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Special Issue Article

Impact of yeast starter formulations on the production of volatile compounds during wine fermentation

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Abstract

The most diffused starter formulation in winemaking is actually represented by active dry yeast (ADY). Spray-drying has been reported as an appropriate preservation method for yeast and other micro-organisms. Despite the numerous advantages of this method, the high air temperatures used can negatively affect cell viability and the fermentative performance of dried cells. In the present study, 11 wine *S. cerevisiae* strains (both indigenous and commercial) were submitted to spray-drying; different process conditions were tested in order to select the conditions allowing the highest strain survival. The strains exhibited high variability for tolerance to spray-drying treatment. Selected strains were tested in fermentation at laboratory scale in different formulations (free fresh cells, free dried cells, immobilized fresh cells and immobilized dried cells), in order to assess the influence of starter formulation on fermentative fitness of strains and aromatic quality of wine. The analysis of volatile fraction in the experimental wines produced by selected strains in different formulations allowed identification of > 50 aromatic compounds (alcohols, esters, ketones, aldehydes and terpenes). The results obtained showed that the starter formulation significantly influenced the content of volatile compounds. In particular, the wines obtained by strains in dried forms (as both free and immobilized cells) contained higher numbers of volatile compounds than wines obtained from fresh cells. Copyright © 2014 John Wiley & Sons, Ltd.

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Keywords: **spray-drying; *Saccharomyces cerevisiae*; drying tolerance; starter formulation; volatile compounds**

Introduction

In the wine sector, the use of starter cultures has been widely increased in recent decades. Most oenologists currently tend to inoculate musts with commercial active dry yeasts (ADYs), mainly belonging to the species *Saccharomyces cerevisiae*, in order to improve wine quality and stability. Due to the demanding nature of modern winemaking practice, there is a continuously growing quest for specialized *S. cerevisiae* strains (Capece *et al.*, 2012) possessing a wide range of optimized or novel oenological properties. An optimal formulation is necessary to distribute such strains successfully to winemakers devoted to the use of specialized strains. The industrial companies generally do not produce small amounts of specialized strains as ADYs and, in this context, the possibility of setting up a system to dry yeast strains in small volumes, in suitable form, can assume great interest for winemakers dedicated to the use of 'specialized strains'.

Spray-drying has been reported to be an appropriate preservation method for yeasts and other micro-organisms (Luna *et al.*, 2000; Abadias *et al.*, 2005; Cañamás *et al.*, 2008). This is a unit operation in which a liquid product is atomized in a hot gas current to instantaneously obtain a powder. In fact, it is obvious that the exposure to

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the high air temperatures required to facilitate water evaporation during the passage of micro-organisms in the spray-drying system exerts a negative impact on cell viability. At the temperatures needed to produce powders with a moisture content of ca. 4%, required for powder stability and spoilage prevention (Masters, 2002), low microbial survival can often occur. During spray-drying, different factors influence the resistance of micro-organisms and, consequently, the subsequent viability of the cells. The drying temperature and rate can be critical for the resistance of yeasts to dehydration and rehydration. Some authors (Luna *et al*., 2003, 2005) have reported the influence of process variables and processing aids on the viability of brewer's yeast submitted to spray-drying. Abadias *et al*. (2005) evaluated the impact of different factors, such as drying temperatures, carrier, growth and rehydration medium, on the viability of *Candida sake* cells after spray-drying; it was concluded that one of the principal factors that affect microbial survival during spray-drying is the ability of a strain to withstand high temperatures. The intrinsic sensitivity of a given strain to heat appears to be an important factor in determining cell survival (To and Etzel, 1997; Gardiner *et al*., 2000; Simpson *et al*., 2005) and the differences in survival rate reflect strains' intrinsic tolerance to heat (Lian *et al*., 2002). In addition to maintaining the viability of dried microbial cultures, it is important that principal microbial properties are maintained following the spray-drying process. Different authors (Gardiner *et al*., 2000; Silva *et al*., 2002) have analysed the effect of spray-drying process variables, not only over final moisture but also on the biological or biochemical activity of dehydrated products. The spray-drying of wine yeasts has to produce dried cultures characterized by storage stability and acceptable fermentative fitness.

In traditional fermentation systems the starters are inoculated as free cell suspensions, but numerous biotechnological processes are advantaged by the use of immobilized cell systems. This technique offers technical and economic benefits compared to the traditional system, including enhanced fermentation productivity, feasibility of continuous processing, lower costs of recovery and recycling and cell stability (Kourkoutas *et al*., 2004). Since *S. cerevisiae* cells are found attached to each other or to a surface in their natural habitat (e.g. a grape), immobilization may be regarded as a natural growth form, which may offer cell protection from certain stresses (Verstrepen and Klis, 2006). The immobilization of spray-dried cells in calcium alginate beads, which are considered the most suitable system for alcoholic fermentation (Colagrande *et al*., 1994), might represent a new biocatalyst, very attractive for modern winemaking.

In the present study, different *S. cerevisiae* wine strains were assessed for their tolerance to spray-drying treatment. Selected strains were tested during fermentation at laboratory scale in different formulations (as free fresh cells, free dried cells, immobilized fresh cells and immobilized dried cells) in order to assess the influence of starter formulation on fermentative fitness of strains and final quality of wine.

Materials and methods

Yeast strains and cultivation conditions

In this study, 11 *S. cerevisiae* strains were used (Table 1). Some were indigenous yeasts, isolated during spontaneous fermentation of grapes and previously selected, whereas two were commercial starters. The strains were maintained on YPD medium (1% w/v yeast extract, 2% w/v peptone, 2% w/v glucose, 2% w/v agar).

Spray-drying of yeast strains

Stationary phase cultures of the *S. cerevisiae* strains were prepared for spray-drying treatment. A loopful of fresh culture of each strain was inoculated in 200 ml YPD broth and maintained on a rotary shaker at 180 rpm. The cultures were incubated at 26°C for 72 h. After centrifugation at 10 000 × *g* for 10 min at 4°C, the cells were resuspended in 100 ml liquid YPD, with skimmed milk and maltose added as protectant agents (3% v/v). The samples were spray-dried in a laboratory-scale apparatus (Mini Spray-drier B-191, Buchi).

