequently, yeast cells were harvested (1500 rpm x 15 min), washed three

102 times with 0·2 M phosphate buffer (pH 7·0), and resuspended into 3 ml of fermentation
103 medium. Experiments were inoculated at ≈ 5·0 $\log_{10}$ CFU ml^{-1}.
104
105 **Experimental design and growth media**
106 In this work, a complete factorial design resulting of the combination of 2 yeast strains
107 and 4 growth media was carried out in triplicate. Table 1 summarizes the total number
108 of treatments included in the experimental design. Fermentations were performed in a
109 synthetic must developed by Varela *et al.* (2004). Natural musts show a variable
110 composition from vintage to vintage that can influence the yeast growth. For this
111 reason, a defined synthetic must was chosen in this work as the most appropriate growth
112 medium to overcome this variation. In the present study, the basal must was modified
113 by adding aseptically different assimilable nitrogen concentrations in the form of amino
114 acids and ammonium salt (must S, 50 mg l^{-1}; and must N, 300 mg l^{-1}; for a complete
115 description of the different sources of nitrogen used see Varela *et al.* 2004) and
116 glucose/fructose ratios (must G, 100 g l^{-1}glucose + 100 g l^{-1} fructose; must F, 80 g l^{-1}
117 glucose + 120 g l^{-1} fructose). Fermentations were carried out at 18ºC, which is a normal
118 temperature for white must fermentations, without shaking in 500 ml of must air fitted
119 with a side-arm port sealed with a rubber septum for sampling and closed with airlocks.
120 Experiments were monitorized during 900 h. At variable time intervals, must samples
121 were taken and diluted in a sterile saline solution and plated onto YEPG agar plates.
122 Then, plates were incubated aerobically at 25ºC for 48 h. Counts were expressed as
123 $\log_{10}$ CFU ml^{-1}.
124
125 **Chemical analysis**
126 Final ethanol and volatile acidity productions, as well as the residual sugar content in

the must, were quantified according to the Official EU Methods for wine analysis (EC 2000). Glycerol was determined with an enzymatic/colorimetric commercial kit especially designed for wines (Roche Applied Science, Mannheim, Germany) following the manufacturer's instructions.

The production of glycerol along the fermentative process was fit with the reparameterized Gompertz equation proposed by Zwietering *et al.* (1990):

$$y = G*\exp\{-\exp[((G_r*e)/G)*(\lambda-t))+1]\} \quad (1)$$

where y (dependent variable) is the glycerol concentration at time t, G is the maximum glycerol production reached (g l^{-1}), G_r is the maximum glycerol production rate (g h^{-1}), and λ is the lag phase period for glycerol production (h). The fit was accomplished using the non-linear module of Statistica version 7.0 (Statsoft Inc, Tulsa, USA), minimizing the sum of squares of the difference between experimental data and the fitted model, i.e., loss function (observed-predicted)^2. Fit adequacy was checked by the proportion of variance explained by the model (R^2) respect to experimental data.

Microbiological analysis

The microbial growth and decay observed in the different treatments was described by the model developed by Peleg (1996) based on the continuous logistic equation (which accounts for growth) on which a Fermi's term (for decay) was superimposed. It has the form:

$$N(t) = \frac{N_0 + \dfrac{N_s - N_0}{(1+\exp[k_g(t_{cg}-t)])}}{1+\exp[k_l(t-t_{cl})]} \quad (2)$$

where N(t) is the number of yeasts at time t, N_0 the initial number of yeasts, N_s the maximum number that the environment can support, k_g a growth rate constant, t_{cg} a characteristic time indicating the time required to reach half the environmental capacity

(i.e. $N(t_{cg})/N_s = 0{\cdot}5$), k_l a lethality or decline rate constant and t_{cl} the time to reach 50% survival. Since N_0 is usually known, the equation may be reduced to one with only five adjustable parameters. To facilitate the fit at the normal plot of $\log_{10}$ CFU ml^{-1} vs time used in microbiology, the $\log_{10}$ transformation at both sides of the equation was achieved. This task was also accomplished using the non-linear regression module of Statistica version 7.0.

