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Abstract

Starmerella bacillaris has been proposed as a potential non-*Saccharomyces* species candidate to be used in mixed fermentations with *Saccharomyces cerevisiae* for the production of wine. Among the prospective applications, reduction of ethanol content, but also reduction in the acetic acid produced from high sugar musts, have attracted particular attention. In this study, we sought to describe the fermentation behavior of 6 strains of *S. bacillaris* in grape must with varying initial sugar concentration that ranged from 200 to 330 g/L. Further, time (days of fermentation) was a second variable that was monitored for its influence on fermentation. A response surface methodology was then employed to model the behavior of the strains. The six strains generally behaved uniformly. Residual sugar concentration as well as ethanol, glycerol and acetic acid production mainly depended on time. Residual glucose also partly depended on initial sugar concentration being higher when musts with higher initial sugar concentration were used. Similarly, malic acid consumption showed a dependence on both time and sugar concentration and was inhibited in higher sugar musts. The behavior of *S. bacillaris* strains can be considered compatible with enological practices that could involve mixed fermentation with *S. cerevisiae*.

Keywords: alcoholic fermentation, non-*Saccharomyces*, response surface methodology, *Starmerella bacillaris.*

Introduction

Wine quality is influenced by the yeast populations residing on the surface of the grapes but more importantly by those that are present and metabolically active during the subsequent alcoholic fermentation of the must (Fleet 2003). Apart from *Saccharomyces cerevisiae*, other non-*Saccharomyces* yeast species participate in the transformation of grape must to wine and their role has long been recognized, especially in spontaneous fermentations. Spontaneous fermentations often result in more complex wines, but they are unpredictable. In order to reconcile these two incompatible enological aspects of spontaneous fermentations, the exploitation of mixed cultures (*S. cerevisiae* and non-*Saccharomyces* species) is gaining ground both among researchers and wine producers (Ciani et al. 2010).

Different yeast species are currently being investigated for their potential application during grape must fermentation. No single species can be proposed as the ideal companion for *S. cerevisiae*: the choice is based on the aim to be achieved in terms of sensorial characteristics and type of the final wine. The best-known positive contributions of non-*Saccharomyces* species to wine relate to enzymatic activities and/or production of volatile compounds that have a sensorial impact (Fleet 2008). Some recently explored attributes of non-*Saccharomyces* yeasts include production of manoproteins, acidification, reduced acetic acid production in high sugar musts (Domizio et al. 2014, Gobbi et al. 2013, Belly et al. 2008, Rantsiou et al. 2012).

Non-*Saccharomyces* can also be employed with the purpose of reducing the ethanol content of the final wine. In the last 20 years an increasing trend in the ethanol content of wines is being registered. This trend is the result of alcoholic fermentation by *S.*

cerevisiae of overripe grapes that are harvested late in order to reach the desired phenolic maturity. Non-*Saccharomyces* can consume grape sugars through respiration (Gonzalez et al. 2013, Contreras et al. 2015, Quiros et al. 2014) or transform them into ethanol but with a lower yield as compared to *S. cerevisiae* (Domizio et al. 2011, Magyar and Tóth 2011, Contreras et al. 2014, Englezos et al. 2016). In this way, grape sugar concentration is reduced and consequently the ethanol content of the final wine is decreased.

Since it was first described, *Starmerella bacillaris* (synonym *Candida zemplinina*) (Duarte et al. 2012) is one of the most frequently isolated non-*Saccharomyces* yeasts from grapes and musts (Csoma and Sipiczki 2008) and is being evaluated for its physiological characteristics of enological interest. *S. bacillaris* is fructophilic, osmotollerant, produces low amounts of acetic acid (<0.7 g/L) and high amounts of glycerol (8-10 g/L). Furthermore, it tolerates moderate concentrations of SO_2 (50 mg/L) and ethanol (8-10%) (Tofalo et al. 2012, Englezos et al. 2015).

In this study we sought to model the fermentation behavior of *S. bacillaris* by employing a Response Surface Methodology (RSM) approach (Bas and Boyaci 2007). The two independent experimental variables chosen to test were the sugar concentration and the time of fermentation. In order to define the experimental domain to be investigated, a Central Composite Design (CCD) was performed to define the values of the two experimental variables and conduct the fermentation trials with pure cultures of *S. bacillaris*. The data of some important wine chemical parameters were then fitted into polynomial equations that describe and predict the behavior of *S. bacillaris*.

Materials and Methods

93 *Yeast strains and culture conditions*

94 The strains of *S. bacillaris* used in this study belong to the yeast culture collection of the
95 Department of Agricultural, Forest and Food Sciences (DISAFA) of the University of
96 Turin, Italy (Englezos et al. 2015). They were previously isolated from four different
97 grapevine cultivars and have been identified to the species level by sequencing of the D1-
98 D2 loop of the 26S rRNA encoding gene. Relevant information regarding the strains used
99 is shown in Table 1.