Two spray-drier conditions were tested: outlet air temperature 60°C/inlet air temperature 120°C; and outlet air temperature 55°C/inlet air temperature 105°C. Yeasts suspensions were sprayed through a pressure nozzle in a climatic chamber where drying was achieved by a flow of totally dried air. After drying, the powder samples (final humidity 8%) were stored under vacuum at 4°C to prevent rehydration.

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Table 1. List of strains used during this study

Strain	Code	Origin
4LB	AV	Wild wine strain, Basilicata region; Capece *et al.* (2011)
AGME	AV1	Wild wine strain, Basilicata region; Capece *et al.* (2011)
F15	CS	Commercial wine strain, Laffort
RHONE 2323	CS1	Commercial wine strain, Lalvin
SC2-37	ST	Wild wine strain, Tuscany region
SB5-18	ST1	Wild wine strain, Tuscany region
SA7-13	ST2	Wild wine strain, Tuscany region
RB3-7sc2	NA	Wild wine strain, Sicily region; Capece *et al.* (2010)
TA8-4sc2	NA1	Wild wine strain, Sicily region
CB1-7sr3	NA2	Wild wine strain, Sicily region; Siesto *et al.* (2013)
CD2-6sc2	NA3	Wild wine strain, Sicily region

To determine the survival rate of the yeast strains, approximately 0.5 g powder from each treatment was rehydrated with 20 ml sterile solution of glucose (5% v/v) at 26°C; the dried cells were shaken at 180 rpm for 30 min. The final concentration of dehydrated cells in spray-dried powder was determined on YPD by the plate count technique. The total viable cell number was expressed as colony-forming units (CFU)/g dry powder. Survival was defined as the ratio of viable cells before and after drying. The percentage of surviving cells was calculated as follows:

$$\text{Survival } \% = (Nf/Ni)´100$$

where *Ni* represents the CFU in the yeast cell suspension before drying, and *Nf* is the CFU in the spray-dried powder. To calculate the dry matter in the initial suspension of yeast cells and the final spray-dried powders, 1 ml or 1 g were placed in duplicate in an aluminium-weighing boat and dried in a convection oven at 105°C for 24 h.

Data related to strain viability after spray-drying treatment were submitted to three-way analysis of variance (ANOVA), considering three factors (protectant agents, temperature combinations and strains) and their interactions. Statistical analysis was performed using R Core Team, Statistical Software (v. 3.0.0). The normal distribution of data and residuals was confirmed using the Shapiro–Wilk normality test, while Levene's test was applied to check the homogeneity of variance.

Cell immobilization

Fresh and dried cells of four selected strains were immobilized in sodium alginate beads, using the procedure described by Ferraro *et al.* (2000). The cells (both in dried and fresh forms) were added to 10 ml water (suspension A) in such a concentration to assure a final concentration of 10^9 cells/g beads. These cellular suspensions were added to 2.5% w/w sodium alginate solution (suspension B) at a ratio of 5% (wet weight). This mixture was added dropwise to a 0.1 M $CaCl_2$ crosslinking solution, using a syringe. The beads were cured for 1 h at room temperature and washed with sterile solution. After 1 h the beads were washed several times with sterile water and used immediately. The beads obtained from 5 ml suspension B were used to inoculate 100 ml grape must.

Inoculated fermentations at laboratory scale with *S. cerevisiae* in different formulations

The selected *S. cerevisiae* strains, prepared in different formulations, were tested in laboratory-scale fermentations. Inoculated fermentation assay was performed in 130 ml Erlenmeyer flasks filled with 100 ml sulphited natural grape must (50 mg/l). Each strain was inoculated in grape must in four different formulations: fresh and dried cells in free suspension and in immobilized form; for each formulation, a final concentration of 10^7 viable cells/ml was inoculated. The dried cells, both free and immobilized, were added directly in the grape must without rehydration. The fermentation was performed at 26°C and the fermentative course was monitored daily by measuring weight loss, determined by carbon dioxide evolution during the process. At the end of the process, the wine samples were refrigerated at 4°C to clarify the wine, racked and stored at –20°C until required for analysis. All the experiments were performed in duplicate.

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Analysis of volatile compounds

Higher alcohols (*n*-propanol, isobutanol, amyl alcohols), acetaldehyde, ethyl acetate and acetic acid were analysed by direct injection gas chromatography of 1 μl experimental wines, following the method described in Capece *et al*. (2013). Levels of these compounds were quantified by internal standardization (calibration curves), using Agilent ChemStation software, and submitted to three-way ANOVA considering three fixed factors, which were cell state (fresh or dried), fermentation type (free or immobilized) and strain, and their interactions. ANOVA analysis was performed using R Core Team, statistical software (v. 3.0.0). The normal distribution of data and residuals was confirmed by Shapiro–Wilk normality test, while Levene's test was applied in order to check the homogeneity of variance.

Other volatile compounds, such as acetates and ethyl esters, volatile fatty acids and terpenes, were analysed by SPME–GC–MS, following the procedure described by Calabretti *et al*. (2012). Data of volatile compounds were submitted to principal component analysis (PCA), using the statistical package PAST v. 1.90 (Hammer *et al*., 2001).

Results

Survival of *S. cerevisiae* strains in response to spray-drying treatment

Eleven *S. cerevisiae* wine strains (nine wild and two commercial) were submitted to spray-drying treatment by testing different conditions, namely two protectant agents (skimmed milk and maltose) and two combinations of outlet/inlet temperatures (60/120°C and 55/105°C). For each strain, three independent experiments were performed. The experimental results for cell survival of the 11 strains after drying at different conditions are shown in Table 2. For each treatment, high strain biodiversity in the surviving rate was found. The strain exhibiting the highest survival was ST, which in each condition showed the highest level of viability. In each experiment, this strain was also found to be more resistant to spray-drying than the two commercial strains used in this study. Regarding the different protectant agents, the addition of skimmed milk assured higher cell survival of the majority of analysed strains than maltose at both the temperature combinations tested. For the main percentage of strains (8), no significant differences in cell survival rate were found between the two different combinations of temperature by using maltose as the protectant agent. Otherwise, by using skimmed milk, all the strains (except NA and ST), after treatment at 60/120°C, exhibited a significantly different survival from that obtained at the temperature combination of 55/105°C. The lowest cell survival was exhibited by strains NA3 and AV1, which can be considered to have had no survivors with all the treatments tested.

