

Effect of *Schizosaccharomyces pombe* on Aromatic Compounds in Dry Sherry Wines Containing High Levels of Gluconic Acid

RAFAEL A. PEINADO,[†] JUAN C. MAURICIO,^{*,‡} MANUEL MEDINA,[†] AND
 JUAN J. MORENO[†]

Departamento Química Agrícola y Edafología, Edificio C-3, and Departamento Microbiología, Edificio C-6, Universidad de Córdoba, Campus Universitario Rabanales, 14014 Córdoba, Spain

Volatile compounds have been determined in control dry sherry wines and those supplemented with gluconic acid, which were inoculated with the *Schizosaccharomyces pombe* 1379 (ATCC 26760) yeast strain. These compounds were grouped, according to volatiles exhibiting the identical odor quality, into nine groups of the same odor character (aromatic series) as a way of establishing the aroma profile for the studied wines. Control and supplemented wines showed changes in the balsamic, spicy, roasty, and fruity aromatic series, and tasters judged the aroma as typical of wines subjected to biological aging. This fission yeast may be used as a treatment to reduce gluconic acid contents in wines obtained from rotten grapes, making feasible the incorporation of these wines into the biological aging process. In addition, this procedure may also help to accelerate the traditional biological aging in sherry winemaking due to the contribution of some specific compounds by *S. pombe* to the wine.

KEYWORDS: Aromatic compounds; gluconic acid; sensory analysis; *Schizosaccharomyces pombe*; wine

INTRODUCTION

Gray rot is a grape disease caused by fungi such as *Botrytis cinerea*. Their development is clearly dependent on climatic factors, grape variety, and viticultural practices (1). The disease causes substantial losses to vine growers, as it decreases the quality and size of crops, and to winemakers, as a result of microbiologically induced changes in grape composition. Tartaric acid, malic acid, and available nitrogen decrease their contents; polysaccharides, glycerol, and gluconic acid increase their concentrations in grapes affected by *B. cinerea* (2). On the other hand, *B. cinerea* produces laccase, an exocellular polyphenoloxidase enzyme that oxidizes the phenolic compounds, with toxic effects on the fungus, into quinones; the quinones polymerize to form brown-colored compounds, which have an adverse effect on wine color. Also, it is well-known that when grapes are affected by *B. cinerea*, high levels of gluconic acid are observed (3); besides, *B. cinerea* creates fissures in the berry and acetic acid bacteria (*Acetobacter* and *Gluconobacter*) proliferate using the sweet juice that escapes from the berry, which increases the gluconic acid concentration above 3 g L⁻¹ (4) and the volatile acidity of musts.

Although the climatic conditions in the Montilla-Moriles and Jerez regions are dry during the maturation of the grape, several

factors can encourage the development of Botrytis in vineyards as was previously reported by Pérez et al. (3).

Biological aging is one of the most important phases in sherry production and of great enological significance as well. At this stage wine is submitted to an intensive action of microorganisms (mainly flor yeasts that cover the surface of the wine) during a very long period of time, at least 4 years of aging. During this stage, many wine components are metabolized and others are formed in such a way that the wine acquires its characteristic structure, unique to sherry, both in analytical and organoleptic characteristics (5). The high content in gluconic acid affect the biological aging process of the dry sherry wine, because heterolactic fermentations, due to lactic bacteria metabolism, appear with certain intensity, producing high concentrations of lactic acid and volatile acidity; this last parameter affects the quality of the wine (3). Therefore, young wine with a high gluconic acid content is not destined to biological aging in the studied area, and as a consequence the elimination or reduction of this acid is very desirable.

Recently, previous studies have shown that a flor yeast strain, viz. *Saccharomyces cerevisiae* var. *capensis* G1 (ATCC No. MYA-2451), which is typical of biological aging process in southern Spain, assimilates gluconic acid, both in the traditional form of a film (velum) on the wine surface (6), and in submerged cultures (7).