100

101 *Experimental must preparation*

102 Grape must of *Barbera* cv. was obtained from a local winery during the harvest 2013.
103 The total sugars (glucose and fructose) concentration was standardized to 200 g/L with
104 distilled water and distributed into five glass-bottles. Yeast assimilable nitrogen (YAN)
105 was adjusted to 160 mg/L for all trials using the commercial product Fermaid O® from
106 Lallemand Inc. (Montreal, Canada) in order to provide an unified starting point for the *S.*
107 *bacillaris* strains. Then, commercial sugar (saccharose) was used in order to obtain five
108 different sugar concentrations (i.e. 200, 219, 265, 311 and 330 g/L). The sugar-adjusted
109 musts were pasteurized in a water bath at 60˚C for 1 hour and the absence of viable
110 population was subsequently evaluated by plating on WLN (Oxoid, Milan, Italy) medium
111 and incubating at 28˚C for 5 days. The successful hydrolysis of saccharose to glucose and
112 fructose was checked by HPLC.

113

114 *Fermentations*

For each strain pre-inocula were prepared from two independent colonies, representing two biological replicates, and incubated for 24 hours in 1 mL of pasteurized must at 25˚C. The pre-inocula were then subcultured in 25 mL of pasteurized must (24 ml of fresh must inoculated with 1 mL of pre-inoculum) in 50 mL tubes for 24 hours at 25˚C. Then the strains were inoculated in 25 mL of sugar-adjusted must at a final concentration of 10^6 viable cells/mL, as determined by methylene blue staining and direct microscope count. Fermentations were carried out in 50 mL tubes with loose screw-cap under static conditions at 25˚C.

Microbiological analysis

Microbiological analyses were performed the 1^{st}, 2^{nd}, 4^{th}, 8^{th}, 16^{th} and 23^{rd} day on replicate fermentations set up in order to monitor the population dynamics. Serial dilutions were performed with sterile Ringer's solution (Oxoid, Milan, Italy) and the number of colony-forming units per milliliter (CFU/mL) was determined by plating 100 µL of the last three dilutions on WLN medium (Oxoid, Milan, Italy) and incubation at 28˚C for 5 days.

Chemical analysis

Organic acids (acetic and malic), sugars (glucose and fructose), ethanol and glycerol were quantified by HPLC (Thermo Electron Corporation, Waltham, MA, USA) equipped with a UV detector (UV100) set to 210 nm and a refractive index detector (RI-150). The analyses were performed isocratically at 0.8 mL min^{-1} and 65 °C with a 300x7.8 mm i.d. cation exchange column (Aminex HPX-87H) and a Cation H^+ Microguard cartridge (Bio-

Rad Laboratories, Hercules, CA, USA), using 0.0026N H_2SO_4 as mobile phase (Rolle et al. 2012).

Response-surface methodology

The main chemical components of the must were modeled with a response surface estimated with a second order polynomial equation as follows:

$$Y = \beta_o + \beta_1 X_1 + \beta_{11} X_1^2 + \beta_2 X_2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2,$$

where Y is the predicted response of the dependent variable; X_1 and X_2 are the independent variables (sugar concentration and time of fermentation) influencing the response; β_0 is the mean/intercept term; β_1 and β_2 are the linear regression coefficient of each independent variable; β_{11} and β_{22} are the quadratic regression coefficient of each independent variable; β_{12} is the regression coefficient of interactions between two independent variables. The positive or negative sign of the regression coefficient indicates an increase or a decrease of the main effects, respectively. The regression coefficients of the squared terms influence the direction of the curvature of the response surfaces.

Statistical analysis

The results were analyzed using the software Statistica version 8.0 (Statsoft Inc., USA). The linear and quadratic effects of the factors as well as their linear interaction were calculated and their significance was evaluated by analysis of variance (ANOVA). A three-dimensional surface, described by a second-order polynomial equation was fitted to each set of experimental data points. First- and second-order coefficients were generated

160 by regression analysis. The fit of the models was evaluated by the determination
161 coefficients (R^2) and only the regression with a satisfactory value (R^2 >0.90), were used.
162

Results

Central composite design

In order to study the influence of two parameters, namely sugar concentration and duration, on the fermentation behavior of *S. bacillaris*, a central composite design (CCD) approach was employed (Bas and Boyaci 2007; Englezos et al. 2016). For *n* factors the CCD is composed of a factorial design (2^n) expanded with star-points (α) situated at $\pm 2^{n/4}$ factorial units of the center, giving five levels per factor (-α, -1, 0, +1, +α), including three repetitions of the center point in order to evaluate the pure error. In this study two factors (sugar concentration and duration) were tested giving $|\alpha| = 1.41$ that defined the limits of the CCD i.e. 200 and 330 g/L for the sugar concentration and 0 and 21 days for the duration. The intermediate values for the sugar concentration were 219, 265 and 311 g/L while for the duration 3, 11 and 18 days.