The use of skimmed milk as the protectant agent and the combination of 60/120°C was considered the most successful condition, being the only one that allowed a survival > 50% for two strains (CS and ST).

Three-way ANOVA (Table 3) revealed significant differences in the survival of strains in response to spray-drying treatment for the variables protectant

Table 2. Survival (%) after spray-drying of 11 *S. cerevisiae* strains by using skimmed milk and maltose as protectant agents and two temperature combinations: results are mean ± SD of three independent spray-drying trials

Strains	Skimmed milk 120–60°C	Skimmed milk 105–55°C	Maltose 120–60°C	Maltose 105–55°C
AV	6.71 ± 0.13	16.58 ± 1.15	2.48 ± 0.11	1.65 ± 0.04
AV1	0.22 ± 0.02	0.57 ± 0.01	1.60 ± 0.14	0.90 ± 0.01
CS	50.67 ± 1.98	38.22 ± 2.46	10.13 ± 2.73	14.72 ± 1.01
CS1	38.13 ± 1.89	23.18 ± 1.50	14.00 ± 0.50	9.70 ± 0.44
ST	62.39 ± 5.05	61.65 ± 0.71	33.71 ± 0.89	29.71 ± 1.23
ST1	0.39 ± 0.04	5.97 ± 0.05	6.99 ± 0.65	6.79 ± 0.21
ST2	13.39 ± 0.04	28.08 ± 1.40	16.52 ± 0.48	10.25 ± 0.10
NA	23.13 ± 0.81	25.58 ± 0.89	2.80 ± 0.07	1.91 ± 0.05
NA1	7.59 ± 0.23	13.69 ± 0.40	12.20 ± 1.46	6.86 ± 0.32
NA2	25.14 ± 1.37	18.46 ± 0.14	1.79 ± 0.13	1.51 ± 0.03
NA3	0.02 ± 0	1.54 ± 0.01	0.00	0.00

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Table 3. Results of three-way ANOVA for cell survival after spray-drying of 11 *S. cerevisiae* strains, using two protectant agents and two temperature combinations

Source of variation	*df*	MS	*V*%	*F*
S	10	2255.2	27.17	2741.4***
P	1	5433.8	65.48	6605.3***
T	1	6.4	0.08	7.8**
S × P	10	505.8	6.10	614.9***
S × T	10	53.7	0.65	65.3 ***
P × T	1	43.4	0.53	52.8***
S × P × T	10	81.3	0.98	98.8***
Residuals	88	0.8		

S, strains (AV, CS, ST, NA); P, protectant agents (skimmed milk, maltose); T, = inlet–outlet temperatures (60–120°C; 55–105°C); *df*, degrees of freedom; MS, mean square; *F*, variance ratio; *V*%, percentage of variance explained.
***$p < 0.001$;**$p < 0.01$.

agents ($p < 0.001$), temperature ($p < 0.01$) and strains $p < 0.001$). All the interactions among the independent variables were highly significant ($p < 0.001$). However, the protectant agent exerted the highest impact (65.48% of variance), followed by strain impact (27% of variance), whereas the temperature and the interactions had a low influence.

Survival kinetics of spray-dried cells during storage

The cells obtained after spray-drying at 60/120°C using skimmed milk were maintained at 4°C in order to evaluate the influence of storage on cell viability. Survival of all the strains decreased during the storage time at 4°C, although at different levels in different strains (Figure 1). The survival curve of strain NA3 showed the highest decrease during storage and no survival was observed at the end of month 4 of storage at 4°C. Three strains (AV, AV1 and NA2) did not survive after 6 months of storage, whereas the cells of the remaining strains were viable after storage of 8 months. The viability of these latter strains, evaluated at the last analysed time (8 months), was in the range 6×10^6 (strain ST)–8×10^2 (strain NA1) cells/ml.

On the basis of these results, for each isolation origin, the strains exhibiting the highest cell survival during storage were chosen for further characterization; the selected strains were AV, NA, ST and CS.

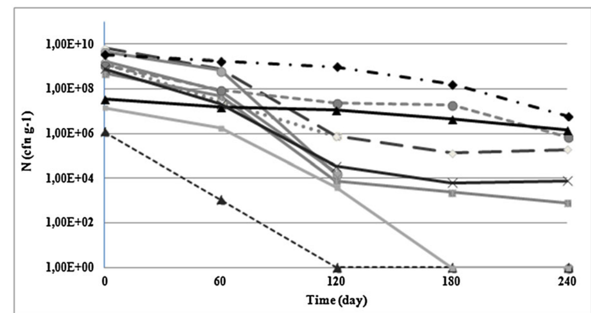

Figure 1. Survival (CFU/g at corresponding evaluation time) of spray-dried *Saccharomyces cerevisiae* strains during storage at 4°C: AB; AV1; CS; CS1; NA; NA1; NA2; NA3; ST; ST1; ST2

Fermentative performance

The effect of spray-drying on strain fermentative performance was investigated. The four selected strains, as free and immobilized cells in dried form, were tested during inoculated fermentation at laboratory scale. Untreated ('fresh') cells, both free and immobilized, were used as controls.

The fermentation process was considered complete when the weight loss became non-variable (the criterion for stopping the experiment). All the strains completed the process after 14 days (Figure 2A–D). When the strains were inoculated in the free forms, both as fresh and dried cells, a similar trend was found for all the strains tested, with the maximum CO_2 production at day 7. A similar result was exhibited by strains inoculated as dried free cells. The highest differentiation among the fermentation kinetics of the four strains was found when the strains were inoculated as dried immobilized cells. In this formulation, only strain ST (Figure 2C) showed a fermentation rate similar to those detected in the other three formulations, whereas the strain CS (commercial strain; Figure 2A) reduced the fermentation rate greatly. The immobilization of dried cells determined a reduction of CO_2 production until the middle stage of fermentation (i.e. until day 7), whereas these differences were reduced at the end of the process.

At the end of the fermentation process, the wine samples were analysed for content of by-products related to wine aroma, in order to evaluate the influence of strain formulation on its metabolic behaviour.