On the other hand, *Schizosaccharomyces pombe* yeast has been widely used for the biological deacidification of musts because this yeast metabolizes malic acid (8–10). However,

* Corresponding author [e-mail milgamaj@uco.es; telephone (+34) 957218640; fax (+34) 957218650].

[†] Departamento Química Agrícola y Edafología.

[‡] Departamento Microbiología.

musts fermented with this fission yeast exhibit off-flavor (11, 12). *S. pombe* is known to be able to grow with gluconic acid containing media as the sole carbon source (13, 14). Specifically, D-gluconate can be used by fission yeast cells as an alternative carbon and energy source for growth during glucose starvation or when cultured on glycerol-containing medium; this use is rapidly inhibited by the addition of D-glucose (15).

The aim of the present study was to examine the evolution of aromatic compounds during treatment of dry sherry wines by *S. pombe* in the presence of gluconic acid. This work is a continuation of a previous paper (16), to confirm *S. pombe* does not produce off-flavor under studied conditions in dry sherry wines. Therefore, we have carefully evaluated aromatic compounds and also sensory characterization by means of groups of the same odor character (namely "aromatic series", 17–19).

MATERIALS AND METHODS

Yeast Strain and Inocula. In this study, pure cultures of a *S. pombe* 1379 (ATCC 26760) strain were used. The cells were cultured on YM medium (0.3% yeast extract, 0.3% malt extract, and 0.5% peptone, pH 6.5) containing 3% gluconic acid as the carbon source. The inocula were incubated at 27 ± 2 °C with shaking for 72 h. Yeast cells were collected by centrifugation at 3500g and used to inoculate the different wines with a population of 10^7 live cells mL⁻¹.

Wine. Wine from healthy Pedro Ximénez grapes grown in the Montilla-Moriles region (Córdoba, southern Spain) after malolactic fermentation was used for the control wine versus the same wine supplied with 5.03 g L⁻¹ gluconic acid (it is the highest quantity of acid reported in the study area). Both wines were sterilized by passage through Supra EK filters (Seitz, Germany).

Culture and Experimental Conditions. All tests were carried out in 5-L Erlenmeyer flasks sterilized by autoclave containing 4 L of sterilized wine by filtration, and the sterility was maintained during the incubation period. Flasks were continuously stirred and thermostated at 23 ± 2 °C. Changes in the concentration of gluconic acid and volatile compounds were studied in 6 flasks. Three flasks were supplied with the same amount of gluconic acid mentioned above (wine + gluconic acid), and the other three ones were without gluconic acid and were used as controls (control wine). Control wines and wines supplied with gluconic acid were examined at 0 and 20 days.

Analytical Methods. Determinations of number of living cells, ethanol, titratable acidity, pH, volatile acidity, residual sugars, sulfur dioxide, and mayor volatile compounds and polyols were reported in the previous paper from Peinado et al. (16).

Volatile compounds were determined by capillary-column gas chromatography–mass spectrometry following continuous extraction of 100 mL of wine sample with 100 mL of Freon-11 for 24 h. Wine was adjusted to pH 3.5 and 5 mL of a 30 mg L⁻¹ solution of 2-octanol was added as an internal standard. The Freon extract containing the volatile compounds was concentrated to 0.2 mL in a Kuderna-Danish microconcentrator and 1.5 µL was injected into a Hewlett-Packard HP-6890 gas chromatograph equipped with an HP MS 5972A mass detector (Agilent Technologies, Palo Alto, CA). An HP–Innovax fused silica capillary column (60 m × 0.32 mm i.d., 0.25 µm film thickness) was used. The temperature program was as follows: initial temperature 40 °C, held for 10 min, and 1 °C min⁻¹ ramp to 180 °C, held for 35 min. Helium at a constant flow rate of 0.9 mL min⁻¹ was used as the carrier gas, and a 30:1 split ratio was employed at the injection port. The mass detector was used with a voltage of 1612 V in the scan mode to span a mass range from 39 to 300 amu.