Population dynamics during fermentation

The results of the microbiological analysis of the musts, performed in order to monitor the viable populations of *S. bacillaris* during fermentation, are shown in Table 2. For all the strains tested in the different sugar concentrations, populations reached a concentration of about 8.5 Log_{10} CFU/mL (range 8.3-8.6) the second day of fermentation and this density remained stable up to the 8th day. After the 8th day, in all cases we observed a decline of the viable populations. Such trend was common for the six strains, but the degree of decline depended on the strain but also on the initial sugar concentration. In the must with the highest sugar concentration (330 $g^{-}L^{-1}$) *S. bacillaris*

185 could not be detected at 23 days of fermentation, while in the musts with lower sugar
186 concentration a residual population was present, ranging from 1 to 6.2 Log_{10} CFU/mL.
187
188 *Application of the response-surface methodology*
189
190 Residual sugars
191 The polynomial equations that model the residual glucose and fructose concentration for
192 the six *S. bacillaris* strains are shown in Table 3. The main effects influencing the
193 residual glucose and fructose concentration were similar for all the strains.
194 Regarding residual glucose, there was primarily a positive linear effect and a smaller
195 quadratic effect of the initial sugar concentration, and a negative linear effect of the time
196 (Figure 1). This means that an increase in the initial total sugar concentration had a
197 negative impact on glucose consumption (increased residual glucose). A low residual
198 glucose (< 5 g/L) was observed after 10 days of fermentation only for an initial sugar
199 concentration below 219 g/L.
200 On the other hand, the initial sugar concentration had a minor effect on the residual
201 fructose, while there was a principal negative linear effect of time on the consumption of
202 fructose and to a lesser extend a positive quadratic effect of time, since the fructose was
203 completely fermented in all cases after 10-18 days (Figure 2).
204
205 Ethanol and glycerol production
206 The polynomial equations that describe the ethanol and glycerol production are shown in
207 Table 4. For all strains, time had an effect on the production of both ethanol and glycerol.
208 More specifically a positive linear and a negative quadratic effect was observed. As

shown in Figures 3 and 4 an increase in the concentration of these two compounds over time was followed by a plateau.

Strain PE3WA showed the most important negative linear effect of the sugar on the ethanol content while there was a positive quadratic effect observed for the strains Cz03 and EER3C.

The initial sugar concentration had a positive effect on the fermentation performed with Cz03 and EER3C to obtain a wine with higher content of glycerol (about 13 g/L) (Figure 4).

Acetic acid production and malic acid consumption

Acetic acid production and malic acid consumption, as described by the polynomial equations for the different *S. bacillaris* strains are shown in Table 5.

For all strains tested, time had a significant effect (positive linear and negative quadratic) on the production of acetic acid. The acetic acid was produced in a time-dependent manner and in most cases was not dependent on the initial sugar concentration. Production of acetic acid showed a linear increase with time followed by a plateau (Figure 5). Only for strain EJ1 a quadratic positive effect of initial sugar concentration was observed. This result means that in musts with increased sugar concentration the acetic acid production will be higher. Similarly, for strain EER3C a higher linear and quadratic (lower negative) effect of sugar was observed, to obtain high final concentration (+ 0.1 g/L) in particular for an initial sugar concentration greater than 250 g/L.

Regarding malic acid consumption, both sugar and time had an effect. More specifically, there was a negative linear effect and positive quadratic effect of time, a positive linear effect of the sugar (except for strain Cz03) and positive interaction effect between sugar and time (Figure 6). Between the six strains tested, Cz03 and FC54 were capable to metabolize a higher quantity of malic acid independently of the initial sugar. EER3C, PE3WA AND EIF7LB had a good capacity to consume malic acid when the fermentation was at low initial sugar concentration. The strain EJ1 had a low impact of the malic acid metabolism with a residual malic acid in the fermentation with low initial sugar grater than 1.5 g/L

Prediction fermentation behavior

The polynomial equations that describe the fermentation behavior of the strains of *S. bacillaris* were used to predict the range of concentration of the most important chemical compounds when must with varying sugar content was used. The results are shown in Table 6. As can be seen, important deviation was observed in the concentration of the residual sugar. This trend could be associated with the amount of ethanol produced; in all cases between 8.8 and 10.6% vol. In addition, the malic acid concentration range was considerable.

Discussion

RSM is a multivariate statistic approach that consents the modeling of experimental data when more than one variables are tested simultaneously. In this process, possible interactive effects that may exist between the variables tested are taken into