Statistical data analysis

An analysis of variance was performed by means of the factorial ANOVA module of Statistica software version 7.0, using "yeast strains" and "growth media" as categorical predictor variables. Dependent variables introduced for the analysis were the maximum glycerol production reached (G), the maximum glycerol rate production (G_r), the final ethanol concentration produced (E), the maximum volatile acidity obtained (V), as well as the growth\decay biological parameters estimated with the Peleg model (1996). To check for significant differences between treatments and to form homogeneous group, a post-hoc comparison test was applied by means of the Scheffe test, which is considered to be one of the most conservative post-hoc tests (Winer 1962). An alternative advantage of the Scheffé test is that it can also be used with unequal sample sizes. In this way, when statistical significance is obtained in an ANOVA analysis ($p \leq 0{\cdot}05$), we can reject the null hypothesis of no differences between means exist, and accept the alternative hypothesis that the means are different from each other.

Results

Yeast growth/decay modeling

176 *S. cerevisiae* and *S. paradoxus* showed a first phase of growth, and subsequent decay,
177 during the 900 h that fermentations were monitorized. After the maximum population
178 was reached, the number of yeasts was progressively falling until no viable cells were
179 detected. This behavior could be well fitted by means of the Peleg model (1996),
180 obtaining diverse growth and decline biological parameters of yeast population in the
181 different media (Table 2). An example of this fit is shown in Figure 1 for both yeasts,
182 obtained using 10 samples (marked as circles in the figure) taken along the fermentative
183 process. The proportion of variance explained by the models (R^2), indicative of the fit
184 adequacy, was high and ranged from 94·5 to 99·6% (Table 2).

185 Growth rate (k_g) and maximum yeast population obtained (N_s), both parameters
186 of the initial growth phase, depended on the media and yeasts tested, and diverse
187 homogenous groups were obtained according to the Scheffé test (see Table 2). N_s
188 ranged from 5·70 (*S. cerevisiae* yeast in SF must) to 8·30 $\log_{10}$ CFU ml^{-1} (*S. paradoxus*
189 in both NF and NG musts and *S. cerevisiae* in NG must), resulting both extreme values
190 statistically different. In general, there was a slight tendency in *S. paradoxus* to reach
191 higher population levels than *S. cerevisiae* in the different media (except in NG must
192 where values were exactly identical). Media enriched with higher initial nitrogen
193 concentrations (NG and NF musts) showed also higher N_s for both yeasts. For the
194 specific case of *S. cerevisiae*, those media with higher glucose concentrations (G)
195 showed higher N_s than media enriched with fructose (F) (comparing NG and SG respect
196 to NF and SF musts, respectively), but with no significant differences. However, for *S.*
197 *paradoxus*, there was not a clear relation of the influence of the glucose/fructose ratio
198 on this parameter.

199 The growth rate (that is the increase in the number of yeasts, in logarithmic
200 scale, per time unit) ranged from 0·021 h^{-1} for *S. cerevisiae* in SF must to 0·868 h^{-1} for

S. cerevisiae in SG must. It was very difficult to obtain any conclusions about the influence of the yeast species or must type on this parameter, although three different homogeneous groups were obtained after the post-hoc comparison. For *S. paradoxus*, the highest k_g was obtained in NG must (enriched with nitrogen and a glucose/fructose ratio of 1). However, for *S. cerevisiae*, the highest k_g was obtained in SG must but with values very similar to the NF must.

Finally, the decline rates (parameter of the decay phase) were very similar among the different runs, and non-significant differences were found according to the ANOVA analysis, ranged from 0·007 (*S. paradoxus* in NF must) until 0·013 h^{-1} (*S. cerevisiae* in SG must). Therefore, the number of viable cells decreased more slowly for *S. paradoxus* in NF must than for *S. cerevisiae* in SG must. Table 2 also shows the values of time required to reach half the environmental capacity (included between 2·15 and 120·5 h) and time to reach 50% of survival (between 217·5 and 420·0 h). In the case of t_{cg}, no significant differences were found among treatments, but for t_{cl}, three different homogeneous were formed.