Retention times, spectral libraries supplied by Wiley, and pure chemicals obtained from Merck, Sigma-Aldrich, Riedel de Haën, and Fluka were used to identify and confirm the volatile compounds studied, and to prepare their standard solutions. Each compound was quantified from its response factor, which was obtained by using standards solutions of known concentration subjected to the same treatment as the samples and using the target ions and qualifier ions selected for each compound by a Hewlett-Packard Chemstation. (Palo Alto, CA).

Statistical Treatment. To study the effect of the fission yeast (yeast effect) in wine, a one-way analysis of variance (ANOVA) comparing data from the initial control wines (0 days) and final control wines (20 days) was performed. In the same way, the effect of the gluconic acid assimilation by *S. pombe* (gluconic acid effect) was studied by means of an ANOVA comparing data from the final control wines (20 days) and final wines supplied with gluconic acid (20 days). On the other hand, an ANOVA to study the gluconic acid effect and the yeast effect on the aromatic series was performed. The statistical software package Statgraphics Plus v. 2, from STSC, Inc. (Rockville, MD), was used to perform the analysis of variance (ANOVA).

All experiments were carried out in triplicate.

Odor Perception Threshold Determination. The odor perception threshold is defined as the lowest concentration capable of producing a sensation. That sensation has to be detected by at least 50% of the judges in the taste panel (20, 21). Different solutions of ascending concentrations of each compound were used. Starting from the lowest concentration solution, the judges have to indicate the solution whose stimulus was different to that perceived in the control. Control consists of a solution of 1/10 ethanol water without the compound. The judges trained but not selected for the test consists of 30 people of both sexes and between 20 and 55 years old. On the other hand, the judges gave the odor descriptor of the studied compounds in the solution in which the odor descriptor was perceived in a clear way.

Sensory Analysis. A paired comparison test was used to identify differences in color, odor, and taste between the initial and treated wines. Dark, coded glasses for aroma and taste, and transparent glasses for color, were used. Twenty experienced judges were chosen to serve on the panel. The minimum numbers of correct judgments required to establish significant differences were obtained from AENOR (22).

RESULTS AND DISCUSSION

The effect of gluconic acid consumption by *S. pombe* on winemaking variables and major volatile compounds was examined in a previous work, as has been reported above. The yeast was found to use about 40% of the initial contents of the gluconic acid and an increase in acetaldehyde and its derivative 1,1-diethoxyethane concentrations about 200 and 10 mg L⁻¹, respectively was observed (16).

In the present work, only 13 of the 36 volatile compounds quantified (Table 1) exhibited significantly different concentrations between the control wines (viz. those containing no gluconic acid) at 0 and 20 days (yeast effect). The levels in ethyl esters as propanoate, isobutanoate, butanoate, hexanoate, and succinate decreased by about 50%, whereas those in ethyl 3-hydroxybutanoate, isoamyl acetate and γ -butyrolactone decreased by less than 20%. Other esters such as ethyl furoate and isobutyl acetate increased in concentration relative to the initial wine. By contrast, only furanmethanol was produced by *S. pombe* by degradation of gluconic acid.

Monoterpenes and monoterpenic alcohols play an important role in the aroma and flavor of wines; however, the major fractions of those occur in the grape as glycosidically bound forms (23), which render them nonvolatile and flavorless. In the present study, neral, citronellol, and 4-ethylguaiacol compounds were found to increase during the 20 days wine aged in the presence with *S. pombe*. This would certainly reflect a hydrolysis of glucosidic precursors by *S. pombe* enzymatic activities. This effect was completely independent of the presence of gluconic acid in the wine. This finding could represent an important result, because the use of *S. pombe* in the wine might have a greater potential to contribute to the liberation of some aglycones from the flavorless precursor glycosides in the wine.