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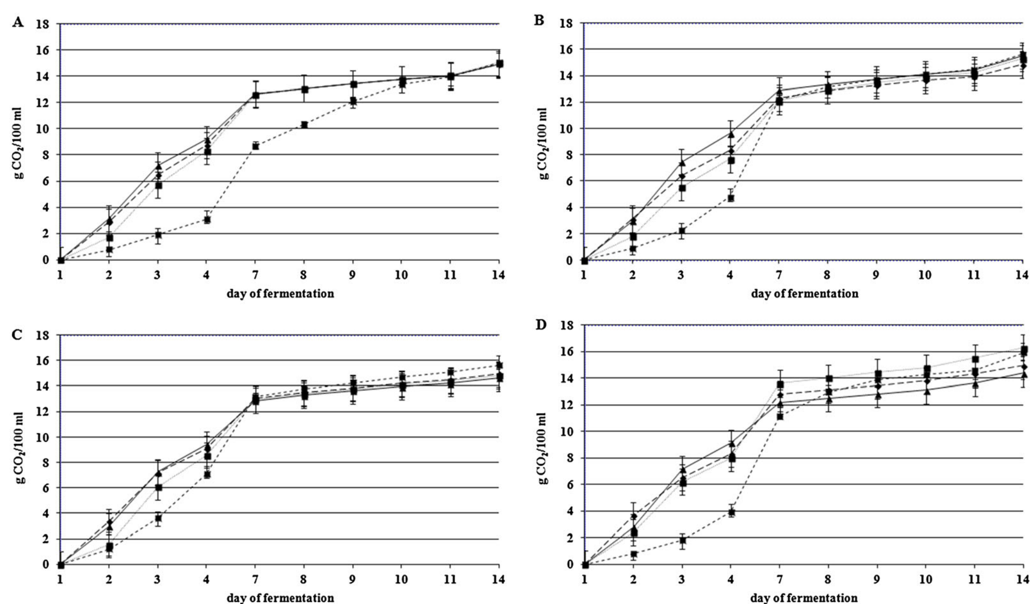

Figure 2. (A–D) Fermentation kinetics of four *S. cerevisiae* strains in different starter formulations: A, CS; B, NA; C, ST; D, AV; —◆–, fresh free; ····■····, fresh immobilized; ——▲——, dried free; – ■–, dried immobilized

The amounts of secondary compounds usually present in high concentrations in wines, such as acetaldehyde, *N*-propanol, isobutanol, amyl alcohols, ethyl acetate and acetic acid, are reported in Table 4 as the means of two different replicates for each strain in the different formulations. Three-way ANOVA was applied to investigate the influence of all independent variables, represented by the state of cells (fresh or dried), type of inoculum (free or immobilized cells) and strains and their interactions (Table 5). For almost all the compounds analysed, the production levels proved to be significantly affected by all the variables and possible interactions of these effects (almost all the effects and possible interactions produced $p < 0.001$). Only the interaction between state of cells and type of inoculum did not significantly affect the production level of isobutanol.

Table 4. By-products in experimental wines produced by the four *S. cerevisiae* strains in the different cell formulations

Strains	SF*	Acetaldehyde	Ethyl acetate	*N*-Propanol	Isobutanol	D-Amyl alcohol	Isoamyl alcohol	Acetic acid
AV	F	14.09 ± 0.53	58.03 ± 0.23	57.42 ± 0.43	39.36 ± 2.67	62.32 ± 5.42	231.74 ± 3.13	241.68 ± 19.19
	FI	64.91 ± 3.01	56.61 ± 0.90	59.74 ± 0.52	32.63 ± 3.69	27.80 ± 3.79	136.81 ± 15.76	651.91 ± 67.54
	D	19.45 ± 1.59	50.70 ± 0.74	64.40 ± 5.15	65.84 ± 10.56	65.13 ± 1.12	253.21 ± 19.73	391.58 ± 75.05
	DI	42.56 ± 3.80	56.75 ± 0.69	73.51 ± 0.25	50.63 ± 0.31	54.22 ± 1.63	197.26 ± 3.59	388.02 ± 7.13
CS	F	43.66 ± 1.04	55.25 ± 0.11	61.35 ± 0.93	48.86 ± 2.43	41.48 ± 6.27	174.47 ± 25.72	478.55 ± 117.05
	FI	67.11 ± 2.36	55.10 ± 0.16	65.33 ± 0.64	43.60 ± 1.25	26.78 ± 0.35	122.01 ± 1.18	497.46 ± 34.77
	D	75.08 ± 5.37	58.02 ± 1.07	73.62 ± 0.11	91.65 ± 1.66	66.11 ± 7.83	239.48 ± 5.47	795.59 ± 63.46
	DI	49.22 ± 4.03	62.22 ± 2.55	90.53 ± 3.10	100.50 ± 4.12	52.00 ± 0.11	203.51 ± 6.00	463.92 ± 51.31
NA	F	34.13 ± 0.64	56.33 ± 0.06	58.57 ± 0.40	42.89 ± 1.70	50.06 ± 4.02	226.73 ± 11.94	428.91 ± 10.89
	FI	75.68 ± 2.31	60.79 ± 2.21	65.91 ± 0.80	31.12 ± 1.23	25.83 ± 0.52	124.87 ± 3.54	868.99 ± 77.77
	D	35.71 ± 1.01	56.04 ± 0.01	63.17 ± 0.35	67.07 ± 2.86	51.95 ± 1.38	228.00 ± 2.98	654.19 ± 36.76
	DI	43.55 ± 6.80	59.76 ± 1.78	80.54 ± 1.12	52.99 ± 2.98	43.05 ± 2.14	190.56 ± 14.96	784.99 ± 32.81
ST	F	24.94 ± 4.44	61.99 ± 0.02	65.49 ± 2.54	64.37 ± 2.96	67.54 ± 4.00	263.56 ± 14.42	251.37 ± 42.46
	FI	42.2 ± 0 0.98	60.60 ± 0.66	65.57 ± 1.91	43.83 ± 5.58	32.79 ± 2.20	146.96 ± 11.18	759.28 ± 26.15
	D	28.86 ± 10.21	62.66 ± 2.55	67.18 ± 2.74	74.29 ± 8.38	64.33 ± 5.44	263.97 ± 25.96	350.64 ± 58.74
	DI	35.59 ± 4.59	62.40 ± 0.73	72.45 ± 2.74	62.32 ± 4.24	48.87 ± 1.35	226.51 ± 9.27	659.47 ± 88.08