254 consideration. This approach has been recently employed to describe the effect of
255 glucose, ethanol and SO_2 on growth and volatile phenol production by *Brettanomyces*
256 *bruxellensis* in wine (Chandra et al. 2014), to model the effect of the same variables on
257 growth of 3 non-*Saccharomyces* species in wine (Chandra et al. 2015) and to describe the
258 effect of time of *S. cerevisiae* inoculation as well as fermentation time in *S. bacillaris*-*S.*
259 *cerevisiae* mixed fermentations (Englezos et al. 2016).
260 In particular, the focus of this study was to describe the fermentation behavior of *S.*
261 *bacillaris* in grape must with varying initial sugar concentration. The main chemical
262 components of enological interest were analyzed and by applying the response surface
263 methodology it was possible to determine the effect of sugar and time on each
264 component. In order to capture the intra-species biodiversity, 6 strains of *S. bacillaris*,
265 were tested. The 6 strains had been previously characterized both genetically and
266 phenotypically. They were genetically different and possessed different characters of
267 possible enological interest (Englezos et al. 2015). All strains were able to reach high
268 density in the must in a short time (within 2 days) and viability was maintained,
269 independently of the strain and the initial sugar concentration, up to the 8^{th} day of
270 fermentation but declined afterwards. The results obtained indicate that the initial sugar
271 concentration does not have an effect on the maximum cell density reached but may
272 influence the viability during the later stages of the fermentation. Differences were
273 observed between the strains that may be the result of varying osmotolerance since the
274 amount of ethanol produced was not changed for musts with increasing initial sugar. It
275 should however be underlined that metabolically active populations of *S. bacillaris*, that
276 cannot be detected by viable count may exist during alcoholic fermentation of high sugar

277 must (Mills et al. 2002). Therefore, it is speculated that the fermenting must undergoes
278 chemical changes even when a viable population is not detected by plating.
279 The initial sugar concentration appeared to have limited or no effect at all in the
280 consumption of sugars. As expected on the other hand, fermentation duration impacted
281 sugar consumption. Fructose was preferentially but not exclusively consumed. Glucose
282 consumption was influenced by the initial sugar concentration; more glucose was
283 consumed when the total sugar concentration was lower. Ethanol was also mainly
284 influenced by time and not by the sugar concentration. The ethanol yield ranged between
285 18.78 and 19.21 $g \cdot \%vol^{-1}$, confirming reduced efficiency in the transformation of sugars
286 into ethanol, when compared with *S. cerevisiae* (Magyar and Tóth 2011, Englezos et al.
287 2015). Similarly, glycerol, a major product of the alcoholic fermentation, was produced
288 in significant amounts in a time-dependent manner. Production of acetic acid has been
289 shown to be produced in wine yeasts as a response to osmotic stress. Interestingly, acetic
290 acid production by *S. bacillaris* (with the exception of one strain) showed a sugar-
291 independent trend and was mainly influenced by the fermentation duration. The strains
292 tested showed also malic acid consumption. Malic acid was consumed in a time
293 dependent manner and resulted to be dependent also on the initial sugar concentration.
294 Consumption was faster and greater at the lowest concentration of sugars. The results
295 obtained suggest a sugar-dependent inhibition of malic acid consumption.
296 The ultimate goal is to understand if *S. bacillaris* can accompany *S. cerevisiae* in mixed
297 fermentations in order to decrease the ethanol content of the final wine. By employing the
298 RSM it was possible to model the fermentation behavior of 6 strains of *S. bacillaris*. The
299 behavior of the different strains was uniform and the results are promising; *S. bacillaris*

proved to ferment grape musts in a predictable manner. Predictions regarding sugar consumption are compatible with the use of this species in combination with *S. cerevsiae* since preferential consumption of fructose will reduce the sugar available to *S. cerevisiae*. In all six fermentations monitored *S. bacillaris* strains were capable to ferment with an ethanol production of about 8.8-10.6% vol., preferentially using fructose .The residual glucose observed at high initial sugar concentration is a situation that will not be verified under enological conditions. The amounts of glycerol and acetic acid produced are well-suited for the application of this species in mixed fermentations. Interesting aspect that requires further investigation and could have practical implications for wines with low pH is the ability of *S. bacillaris* to consume malic acid, albeit mainly in musts with moderate sugar concentration.

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Figure legends

Figure 1. Response surface curve, showing the effect of time of fermentation (days) and initial sugar concentration (g/L) on Glucose content (g/L) for strain FC54. Similar curves were obtained for the other 5 *S. bacillaris strains* (data not shown).

Figure 2. Response surface curve showing the effect of time of fermentation (days) and initial sugar concentration (g/L) on Fructose content (g/L) for strain FC54. Similar curves were obtained for the other 5 *S. bacillaris strains* (data not shown).

Figure 3. Response surface curves showing the effect of time of fermentation (days) and initial sugar concentration (g/L) on Ethanol content (% v/v) for the six *Starmerella bacillaris* strains.

Figure 4. Response surface curve showing the effect of time of fermentation (days) and initial sugar concentration (g/L) on Glycerol content (g/L) for strain FC54. . Similar curves were obtained for the other 5 *S. bacillaris strains* (data not shown).

Figure 5. Response surface curves showing the effect of time of fermentation (days) and initial sugar concentration (g/L) on acetic acid content (g/L) for strains FC54 and EJ1 (showing a positive quadratic effect of initial sugar concentration on acetic acid production).

Figure 6. Response surface curve showing the effect of time of fermentation (days) and initial sugar concentration (g/L) on malic acid content (g/L) for strains FC54 and EJ1 (for which no positive linear effect of initial sugar concentration was observed).