Studies on minor volatile compounds in wine and their contribution to the aroma of wines biologically aged under flor yeasts have revealed that the most salient features of the process are

Table 1. Volatile Compounds $\mu\text{g L}^{-1}$, in Control Wines and Those Supplied with 5.03 g L^{-1} Gluconic Acid (GA) Stirred and Inoculated with *S. pombe*^a

volatile compound	0 days ^b		20 days		yeast effect ^c	GA effect ^c
	control wine	control wine	control wine	wine + GA		
1-butanol	3248 ± 252	2939 ± 242	3294 ± 296			
2-butanol	1749 ± 218	1720 ± 140	1759 ± 153			
1-hexanol	442 ± 36	429 ± 26	412 ± 21			
1-octanol	98 ± 9	105 ± 8	108 ± 11			
Z-3-hexenol	54 ± 6	54 ± 5	49 ± 4			
2-methyl-1-pentanol	26 ± 3	25 ± 3	30 ± 5			
3-ethoxy-1-propanol	738 ± 65	678 ± 61	668 ± 20			
benzyl alcohol	2780 ± 161	2862 ± 286	2731 ± 132			
methionol	4531 ± 303	4523 ± 408	4822 ± 417			
furanmethanol	853 ± 73	977 ± 78	1195 ± 73		*	*
ethyl propanoate	1200 ± 107	553 ± 139	616 ± 52		*	
ethyl isobutanoate	561 ± 80	195 ± 29	200 ± 16		*	
ethyl butanoate	450 ± 56	263 ± 13	260 ± 5		*	
ethyl 3-hydroxybutanoate	1150 ± 25	938 ± 60	958 ± 26		*	
ethyl hexanoate	139 ± 48	50 ± 8	59 ± 20		*	
ethyl octanoate	472 ± 45	515 ± 66	530 ± 35		*	
ethyl furoate	25 ± 3	33 ± 5	32 ± 2		*	
ethyl succinate	875 ± 137	566 ± 137	448 ± 11		*	
diethyl malate	2023 ± 133	2156 ± 206	2080 ± 61		*	
isobutyl acetate	144 ± 22	185 ± 9	178 ± 13		*	
isoamyl acetate	303 ± 20	110 ± 5	104 ± 9		*	
2-phenylethyl acetate	57 ± 4	57 ± 2	56 ± 3		*	
isobutanoic acid	6798 ± 505	7590 ± 514	7908 ± 231		*	
butanoic acid	3481 ± 417	3521 ± 147	3643 ± 112		*	
2- and 3-methylbutanoic acid	5971 ± 488	6455 ± 493	6378 ± 130		*	
hexanoic acid	1605 ± 86	1666 ± 123	1655 ± 81		*	
octanoic acid	1266 ± 31	1184 ± 83	1196 ± 31		*	
neral	49 ± 8	69 ± 6	63 ± 4		*	
limonene	120 ± 50	145 ± 12	123 ± 20		*	
β -citronellol	0 ± 0	13 ± 3	10 ± 0		*	
γ -butyrolactone	40303 ± 1156	32554 ± 2262	33431 ± 1715		*	
γ -hexalactone	21 ± 1	22 ± 2	21 ± 1		*	
Z-whiskey lactone	110 ± 12	138 ± 11	139 ± 6		*	
pantolactone	2592 ± 223	2228 ± 179	1982 ± 168		*	
4-ethyl phenol	87 ± 7	94 ± 7	91 ± 3		*	
4-ethylguaiacol	103 ± 6	176 ± 20	168 ± 8		*	

^a Analysis of variance to study yeast effect and gluconic acid effect. ^b Data at time 0 were obtained without yeast. Results of wine + gluconic acid at time 0 are not shown because no detectable change by gluconic acid addition was observed in the studied compounds at 0 days. ^c *p* values obtained by ANOVA for yeast effect and gluconic acid effect (*, *p* ≤ 0.05).

an increase in the concentrations of acetaldehyde and its derivatives, and in the C-4 acids, as well as a decrease in glycerol, ethyl acetate, and acetic acid (24). The use of *S. pombe* changes the wine composition similarly to the effect flor yeasts during biological aging; also, the use of gluconic acid by this yeast has no appreciable effect on the compounds studied in the wine.