SF*, strain formulation; DI, dried immobilized; FI, fresh immobilized; D, dried free; F, fresh free.Data are mean ± SD of two independent experiments

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Table 5. Results of three-way ANOVA for by-product levels determined in wines produced by the four *S. cerevisiae* strains in the different cell formulations

Source of variation	df	Acetaldehyde		Ethyl acetate		n-Propanol		Isobutanol		Acetic acid		Amyl alcohol		Isoamyl alcohol	
		F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS
S	1	25.57***	292.2	189.22***	414.25	504.51***	1474.64	815.85***	9103.6	11.73**	20783	245.43***	2186.2	231.28***	30645
F	1	350.61***	4006.4	352.97***	772.73	230.65***	674.18	100.32***	1119.4	238.47***	422363	504.63***	4495.0	400.92***	53122
SS	3	132.38***	1512.8	436.48***	955.54	60.75***	177.58	139.08***	1551.9	88.15***	156137	34.61***	335.1	29.53***	3913
S × F	1	258.56***	2954.6	309.94***	678.53	68.66***	200.70	0,07	15.5	176.97***	313436	85.12***	758.3	50.37***	6674
S × SS	3	22.38***	255.7	330.50***	723.53	30.72***	89.81	61.02***	680.9	12.39***	21961	21.16***	188.5	6.51**	863
F × SS	3	74.37***	849.9	219.88***	481.36	23.63***	69.08	17.30***	193.1	99.00***	175342	9.79***	87.3	4.33*	574
S × F × SS	3	17.02***	195.1	266.60***	584.30	4.31*	12.60	6.91**	77.1	3.33*	5904	8.84***	78.8	3.50*	465
Residuals	32		11.4		2.19		2.92		11.2		1771		8.9		132

S, cell state (fresh and dried); F, fermentation type (free or immobilized); SS, strains (AV, CS, ST, NA); *df*, degrees of freedom; MS, mean square; *F*, variance ratio.*$p < 0.05$;**$p < 0.01$;***$p < 0.001$.

Isoamyl alcohol was always produced at the lowest amounts when the strain was inoculated in the fresh immobilized state, whereas free dried cells generally produced the highest amounts (Table 4). With regard to acetic acid, generally the lowest amount was detected in wines produced by strains in fresh free form, whereas the highest level was detected in wines produced by inoculating the strains as fresh immobilized cells (except CS, which produced the highest amount of acetic acid in dried free form). However, only strain NA, inoculated as fresh immobilized cells, produced considerable amounts (>800 mg/l), which can negatively affect the organoleptic quality of the wine.

Analysis of the volatile fraction by SPME–GC–MS of the experimental wines, obtained by inoculating the four strains in four different formulations, allowed the identification of about 50 compounds, belonging to different chemical classes, such as acetate esters, ethyl esters, higher alcohols, carbonyl compounds and volatile fatty acids. The amounts (mg/l) of compounds determined in each wine are reported in Table 6. Among the esters, the compounds present in the highest amounts were represented by methyl acetate, ethyl propanoate, ethyl 2-methylbutanoate and ethyl octanoate, whereas 2,3-butanediol was the higher alcohol present in the highest concentration.

For each formulation, strain ST produced the wine with the highest number of compounds, whereas the commercial strain CS produced wines with the lowest number of volatile compounds in almost all the formulations. The wines obtained by strains in fresh forms (both as free and immobilized cells) contained a lower number of volatile compounds than wines from strains in dried forms (both as free and immobilized cells). Some compounds were found only (hexyl acetate and 2-phenylethanol) or mainly (ethyl decanoate, ethyl hexadecanoate, 3-methylbutyl octanoate, ethyl dodecanoate) in wines obtained by using strains in dried forms.

The immobilization also seems to influence the production of volatile compounds. For almost all the strains, a higher number of volatile compounds was present in wines obtained by strains in free form (both fresh and dried cells) than in wines produced with immobilized cells, both in fresh and dried state (i.e. wines from strain AV free cells in fresh form were characterized by a higher number of volatile compounds than wines from fresh cells of strain AV in the immobilized state, and the same behaviour was observed for dried cells of this strain).

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Table 6. Volatile compounds (mg/l) determined by SPME–GC–MS in experimental wines produced by the four *S. cerevisiae* strains in different cell formulations