454 Table 1. Strains used in this study

Strain code	Geographical region of isolation	Grapevine cultivar
FC54	Friuli Venezia Giulia (Italy)	Picolit
Cz03	Piedmont (Italy)	Barbera
EER3C	Piedmont (Italy)	Erbaluce
PE3WA	Piedmont (Italy)	Erbaluce
EIF7LB	Piedmont (Italy)	Erbaluce
EJ1	California (USA)	Chardonnay

455

456 Table 2. *Starmerella bacillaris* viable count as a function of initial sugar concentration of must and time
457

	200 g/L initial sugar						219 g/L initial sugar						265 g/L initial sugar						311 g/L initial sugar						330 g/L initial sugar					
	Days of fermentation						Days of fermentation						Days of fermentation						Days of fermentation						Days of fermentation					
Starmerella bacillaris	1	2	4	8	16	23	1	2	4	8	16	23	1	2	4	8	16	23	1	2	4	8	16	23	1	2	4	8	16	23
FC54 (Log_{10}CFU/mL)	7.3	8.3	8.4	8.3	6.7	2.6	7.3	8.4	8.4	8.4	7.2	<1	7.4	8.5	8.3	8.4	7.3	3.0	7.4	8.3	8.4	8.3	7.1	2.6	7.2	8.5	8.3	8.4	6.8	<1
Cz03 (Log_{10}CFU/mL)	7.2	8.3	8.4	8.3	7.4	<1	7.3	8.4	8.3	8.3	7.6	2.3	7.1	8.2	8.3	8.4	7.7	5.4	7.1	8.3	8.2	8.3	7.7	4.3	7.3	8.3	8.3	8.3	7.6	<1
EER3C (Log_{10}CFU/mL)	7.3	8.4	8.4	8.4	7.1	5.0	7.5	8.4	8.4	8.4	6.8	<1	7.5	8.5	8.4	8.4	7.2	3.2	7.4	8.3	8.3	8.4	7.0	<1	7.4	8.3	8.3	8.3	6.3	<1
PE3WA (Log_{10}CFU/mL)	7.5	8.3	8.4	8.5	5.3	5.9	7.4	8.4	8.3	8.5	6.6	5.5	7.5	8.4	8.4	8.4	7.5	5.2	7.4	8.4	8.3	8.3	7.0	4.2	7.4	8.3	8.3	8.3	6.6	<1
EIF7LB (Log_{10}CFU/mL)	7.7	8.6	8.6	8.5	7.1	3.5	7.8	8.4	8.4	8.4	7.3	<1	7.8	8.5	8.5	8.4	7.4	2.8	7.6	8.4	8.3	8.3	7.1	1.0	7.4	8.4	8.5	8.4	6.8	<1
EJ1 (Log_{10}CFU/mL)	7.7	8.6	8.5	8.3	6.8	6.2	7.9	8.6	8.5	8.4	6.4	<1	7.6	8.4	8.5	8.4	7.0	3.2	7.5	8.4	8.4	8.4	6.2	<1	7.4	8.4	8.4	8.3	6.0	<1

Table 3. Polynomial equations modeling the residual glucose and fructose as a function of initial sugar concentration (S) and time (t) for the 6 strains of *S. bacillaris*. S, t: linear regression coefficient, S^2, t^2: quadratic regression coefficient, S*t: regression coefficient of interactions between sugar and time

Glucose						
Strain		**S**	**t**	**S^2**	**S*t**	**t^2**
FC54	-7.10E+1	1.17E+0	-1.67E+1	-1.43E-3	3.28E-2	2.44E-1
Cz03	-5.91E+1	1.17E+0	-1.67E+1	-1.58E-3	3.77E-2	2.00E-1
EER3C	-1.72E+1	7.70E-1	-1.91E+1	-6.37E-4	3.24E-2	3.51E-1
PE3WA	-1.15E+2	1.50E+0	-1.79E+1	-2.04E-3	3.81E-2	2.48E-1
EIF7LB	5.24E+1	2.38E-1	-1.97E+1	2.99E-4	3.81E-2	3.17E-1
EJ1	-8.32E+1	1.21E+0	-1.73E+1	-1.41E-3	3.00E-2	2.82E-1
Fructose						
Strain		**S**	**t**	**S^2**	**S*t**	**t^2**
FC54	-2.31E+1	5.75E-1	-8.26E+0	-2.36E-4	-3.28E-2	5.77E-1
Cz03	-2.02E+1	5.38E-1	-7.92E+0	-1.18E-4	-3.46E-2	5.82E-1
EER3C	1.13E+1	3.51E-1	-8.95E+0	1.80E-4	-3.22E-2	5.93E-1
PE3WA	-3.61E+1	6.19E-1	-7.23E+0	-2.23E-4	-3.65E-2	5.76E-1
EIF7LB	-2.75E+1	5.78E-1	-7.63E+0	-1.80E-4	-3.54E-2	5.80E-1
EJ1	-3.30E+1	6.25E-1	-7.80E+0	-2.90E-4	-3.45E-2	5.79E-1