EFFECT ON AROMATIC SERIES

The relationship of volatile compounds and wine aroma can be established by means of an aroma descriptor of each compound. Volatile compounds exhibiting similar odor quality are grouped into so-called aromatic series. The contribution of each volatile compound to a given series and to the overall wine aroma can be quantified via its odor activity value (OAV), which is the ratio of the compound concentration to their odor threshold. This novel procedure has recently been applied by our research group to various types of wine (17–19).

Major volatile compounds in wines are those that habitually show concentrations above 10 mg L^{-1} . We considered both major compounds, which have already been discussed in a previous paper (16), and minor volatile compounds and γ -butyrolactone (Table 1) to define a representative odor profile for the studied wine. Table 2 lists the odor descriptor, odor threshold, and aromatic series into which the volatile compounds determined were grouped. 1,1-Diethoxyethane, 4-ethylguaiacol,

3-ethoxy-2-propanol, isoamyl alcohols, *levo* and *meso* 2,3-butanediol, ethyl lactate, ethyl acetate, acetaldehyde, γ -butyrolactone, butanoic acid, 2- and 3-methylbutanoic acids, methionol, Z-whiskey lactone, and pantolactone were the compounds most markedly contributing to wine aroma, all with OAVs greater than unity.

The OAVs for the esters ethyl 3-hydroxybutanoate, ethyl hexanoate, ethyl butanoate, and isoamyl acetate only exceeded unity in the initial control wine. All other samples exhibited OAVs between 0.5 and 1 for these compounds. The compounds with OAVs 10 times lower than their odor thresholds were 4-ethylphenol, benzyl alcohol, ethyl furoate, furanmethanol, 1-butanol, 2-butanol, Z-3-hexenol, 2-phenylethyl acetate, and ethyl succinate. Other compounds such as limonene and γ -hexalactone were present in levels 1000 times lower than their odor thresholds.

An odor profile for the wines was obtained by grouping the volatile compounds into nine aromatic series (see Table 2). The value for each aromatic series was calculated as the sum of the OAV of the compounds in it. The profiles for the wines are shown in Figures 1 and 2. An ANOVA was performed to identify which aromatic series were exclusively influenced by the *S. pombe* (yeast effect) and which by the gluconic acid consumption (gluconic acid effect).

Figure 1 shows the profile for the initial control wine (0 days) and the final control wine, which was only treated with *S. pombe*

Table 2. Volatile Compounds Quantified in Wines, Odor Thresholds (mg L⁻¹), Odor Descriptions, and Assignment to Aromatic Series of Volatile Compounds Determined in Sherry Wines