Glycerol production modeling

In this work, the production of glycerol along the fermentative process could also be appropriately modeled, but in this case by means of the reparameterized Gompertz equation proposed by Zwietering *et al.* (1990). A graphic example of the fit is depicted in Figure 1 (marked with squared points), while the parameters obtained for the diverse treatments are shown in Table 3.

The production of glycerol in synthetic must was composed by a first lag phase, where the concentration did not increase, a second phase of intense production, and a third phase where the maximum asymptote was reached and the glycerol concentration

remained stable. As can be seen in Figure 1, the maximum release of glycerol in must occurred during the decay phase for both yeasts. Similar results were also found in the other treatments (data not shown). The proportion of variance explained by the models was high and ranged from 90·6 to 99·9% (Table 3).

The maximum production of glycerol obtained ranged from 3·76 (*S. paradoxus* in SG must) to 6·84 g l^{-1} (*S. paradoxus* in NG must). Statistically, the production of glycerol in *S. paradoxus* increased in those media with higher nitrogen levels (N). However, for *S. cerevisiae,* the production of glycerol was not statistically influenced by the type of must (Table 3). Apparently, for *S. paradoxus* the effect of glucose/fructose ratio did not show influence on glycerol production. However, in the case of *S. cerevisiae,* glycerol production slightly decreased in those fructose-enriched media (F), but with no significant differences.

The glycerol production rate was influenced by the yeast species and type of must used, and three different homogeneous groups were detected according to the Scheffé test (Table 3). Glycerol production rates ranged from 0·009 g h^{-1} for *S. cerevisiae* in NG must, to 0·031 g h^{-1} for *S. paradoxus* in SF must. *S. paradoxus* always showed a higher glycerol production rate than *S. cerevisiae* in any must, except in NF, in which *S. cerevisiae* and *S. para*doxus rates were almost identical. In all cases, a lag period was observed for the glycerol production (see Figure 1). This lag period ranged from 7·79 h for *S. cerevisiae* in NG must to 252·07 h for *S. paradoxus* in SF must.

Influence of the must composition on other enological parameters

Table 4 shows the final alcohol, volatile acidity and residual sugar concentrations for the different fermentations conducted by both yeast species. According to Table 4, the final volatile acidity produced by *S. paradoxus* in all fermentations was statistically

lower than that produced by *S. cerevisiae*. Three different homogeneous groups were obtained. One group formed by the fermentations performed with *S. paradoxus* (average $\approx$ 0·21 g l^{-1}), a second group including the fermentation conducted by *S. cerevisiae* in NF must (0·76 g l^{-1}), and a third group including the remaining *S. cerevisiae* fermentations (average $\approx$1·09 g l^{-1}).

The residual sugar concentration was very similar in all treatments, with no significant differences among them. The average residual sugar concentration was 0·41 g l^{-1}, indicating that the fermentative processes were finished in all cases. Finally, the ethanol yield ranged from 10·7% for *S. paradoxus* in NG must to 12·1% for *S. cerevisiae* in SG must. Not significant differences were found among the diverse fermentations according to the ANOVA analysis (Table 4), although a slight tendency to increase the ethanol yield was noticed in those fermentations performed by *S. cerevisiae* (Table 4). In fact, the lowest yields were obtained in the NG and NF must fermentations conducted by *S. paradoxus*.

Discussion

In this paper, we studied the effect that different nitrogen and fructose concentrations had on the fermentative performance of *S. paradoxus*, a species of potential enological interest (Orlić *et al.* 2007), in comparison to that of the classical wine species *S. cerevisiae*. We compared the production of major wine compounds during fermentation such as ethanol, glycerol and acetic acid.

S. paradoxus, the closest species to *S. cerevisiae* (Rokas *et al.* 2003), is not usually isolated from wine environments (Rainieri *et al.* 2003), but Croatian wines fermented by indigenous *S. paradoxus* strains isolated from vineyard showed good enological properties, with a positive influence on final wine quality (Orlić *et al.* 2007).