Table 4. Polynomial equations modeling the production of ethanol and glycerol as a function of initial sugar concentration (S) and time (t) for the 6 strains of *S. bacillaris*

Ethanol						
Strain		**S**	**t**	**S^2**	**S*t**	**t^2**
FC54	7.80E+0	-5.38E-2	1.41E+0	9.86E-5	-2.24E-4	-4.39E-2
Cz03	1.02E+1	-7.48E-2	1.36E+0	1.41E-4	-3.01E-4	-4.16E-2
EER3C	9.84E+0	-6.26E-2	1.21E+0	9.84E-5	8.42E-4	-4.69E-2
PE3WA	1.81E+1	-1.25E-1	1.34E+0	2.23E-4	-2.27E-4	-4.17E-2
EIF7LB	-2.40E-1	7.85E-3	1.55E+0	-1.84E-5	-5.02E-4	-4.69E-2
EJ1	1.13E+1	-6.89E-2	1.26E+0	1.09E-4	4.09E-4	-4.62E-2
Glycerol						
Strain		**S**	**t**	**S^2**	**S*t**	**t^2**
FC54	1.64E+1	-1.07E-1	1.09E+0	1.93E-4	9.78E-4	-4.46E-2
Cz03	1.88E+1	-1.23E-1	8.85E-1	2.19E-4	1.57E-3	-4.19E-2
EER3C	2.21E+1	-1.49E-1	9.53E-1	2.70E-4	1.20E-3	-4.00E-2
PE3WA	2.05E+1	-1.31E-1	8.02E-1	2.29E-4	1.50E-3	-3.87E-2
EIF7LB	9.69E+0	-5.02E-2	9.90E-1	7.94E-5	1.42E-3	-4.58E-2
EJ1	1.20E+1	-6.66E-2	9.29E-1	1.10E-4	1.39E-3	-4.24E-2

Table 5. Polynomial equations modeling the production of acetic acid and consumption of malic acid as a function of initial sugar concentration (S) and time (t) for the 6 strains of *S. bacillaris*

Acetic acid						
Strain		**S**	**t**	**S^2**	**S*t**	**t^2**
FC54	-3.76E-1	3.01E-3	4.58E-2	-5.26E-6	1.12E-5	-1.62E-3
Cz03	-8.58E-1	6.04E-3	6.78E-2	-9.99E-6	-4.58E-5	-1.82E-3
EER3C	-1.68E+0	1.19E-2	9.31E-2	-2.08E-5	-5.19E-5	-2.71E-3
PE3WA	-8.81E-1	6.27E-3	6.91E-2	-1.09E-5	-3.42E-5	-2.00E-3
EIF7LB	-7.99E-1	5.37E-3	7.05E-2	-8.71E-6	-4.52E-5	-2.01E-3
EJ1	2.99E-1	-2.39E-3	4.24E-2	5.06E-6	3.49E-5	-1.69E-3
Malic acid						
Strain		**S**	**t**	**S^2**	**S*t**	**t^2**
FC54	9.09E-1	1.66E-2	-2.26E-1	-3.17E-5	4.31E-4	2.87E-3
Cz03	3.40E+0	-9.44E-4	-2.46E-1	-1.55E-6	5.74E-4	1.55E-3
EER3C	2.65E+0	4.76E-3	-2.49E-1	-1.18E-5	5.09E-4	3.20E-3
PE3WA	2.60E+0	4.23E-3	-2.33E-1	-9.40E-6	4.71E-4	2.95E-3
EIF7LB	2.42E+0	5.53E-3	-2.22E-1	-1.19E-5	4.64E-4	2.31E-3
EJ1	1.93E+0	8.68E-3	-1.69E-1	-1.68E-5	2.88E-4	2.47E-3

Table 6. Predicted values, after 21 days of fermentation, of main compounds of enological interest for the 6 strains of *S. bacillaris*. Concentrations reported are based on the polynomial equations reported in tables 3, 4 and 5, considering an initial sugar concentration at 200 (first value) and 330 g/L (second value).

Strain	Residual glucose	Residual fructose (17 days)	Ethanol	Glycerol	Acetic acid	Malic acid
	g/L	g/L	% v/v	g/L	g/L	g/L
FC54	0.1 - 142.6	<0.1- <0.1	10.3 - 9.5	10.2 - 12.3	0.31 - 0.37	1.3 - 2.5
Cz03	7.2 - 153.1	<0.1- <0.1	9.9 - 9.1	9.7 - 13.1	0.38 - 0.35	1.1 - 2.4
EER3C	1.6 - 146.2	<0.1- <0.1	9.6 - 10.5	10.5 - 12.9	0.41 - 0.39	1.5 - 2.6
PE3WA	<0.1 - 153.6	<0.1- <0.1	10.6 - 9.0	9.5 - 12.4	0.36 - 0.33	1.4 - 2.6
EIF7LB	<0.1 - 153.3	<0.1- <0.1	10.4 - 8.8	9.4 - 12.2	0.33 - 0.31	1.4 - 2.5
EJ1	<0.1 - 131.4	<0.1- <0.1	9.7 - 9.5	9.8 - 12.5	0.32 - 0.45	1.7 - 2.5