volatile compound	odor threshold	odor description	aromatic series ^a
acetaldehyde	110	pungent, ripeness apple	3
ethyl acetate	12	pineapple, varnish, balsamic	1, 3
1,1-diethoxyethane	1	licorice, green fruit	3, 4
methanol	500	solvent	1
1-propanol	306	ripe fruit, alcohol	1, 3
isobutanol	75	alcohol, nail polish, winelike	1
isoamyl alcohols	60	alcohol, nail polish	1
acetoin	150	buttery, cream	5
ethyl lactate	150	buttery, butterscotch, fruity	3, 5
2,3-butanediol <i>levo</i>	150	fruity	3
2,3-butanediol <i>meso</i>	150	fruity	3
diethyl succinate	1250	fruity, floral	2, 3
phenethyl alcohol	200	rose, Honey	2
1-butanol	150	medicinal	6
2-butanol	50	winelike, solvent	1
1-hexanol	1.1	herbaceous, grass, woody	7
1-octanol	0.8	rose, orange, lemon, apple, jasmine	2, 3
Z-3-hexenol	1	herbaceous, fatty, green, bitter	5, 7
3-methyl-1-pentanol	1.1	pungent, cocoa, green, winelike	1, 2
3-ethoxy-1-propanol	0.1	fruity	3
benzyl alcohol	900	burning taste	8
methionol	1.5	cooked potato, cut hay	7
furanmethanol	15	floral	2
ethyl propanoate	1.8	banana, apple	3
ethyl isobutanoate	5.0	fruity	3
ethyl butanoate	0.4	banana, pineapple, strawberry	3
ethyl 3-hydroxybutanoate	1	fruity, grape	3
ethyl hexanoate	0.08	banana, green apple	3
ethyl octanoate	0.58	banana, pineapple, pear, floral	2, 3
ethyl furoate	1	solvent, balsamic	1, 4
ethyl succinate	1200	herbaceous	7
diethyl malate	10	fruity	3
isobutyl acetate	1.6	sweet, fruity, apple, banana	3
isoamyl acetate	0.16	banana, fruity	3
2-phenylethyl acetate	0.25	fruity	2, 3
isobutanoic acid	30	rancid butter	5
butanoic acid	2.2	cheesy, rancid, putrid	5
2- and 3-methylbutanoic acid	1.5	rancid	5
hexanoic acid	3	rancid, fatty, cheese	5
octanoic acid	10	fatty, rancid	5
neral	0.5	fruity	3
limonene	200	fruity	3
β -citronellol	0.1	rose	2
γ -butyrolactone	20	caramel, coconut	3, 8
γ -hexalactone	359	fruity	3
Z-whiskey lactone	0.07	coconut	3
pantolactone	2.2	balsamic, smoky, toasted bread	4, 8
4-ethylphenol	140	pungent, medicinal	6
4-ethylguaiaicol	0.02	smoky, toasted bread, clove	8, 9

^a Aromatic series: 1, solvent; 2, floral; 3, fruity; 4, balsamic; 5, fatty; 6, medicinal; 7, herbaceous; 8, roasty; 9, spicy.

at the end of the experiment (20 days). The fission yeast substantially enhanced the fruity, spicy, roasty, and balsamic series (all with $p \leq 0.01$) as a result of the increased concentrations of acetaldehyde and 1,1-diethoxyethane, which belonged to the fruity series, and 4-ethylguaiaicol, which was grouped in the spicy series, although it is also associated with the roasty series. In addition, the increased 1,1-diethoxyethane concentration was the origin of the increased value for the balsamic series in the control wine to the end. Spicy and roasty aromas extracted from the wood, and fruity and balsamic aromas, are especially valued in sherry wines.

On the other hand, several attempts to reduce the biological aging time by means of submerged cultures (7) or with periodic aeration of sherry wines (25) were carried out by our research group. The changes in aroma series caused by *S. pombe* are similar to those exhibited by dry sherry wines after prolonged biological aging with pure cultures of flor yeasts, in industrial and laboratory conditions (17, 26). Therefore, the findings

provide evidence that the use in wines of *S. pombe* might reduce the biological aging time by increasing aromatic compounds related with biological aging.

The ANOVA obtained for the series in the control wines (20 days) and in gluconic acid containing wine at day 20 (gluconic acid effect) revealed no significant differences for any series (**Figure 2**). This suggests that, even though the acetaldehyde, acetoin, and furanmethanol concentrations increase their contents in gluconic-acid-containing wines, such an increment does not allow wines to be distinguished in terms of odor profile.