276 In this study, *S. paradoxus* was able to finish the fermentation independently of the
277 initial nitrogen or fructose concentrations present in the must (100 and 120 g l^{-1}), which
278 is very important for the utilization of strains of this species as a starters in wine
279 fermentations. Our results confirm those obtained previously by Orlić *et al.* (2007) in
280 Chardonnay wine fermentations, where some *S. paradoxus* strains showed a
281 considerable fermentative vigour.

282 Nitrogen has been described as one of the major limiting yeast growth factors,
283 and assimilable nitrogen concentration around 140-150 mg l^{-1} has been reported to be
284 necessary to complete fermentation (Bell and Henscke 2005). Some authors have
285 reported that must with 60 mg l^{-1} of assimilable nitrogen achieve dryness (Wang *et al*.
286 2003; Beltran *et al*. 2005), but Varela *et al*. (2004) demonstrated that fermentations with
287 50 mg l^{-1} of nitrogen left 16 g l^{-1} of residual sugars. In this work, a total nitrogen
288 concentration of 50 mg l^{-1} was enough for *S. paradoxus*, as well as for *S. cerevisiae*, to
289 complete the fermentation with an initial sugar concentration of 200 g l^{-1}. Wine yeast
290 strains have significantly different nitrogen requirements that are strain specific and
291 mostly appear during the stationary phase (Manginot *et al*. 1998). D'Amato *et al.*
292 (2006) reported that the maximum population of a *S. cerevisiae* strain in synthetic must
293 fermentations was attained at the higher ammonium concentrations assayed (270 mg l^{-1}).
294 It is very interesting to notice that in this work *S. paradoxus* reached higher
295 population levels than *S. cerevisiae* practically in all conditions assayed. In fact, *S.*
296 *paradoxus* reached its highest population levels in media enriched with nitrogen, but
297 their values were not statistically different than those obtained for *S. cerevisiae*.

298 Glycerol represents a very important non-volatile compound for wine quality,
299 and from a technological point of view it is worth to get a better knowledge of the
300 influence of must components on glycerol production. The maximum production of

glycerol was obtained during the decay phase for both yeast species (Figure 1) in all fermentation conditions. Possibly, glycerol is produced by yeasts at the early stage of fermentation in response to osmotic pressure, but only is released during the last phase of fermentation when occur the breakage of the cell wall due to cellular lysis or higher membrane permeability. Apparently, nitrogen seems to have a significant influence on the glycerol synthesis in *S. paradoxus*, which is not observed in the case of *S. cerevisiae*. Glycerol formation is the results of redox balance and stress response (Nevoigt and Stahl 1997) and the observed differences suggest that the two species could have a different osmotic shock response, especially in presence of nitrogen. This hypothesis is also supported by the final production of volatile acids (mainly acetic acid), another significant redox-driven product, which was also different between *S. cerevisae* and *S. paradoxus*. Clearly, *S. cerevisiae* produced higher concentrations of acetic acid than *S. paradoxus* under all fermentation conditions.

Although ethanol yields in fermentations conducted by *S. paradoxus* were not significantly different to those obtained with *S. cerevisiae*, we found that *S. paradoxus* always produced lower ethanol concentrations than *S. cerevisiae*. In addition, for both species, there was a slight tendency to produce higher ethanol levels in musts with lower nitrogen content. These results are not in agreement with those obtained by Vilanova *et al*. (2007), who observed higher ethanol yields in fermentations with 300 mg l^{-1} of nitrogen. However, under lower nitrogen concentrations yeast strains metabolize amino acids as a nitrogen source and as a mechanism for NAD(P)H reoxidation (Valero *et al*. 2003). D'Amato *et al.* (2006) determined that an excess of ammonium could also lead to a modification of the aromatic profile of wines. The reason could be that under these conditions yeasts do not need to metabolize amino acids, and hence, a lower production of higher alcohols and their esters is obtained.