Figure 1

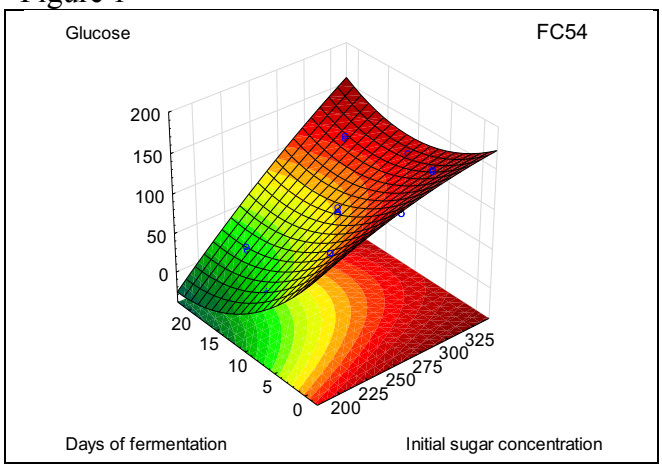

Figure 2

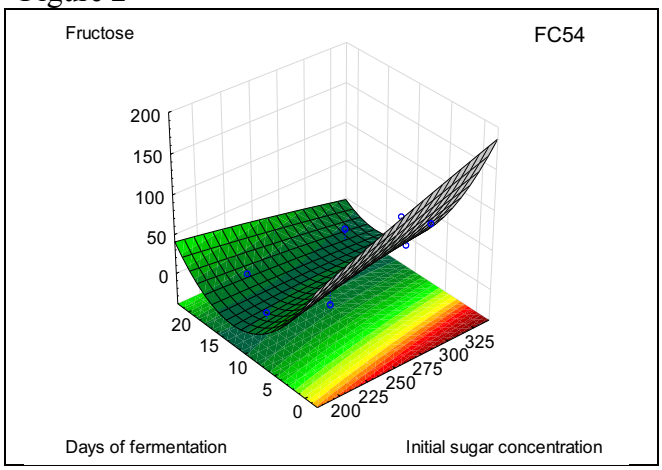

Figure 3

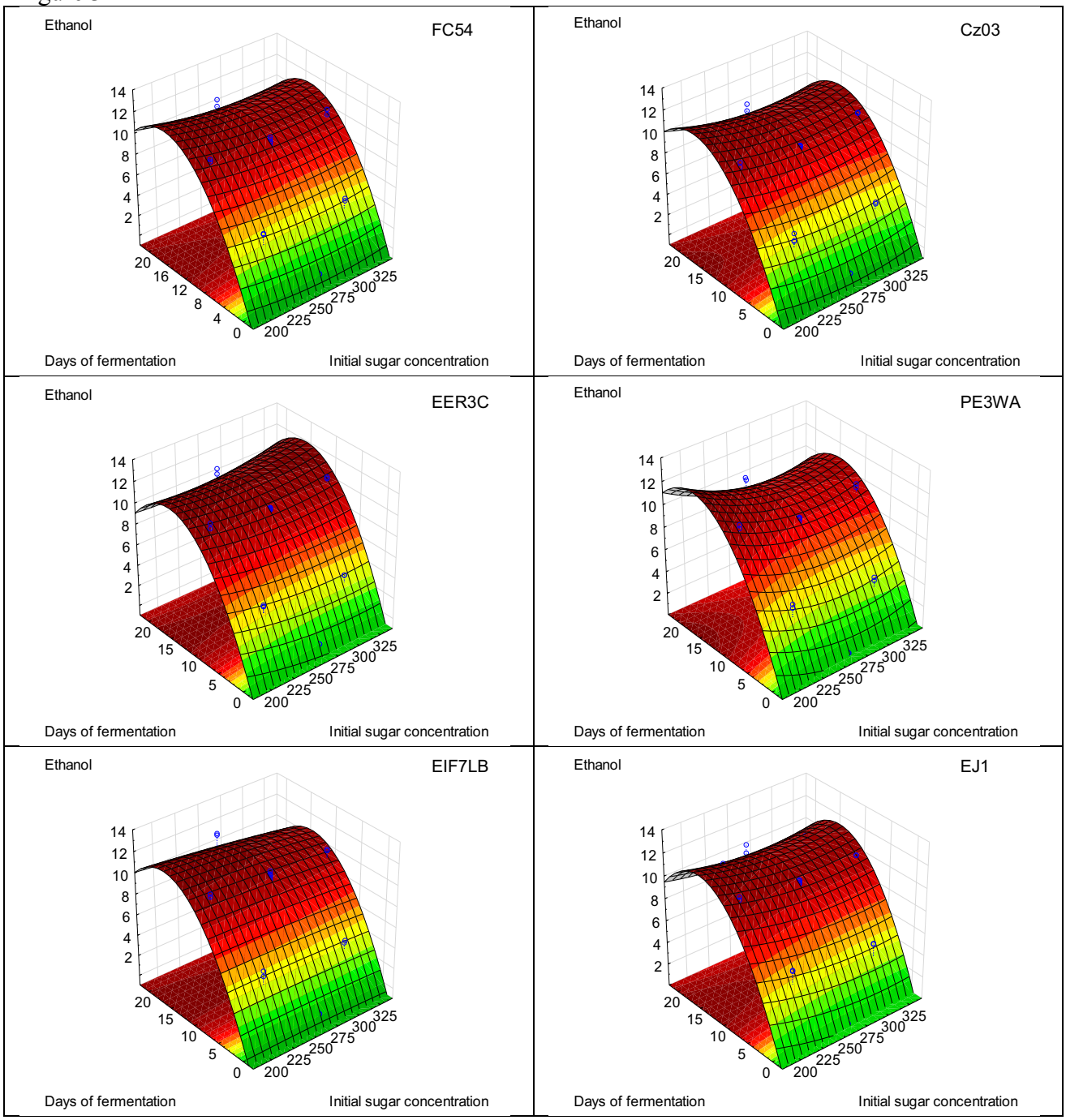
Ethanol
FC54
Days of fermentation
Initial sugar concentration
Ethanol
Cz03
Days of fermentation
Initial sugar concentration
Ethanol
EER3C
Days of fermentation
Initial sugar concentration
Ethanol
PE3WA
Days of fermentation
Initial sugar concentration
Ethanol
EIF7LB
Days of fermentation
Initial sugar concentration
Ethanol
EJ1
Days of fermentation
Initial sugar concentration