Compounds	AV F	AV FI	AV D	AV DI	NA F	NA FI	NA D	NA DI
Acetates								
Methyl acetate	16.76 ± 1.98	15.00 ± 1.63	18.73 ± 1.58	20.90 ± 2.26	16.10 ± 0.80	13.71 ± 1.27	19.86 ± 0.69	20.79 ± 0.22
2-Methylpropyl acetate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Isoamyl acetate	0.49 ± 0.04	0.41 ± 0.04	0.59 ± 0.10	0.70 ± 0.13	0.66 ± 0.00	0.60 ± 0.00	0.83 ± 0.00	0.84 ± 0.00
3-Methylbutyl acetate	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.10	0.10 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
cis-3-Hexenyl acetate	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.03 ± 0.01
2-Phenylethyl acetate	3.55 ± 0.62	0.00 ± 0.00	4.32 ± 0.21	0.00 ± 0.00	3.79 ± 0.57	0.00 ± 0.00	4.68 ± 0.18	0.00 ± 0.00
Hexyl acetate	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.07 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.01	0.05 ± 0.00
Esters								
Ethyl propanoate	15.13 ± 0.15	14.18 ± 0.06	16.47 ± 0.31	17.70 ± 0.21	14.16. ± 0.16	13.02 ± 0.23	17.69 ± 0.48	18.92 ± 0.28
Ethyl butanoate	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.01	0.12 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ethyl 2-methylpropanoate	12.54 ± 0.04	12.01 ± 0.03	13.94 ± 0.26	15.56 ± 0.33	11.16 ± 0.43	10.22 ± 0.48	13.37 ± 0.84	14.70 ± 0.08
Ethyl 2-methylbutanoate	23.36 ± 0.30	21.31 ± 0.54	26.27 ± 0.46	29.71 ± 0.64	0.00 ± 0.00	0.00 ± 0.00	10.92 ± 0.46	11.09 ± 0.03
Ethyl 3-methylbutanoate	0.00 ± 0.00	0.00 ± 0.00	0.83 ± 0.01	0.95 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ethyl pentanoate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.17 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.02
Ethyl hexanoate	18.75 ± 0.29	16.41 ± 0.14	20.56 ± 0.27	25.13 ± 0.33	16.86 ± 0.45	15.74 ± 0.41	21.00 ± 0.26	22.92 ± 0.26
Ethyl heptanoate	1.24 ± 0.04	0.00 ± 0.00	1.60 ± 0.05	0.00 ± 0.00	2.46 ± 0.09	0.00 ± 0.00	3.08 ± 0.15	0.00 ± 0.00
Methyl octanoate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ethyl octanoate	32.22 ± 2.09	0.00 ± 0.00	37.49 ± 2.28	46.56 ± 3.39	34.66 ± 2.06	0.00 ± 0.00	42.43 ± 1.73	45.54 ± 1.90
Methyl decanoate	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.02	0.07 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.00
Isopentylhexanoate	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.00	0.13 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Ethyl decanoate	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.01	0.19 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.03 ± 0.01
Ethyl hexadecanoate	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.01	0.17 ± 0.02
3-Methylbutyl octanoate	0.00 ± 0.00	0.00 ± 0.00	1.13 ± 0.16	1.23 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.81 ± 0.06	0.83 ± 0.02
Ethyl dodecanoate	0.00 ± 0.00	0.00 ± 0.00	0.09 ± 0.01	1.03 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.81 ± 0.06	0.05 ± 0.01
Ethyl tetradecanoate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.00	0.00 ± 0.00
Ethyl pentadecanoate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Higher alcohols								
Propan-2-ol	6.78 ± 0.47	5.17 ± 0.83	7.76 ± 0.80	9.04 ± 0.16	7.13 ± 0.03	6.08 ± 0.38	9.16 ± 0.42	9.58 ± 0.58
Butanol	0.05 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.16 ± 0.02	0.16 ± 0.01
1-Hexanol	2.72 ± 0.52	2.22 ± 0.18	3.32 ± 0.46	4.31 ± 0.50	3.94 ± 0.25	3.30 ± 0.17	4.86 ± 0.25	5.06 ± 0.19
3-Methyl-1-pentanol	0.01 ± 0.00	0.01 ± 0.00	0.11 ± 0.13	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
2-Octanol	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
cis-3-Hexen-1-ol	0.38 ± 0.04	0.38 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.58 ± 0.04	0.52 ± 0.03	0.70 ± 0.04	0.75 ± 0.06
Benzyl alcohol	0.24 ± 0.03	0.21 ± 0.01	0.29 ± 0.01	0.34 ± 0.04	0.18 ± 0.01	0.15 ± 0.01	0.21 ± 0.01	0.21 ± 0.01
2-Phenylethanol	0.00 ± 0.00	0.00 ± 0.00	70.34 ± 1.15	81.05 ± 3.10	0.00 ± 0.00	0.00 ± 0.00	67.43 ± 3.08	70.34 ± 2.57
Nonanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Decanol	0.00 ± 0.00	0.00 ± 0.00	0.92 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.61 ± 0.04	0.00 ± 0.00
1-Dodecanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1-Tetradecanol	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00
2,3-Butanediol	33.35 ± 1.74	28.57 ± 0.57	35.66 ± 3.55	39.61 ± 0.76	32.90 ± 3.83	31.21 ± 3.92	37.11 ± 2.76	42.33 ± 2.51
Carbonyl compounds								
Acetoin	13.64 ± 0.93	0.00 ± 0.00	17.08 ± 0.78	0.00 ± 0.00	8.76 ± 0.93	7.31 ± 0.36	10.96 ± 0.13	12.76 ± 0.75
Nonanal	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Decanal	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01
Undecanal	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
Dodecanal	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
Diacetyl	0.02 ± 0.01	0.00 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.08 ± 0.00	0.00 ± 0.00	0.06 ± 0.02	0.08 ± 0.01
Fatty acids								
Butanoic acid	0.91 ± 0.05	0.82 ± 0.08	1.01 ± 0.14	1.43 ± 0.36	0.70 ± 0.06	0.65 ± 0.06	0.85 ± 0.09	1.22 ± 0.02
Isobutyric acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.01	0.10 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Hexanoic acid	1.36 ± 0.12	0.00 ± 0.00	2.25 ± 0.18	2.76 ± 0.15	1.75 ± 0.13	1.45 ± 0.13	2.33 ± 0.06	2.99 ± 0.16
Octanoic acid	0.00 ± 0.00	2.37 ± 0.00	5.13 ± 0.00	5.99 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Decanoic acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.73 ± 0.03	0.64 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
Number of compounds	21	16	36	32	24	20	34	33

(Continues)

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Table 6. (Continued)