Conclusions

This is the first study carried out to evaluate the fermentative performance of *S. paradoxus* under different nitrogen levels and glucose/fructose ratios in a wine model system. In the present work, we have found that a *S. paradoxus* strain isolated from vineyards possess enological properties of interest for the wine industry, such as significant higher synthesis of glycerol and lower production of volatile acidity than *S. cerevisiae*. These properties together with their excellent behavior under the typical stresses present in fermentation environments and an excellent contribution to the aromatic fraction of wines makes them an alternative to *S. cerevisiae* as wine starters according to the current winemaking trends.

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436 **Figure legends**

437

438 *Figure 1.* Growth/decay plate count data fitted by means of the Peleg model (1996), and
439 glycerol production modeled with the reparameterized Gompertz equation proposed by
440 Zwietering *et al.* (1990) for yeasts a) *Saccharomyces paradoxus* and b) *S. cerevisiae* in
441 NG must (300 mg l^{-1} of assimilable nitrogen; 100 g l^{-1} glucose + 100 g l^{-1} fructose).

Table 1. Fermentations included in the factorial experimental design (2 yeast strains x 4 musts) used in the present work.

Treatment code	Yeast strains	Must composition
Sp – NG	*S. paradoxus* SOY54	300 mg l^{-1} of assimilable nitrogen 100 g l^{-1} glucose + 100 g l^{-1} fructose
Sp – NF	*S. paradoxus* SOY54	300 mg l^{-1} of assimilable nitrogen 80 g l^{-1} glucose + 120 g l^{-1} fructose
Sp – SG	*S. paradoxus* SOY54	50 mg l^{-1} of assimilable nitrogen 100 g l^{-1} glucose + 100 g l^{-1} fructose
Sp – SF	*S. paradoxus* SOY54	50 mg l^{-1} of assimilable nitrogen 80 g l^{-1} glucose + 120 g l^{-1} fructose
Sc – NG	*S. cerevisiae* SOY51	300 mg l^{-1} of assimilable nitrogen 100 g l^{-1} glucose + 100 g l^{-1} fructose
Sc – NF	*S. cerevisiae* SOY51	300 mg l^{-1} of assimilable nitrogen 80 g l^{-1} glucose + 120 g l^{-1} fructose
Sc – SG	*S. cerevisiae* SOY51	50 mg l^{-1} of assimilable nitrogen 100 g l^{-1} glucose + 100 g l^{-1} fructose
Sc – SF	*S. cerevisiae* SOY51	50 mg l^{-1} of assimilable nitrogen 80 g l^{-1} glucose + 120 g l^{-1} fructose

Table 2. Growth/decay biological parameters obtained by means of the Peleg model (1996) for the different fermentations.

Treatment code[†]	R^2	N_s	k_g	t_{cg}	k_l	t_{cl}
Sp – NG	0·977	8·300[a]	0·708[b,c]	24·190[a]	0·009[a]	292·600[a,b,c]
	(0·002)	(0·424)	(0·016)	(0·113)	(0·001)	(20·85)
Sp – NF	0·945	8·300[a]	0·098[a]	23·015[a]	0·007[a]	358·620[a,b,c]
	(0·000)	(0·000)	(0·007)	(1·407)	(0·000)	(2·559)
Sp – SG	0·986	7·300[b,d]	0·177[a]	54·925[a]	0·009[a]	420·010[c]
	(0·009)	(0·141)	(0·010)	(0·247)	(0·002)	(3·464)
Sp – SF	0·987	7·700[a,b]	0·340[a,c]	58·740[a]	0·010[a]	395·615[a,c]
	(0·001)	(0·141)	(0·073)	(0·141)	(0·000)	(1·576)
Sc – NG	0·977	8·300[a]	0·699[b,c]	23·460[a]	0·012[a]	266·520[a,b]
	(0·017)	(0·141)	(0·164)	(0·110)	(0·001)	(30·197)
Sc – NF	0·988	7·700[a,b]	0·864[b]	23·635[a]	0·012[a]	262·875[a,b]
	(0·002)	(0·141)	(0·081)	(0·007)	(0·001)	(5·154)
Sc – SG	0·980	6·400[c,d]	0·868[b]	2·155[a]	0·013[a]	217·545[b]
	(0·022)	(0·141)	(0·070)	(0·219)	(0·003)	(3·330)
Sc – SF	0·996	5·700[c]	0·021[a]	120·57[a]	0·009[a]	359·885[a,c]
	(0·003)	(0·141)	(0·009)	(90·990)	(0·001)	(71·721)