Figure 4

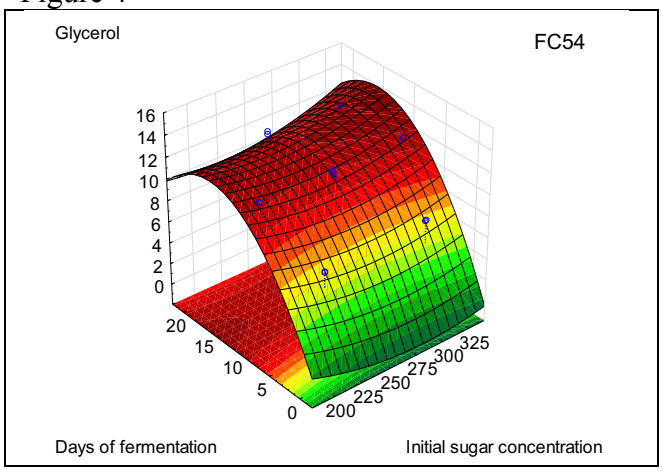

Figure 5

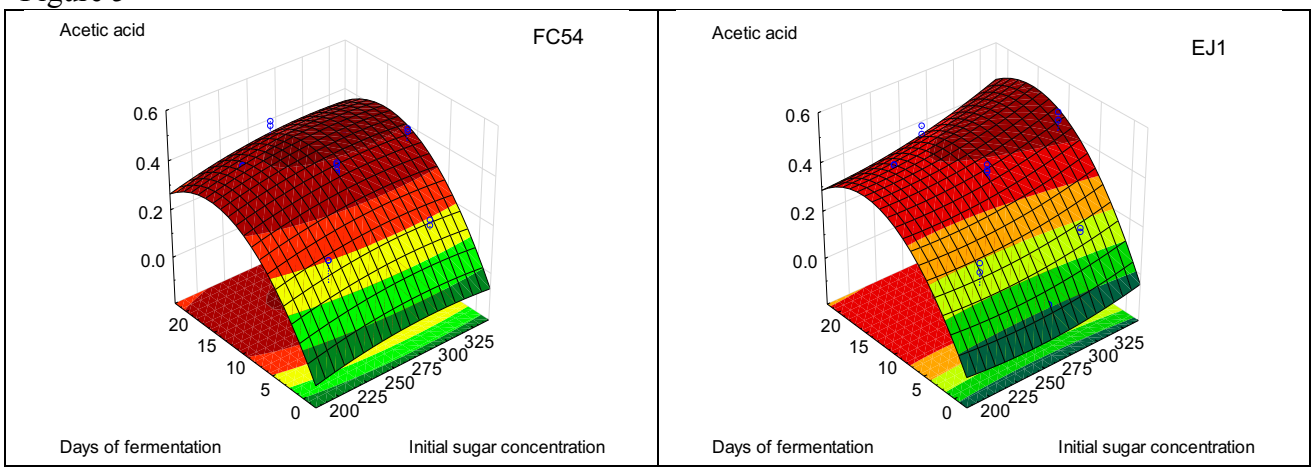

Figure 6