Compounds	ST F	ST FI	ST D	ST DI	CS F	CS FI	CS D	CS DI
Acetates								
Methyl acetate	31.17 ± 2.85	28.05 ± 0.64	35.35 ± 1.65	38.14 ± 1.89	22.21 ± 0.12	0.00 ± 0.00	24.80 ± 1.20	27.94 ± 0.23
2-Methylpropyl acetate	0.05 ± 0.01	0.03 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Isoamyl acetate	0.60 ± 0.08	0.55 ± 0.05	0.69 ± 0.03	0.77 ± 0.03	0.45 ± 0.06	0.46 ± 0.01	0.00 ± 0.00	0.29 ± 0.05
3-Methylbutyl acetate	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.04 ± 0.01
cis-3-Hexenyl acetate	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
2-Phenylethyl acetate	3.56 ± 0.52	0.00 ± 0.00	4.11 ± 0.22	4.53 ± 0.50	5.68 ± 0.93	0.00 ± 0.00	6.42 ± 1.22	7.39 ± 0.65
Hexyl acetate	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.03 ± 0.00
Esters								
Ethyl propanoate	15.96 ± 0.38	15.01 ± 0.08	18.00 ± 0.18	18.95 ± 0.14	16.51 ± 0.47	14.12 ± 0.23	17.88 ± 0.35	19.78 ± 0.52
Ethyl butanoate	0.17 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.18 ± 0.01	0.16 ± 0.01	0.19 ± 0.00	0.24 ± 0.03
Ethyl 2-methylpropanoate	12.60 ± 0.30	11.99 ± 0.12	14.24 ± 0.36	15.57 ± 0.48	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ethyl 2-methylbutanoate	0.00 ± 0.00	0.00 ± 0.00	11.33 ± 0.35	12.53 ± 0.10	24.10 ± 0.46	21.88 ± 0.48	26.41 ± 0.47	30.75 ± 0.52
Ethyl 3-methylbutanoate	1.04 ± 0.08	0.91 ± 0.06	1.17 ± 0.08	1.24 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ethyl pentanoate	1.10 ± 0.13	0.94 ± 0.04	1.27 ± 0.02	1.34 ± 0.07	0.92 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ethyl hexanoate	16.96 ± 0.61	16.14 ± 0.33	18.93 ± 0.13	20.00 ± 0.22	18.17 ± 0.13	16.12 ± 0.34	20.11 ± 0.29	23.38 ± 0.43
Ethyl heptanoate	1.97 ± 0.07	1.74 ± 0.08	2.33 ± 0.03	0.00 ± 0.00	2.59 ± 0.04	0.00 ± 0.00	2.92 ± 0.05	3.26 ± 0.07
Methyl octanoate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.01
Ethyl octanoate	30.84 ± 1.99	0.00 ± 0.00	37.19 ± 1.71	40.86 ± 2.58	37.35 ± 1.68	0.00 ± 0.00	42.97 ± 2.79	48.96 ± 3.82
Methyl decanoate	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.01	0.06 ± 0.03
Isopentylhexanoate	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.02 ± 0.00
Ethyl decanoate	0.17 ± 0.03	0.16 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.14 ± 0.02	0.17 ± 0.01
Ethyl hexadecanoate	0.00 ± 0.00	0.00 ± 0.00	0.14 ± 0.02	0.14 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.12 ± 0.02	0.12 ± 0.01
3-Methylbutyl octanoate	0.00 ± 0.00	0.00 ± 0.00	0.76 ± 0.04	0.85 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ethyl dodecanoate	0.00 ± 0.00	0.05 ± 0.01	0.04 ± 0.01	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.01	0.00 ± 0.00
Ethyl tetradecanoate	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.02	0.04 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ethyl pentadecanoate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01
Higher alcohols								
Propan-2-ol	7.50 ± 0.09	6.91 ± 0.35	9.34 ± 0.35	10.02 ± 1.19	7.59 ± 0.76	6.15 ± 0.11	8.80 ± 0.92	10.27 ± 0.58
Butanol	0.11 ± 0.02	0.10 ± 0.00	0.17 ± 0.02	0.16 ± 0.01	0.07 ± 0.02	0.08 ± 0.01	0.15 ± 0.06	0.15 ± 0.03
1-Hexanol	3.85 ± 0.58	3.20 ± 0.03	4.69 ± 0.66	5.33 ± 1.02	3.03 ± 0.21	2.26 ± 0.25	3.39 ± 0.73	0.00 ± 0.00
3-Methyl-1-pentanol	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01
2-Octanol	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01
cis-3-Hexen-1-ol	0.70 ± 0.04	0.00 ± 0.00	0.85 ± 0.03	0.92 ± 0.04	0.57 ± 0.06	0.53 ± 0.05	0.62 ± 0.10	0.75 ± 0.09
Benzyl alcohol	0.34 ± 0.04	0.27 ± 0.03	0.39 ± 0.04	0.44 ± 0.06	0.21 ± 0.02	0.23 ± 0.03	0.22 ± 0.01	0.27 ± 0.01
2-Phenylethanol	0.00 ± 0.00	0.00 ± 0.00	56.59 ± 1.83	63.91 ± 0.41	0.00 ± 0.00	0.00 ± 0.00	43.55 ± 2.42	49.96 ± 1.85
Nonanol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01
Decanol	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.01	0.04 ± 0.02
1-Dodecanol	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1-Tetradecanol	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.02 ± 0.01
2,3-Butanediol	30.92 ± 2.14	27.85 ± 0.99	35.20 ± 2.13	39.37 ± 3.50	44.38 ± 1.70	38.11 ± 2.60	50.68 ± 2.09	59.43 ± 1.24
Carbonyl compounds								
Acetoin	8.48 ± 0.06	8.08 ± 0.49	9.62 ± 0.66	10.59 ± 0.95	16.02 ± 1.04	0.00 ± 0.00	18.79 ± 0.76	22.62 ± 1.10
Nonanal	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01
Decanal	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.00
Undecanal	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Dodecanal	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.02 ± 0.00
Diacetyl	0.08 ± 0.02	0.59 ± 0.72	0.07 ± 0.01	0.66 ± 0.79	0.11 ± 0.01	0.12 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Fatty acids								
Butanoic acid	1.17 ± 0.25	1.04 ± 0.12	1.37 ± 0.30	1.49 ± 0.24	1.10 ± 0.10	0.94 ± 0.04	1.37 ± 0.25	1.48 ± 0.14
Isobutyric acid	0.10 ± 0.01	0.13 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.01
Hexanoic acid	1.66 ± 0.13	0.00 ± 0.00	1.98 ± 0.12	2.52 ± 0.52	1.75 ± 0.15	0.00 ± 0.00	1.94 ± 0.23	0.00 ± 0.00
Octanoic acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00
Decanoic acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Number of compounds	26	22	40	38	21	14	31	35

F, fresh free cells; D, dried free cells; FI, fresh immobilized cells; DI, dried immobilized cells.Data are mean ± SD of two independent experiments.