[†] Yeast species and types of musts for the different fermentations are shown in Table 1.
Note: N_s, maximum number of yeasts ($\log_{10}$ CFU ml^{-1}) that the fermentation environment can support; k_g, growth rate constant (h^{-1}); t_{cg}, time (h) required to reach half the environmental capacity (N_{tcg}/N_s=0·5); k_l, lethality or decline rate constant (h^{-1}); t_{cl}, time to reach 50% survival (h). R^2, proportion of variance explained by the models. Values followed by different superindexes, within the same column, are significantly different according to Scheffé test. Standard deviations are given between parentheses.

Table 3. Glycerol parameters obtained by means of the Gompertz equation proposed by Zwietering *et al*. (1990) for the different fermentations.

Treatment code[†]	R^2	G	G_r	λ
Sp – NG	0·999 (0·000)	6·846^{a} (0·507)	0·025^{b,c} (0·000)	147·905^{b,c} (9·340)
Sp – NF	0·999 (0·000)	6·676^{a} (0·154)	0·015^{a,b} (0·001)	86·000^{a,b} (9·913)
Sp – SG	0·999 (0·000)	3·763^{b} (0·267)	0·018^{a,b} (0·005)	244·440^{c} (6·299)
Sp – SF	0·999 (0·000)	4·394^{b} (0·045)	0·031^{c} (0·001)	252·075^{c} (2·699)
Sc – NG	0·906 (0·020)	4·785^{b} (0·183)	0·009^{a} (0·001)	7·795^{a} (4·744)
Sc – NF	0·991 (0·001)	4·171^{b} (0·146)	0·014^{a,b} (0·002)	62·444^{a,b} (59·744)
Sc – SG	0·992 (0·002)	4·850^{b} (0·121)	0·010^{a} (0·001)	35·515^{a} (3·839)
Sc – SF	0·999 (0·000)	4·447^{b} (0·059)	0·017^{a,b} (0·001)	95·675^{a,b} (3·075)

[†]Yeast species and type of medium for the different fermentations are shown in Table 1. Note: G, maximum glycerol production reached (g l^{-1}); G_r, maximum glycerol production rate (g h^{-1}); λ, lag phase period for glycerol production (h). R^2, proportion of variance explained by the models. Values followed by different superindexes, within the same column, are significantly different according to Scheffé test. Standard deviations are given between parentheses.

Table 4. Final production of alcohol (%), volatile acidity (g l^{-1}) and residual sugars (g l^{1}) for the different fermentations.

Treatment code[†]	Alcohol	Volatile acidity	Residual sugar
Sp – NG	10·70 (0·28)[a]	0·230 (0·030)[a]	0·333 (0·057)[a]
Sp – NF	10·82 (0·84)[a]	0·140 (0·020)[a]	0·433 (0·057)[a]
Sp – SG	11·35 (0·08)[a]	0·290 (0·030)[a]	0·466 (0·057)[a]
Sp – SF	11·60 (0·00)[a]	0·176 (0·005)[a]	0·366 (0·057)[a]
Sc – NG	11·15 (0·08)[a]	1·140 (0·040)[b]	0·400 (0·100)[a]
Sc – NF	11·60 (0·43)[a]	0·766 (0·057)[c]	0·400 (0·100)[a]
Sc – SG	12·10 (0·14)[a]	1·066 (0·057)[b]	0·466 (0·057)[a]
Sc – SF	11·70 (0·28)[a]	1·072 (0·017)[b]	0·433 (0·057)[a]

[†]Yeast species and type of medium for the different fermentations are shown in Table 1.

Note: Values followed by different superindexes, within the same column, are significantly different according to Scheffé test. Standard deviations are given between parentheses.