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Data related to volatile compounds were submitted to PCA in order to evaluate the relationships among the wines obtained by the analysed starters in different formulations; the scatter plot obtained is shown in Figure 3. The first component accounted for about 75% of the total variance and was correlated mainly with 2-phenylethanol, whereas the second component accounted for 11% of the total variance and was related mainly with ethyl octanoate. The PCA analysis was able to separate the wines in function of starter formulation. All the wines obtained by strains in fresh form were located in the left side of the PCA plot, whereas the wines obtained by dried cells were distributed in the right side (Figure 3). This result can be related to the highest number of volatile compounds detected in wines fermented with dried cells compared with those detected in samples obtained by inoculating fresh cells (Table 6). Furthermore, with regard to the wines obtained by inoculating fresh cells, this analysis allowed to discriminate wines obtained by free cells (upper part of PCA plot) from those produced by immobilized cells (lower part of Figure 3). This result is correlated to ethyl octanoate content, the factor mainly affecting the second component of PCA. In fact, all the wines produced by inoculating fresh free cells contained this compound, which was not detected in any of the wines from fresh immobilized cells.

Discussion

In order to be successful candidates for winemaking applications, wine yeast strains have to be able to withstand the harsh conditions encountered during the production of active dried yeast, the most diffused formulation of wine starter. Industries producing active or instant dry yeasts constantly search for new ways to increase the quality of their products. There are several alternative directions for this research. Undoubtedly, one of these involves the search for new and more dehydration-resistant strains that can be isolated from nature or constructed by genetic approaches. Increased desiccation tolerance was considered among the general targets that may be candidates for genetic engineering (Pretorius, 2000). Although natural habitats provide species that are strikingly highly resistant to dehydration (Khroustalyova *et al.*, 2001) and tolerance to desiccation is considered a desirable characteristic of wine yeasts, until now this parameter was poorly investigated during the selection programme of indigenous strains as starters for winemaking.

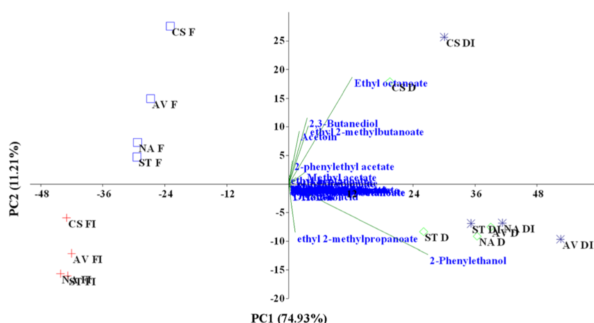

Figure 3. Principal component analysis (PCA) biplot of volatile compounds determined in wines obtained by different strain formulations: F, fresh free cells (□); D, dried free cells (+); FI, fresh immobilized cells (◊); DI, dried immobilized cells (*)

The manufacturers of active dried wine yeast starter cultures can positively influence the degree of cell viability and vitality, as well as the subsequent fermentation performance of their cultures. Different studies have reported the method used for assessing dried yeast quality during and after manufacture (Attfield *et al.*, 2000). Some authors have tested desiccation tolerance in indigenous *S. cerevisiae* strains (Rodríguez-Palero *et al.*, 2013) by using process conditions, which were very far from the parameters used for the production of commercial dried starters (i.e. drying of yeast cells at 30°C until constant dry weight was reached). Otherwise, in this study, the temperatures used during spray-drying were comparable with those utilized during the production of commercial starters. A number of empirical decisions were made in designing our experiments to dry the yeast strains, including decisions on drying time and temperature and the use of different protectant agents. The performance of different *S. cerevisiae* strains during spray-drying and powder storage was compared; conditions allowing the highest strain survival were identified, although considerable heterogeneity for spray-drying survival was observed among wild *S. cerevisiae* strains. At the same spray-drying conditions, the analysed *S. cerevisiae* strains showed distinctly different survival values, confirming that desiccation tolerance in yeasts varies from strain to strain, as previously reported (Lian *et al.*, 2002).

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Spray-drying is a potentially useful process for the large-scale production of dried starters containing high numbers of viable cells. However, for the use of starter cultures for winemaking this is not enough, and whether the fermentative performances of the dried strains are maintained after the treatment must be investigated. Knowledge of the structural, physiological and molecular bases for desiccation tolerance will contribute to our basic understanding of the living cell and its inherent ability to enter into, and return from, a state of complete metabolic arrest. However, these techniques generally show varying degrees of correlation with fermentative performance, and none of them alone can accurately predict the physiological activity of an active dried wine yeast starter culture. To our knowledge, this is the first time that the evaluation of fermentative performance of *S. cerevisiae* strains submitted to spray-drying, under conditions simulating commercial dried yeast production, has been reported. In this study, it was demonstrated that the production of most volatile compounds was significantly influenced by strain formulation. In particular, wines obtained by inoculating strains submitted to spray-drying contained a higher number of volatile compounds than those detected in wines produced by the strains in fresh form. This result could be related to changes in the expression level of genes involved in metabolic pathways related to the production of secondary compounds. Different authors (Singh *et al.*, 2005; Shima and Takagi, 2009; Calahan *et al.*, 2011; Ratnakumar *et al.*, 2011) have searched for genes involved in the response of yeasts to desiccation stress. Nakamura *et al.* (2008) analysed changes in the gene expression of commercial baker's yeast during an air-drying process, which simulated dried yeast production, and found that the genes involved in fatty acid metabolism (in particular in β-oxidation) were constantly upregulated during the air-drying process. Results from the study of Singh *et al.* (2005) indicated that genes related to gluconeogenesis, fatty acid catabolism and the glyoxylate cycle were 'turned on' during desiccation. However, these studies concluded that desiccation tolerance in *S. cerevisiae* is a complex, multifactorial process with contributions from several hundred genes, some of which could be related to metabolic pathways involved in the production of volatile compounds.

Conclusions

In conclusion, the procedure described for spray-drying of *S. cerevisiae* strains represents a useful tool to evaluate the heat tolerance of wild strains at laboratory scale. This trait could be considered the final step of the selection process for wild strains suitable for the production of commercial starter cultures. During this study, some indigenous *S. cerevisiae* strains with a distinctive heat tolerance were identified. These strains exhibited a high initial cell survival after spray-drying, maintaining viability during storage at refrigerated temperatures, and they can therefore be considered to have commercial potential, in particular for winemakers interested in the use of specialized strains.

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