To check the possible presence of defects (off-flavor, color, and taste) caused by *S. pombe* in the inoculated wines, paired comparison tests of odor, color, and taste, between the initial (0 days) and final (20 days) control wines, between the initial (0 days) control wine and final (20 days) gluconic acid-containing wines, and between the final control wine and gluconic-acid-containing wines at 20 days, were performed. The following question was asked of the tasters: Does the wine show

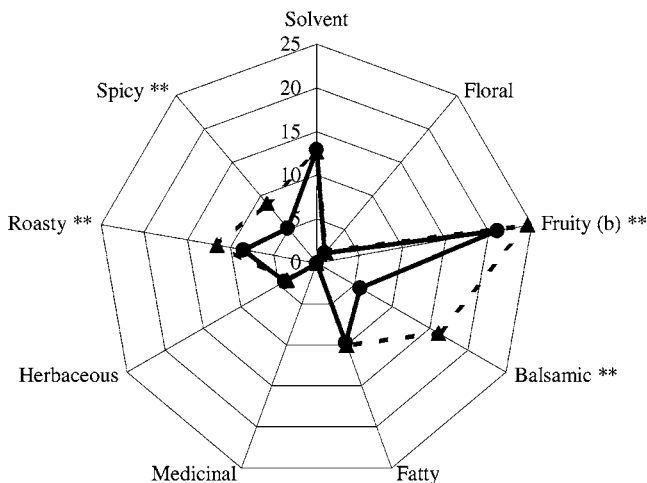


Figure 1. Aromatic series^a calculated by adding the odor activity values (OAVs) of the compounds grouped in the initial control wines at 0 days (●) and final control wines at 20 days (▲). ^a*p* values obtained by ANOVA: **, *p* ≤ 0.01; ^bfor better visualization, values shown for the fruity series are one-half of the real values.

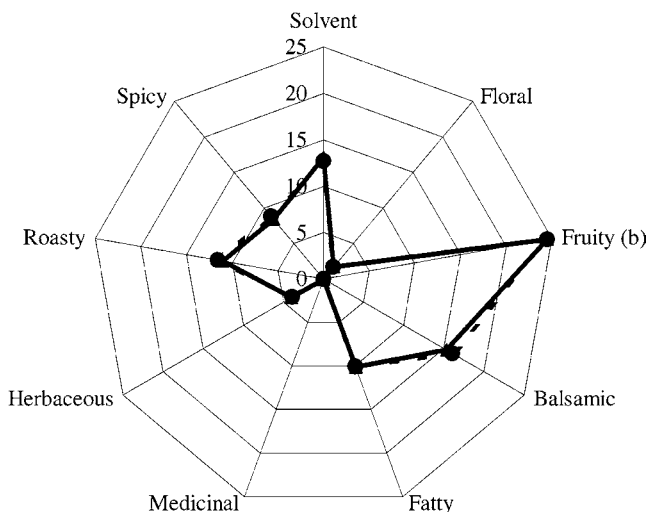


Figure 2. Aromatic series calculated by adding the odor activity values (OAVs) of the compounds grouped in the final (20 days) control wine (●) and the final (20 days) wine supplemented with gluconic acid (▲).

Table 3. Results of the Paired Comparison Test^a for Identifying Differences among the Initial Control Wine (ICW; 0 Days), the Final Control Wine (FCW; 20 Days), and Those Supplied with Gluconic Acid (GA; 20 Days) (Number of "Yes" Answers of 20)

wine	color	odor	taste
ICW vs FCW	6 (ns)	15 (*)	15 (*)
ICW vs GA	6 (ns)	15 (*)	15 (*)
FCW vs GA	3 (ns)	11 (ns)	16 (**)

^a *p* values obtained in the paired comparison test: *, *p* ≤ 0.05; **, *p* ≤ 0.01. ns: not significant differences.

differences in color, odor, or taste? If so, they should give each wine a score from 1 to 10 for color, odor, and taste.

Table 3 shows the results provided by a panel of 20 experienced tasters. As can be seen, there were no significant differences in color among the three wines tested. However, differences were detected in odor between the initial and final control wines and between the initial control wine and the final gluconic-acid-containing wine, although no significant differences were detected by the gluconic acid addition (gluconic acid

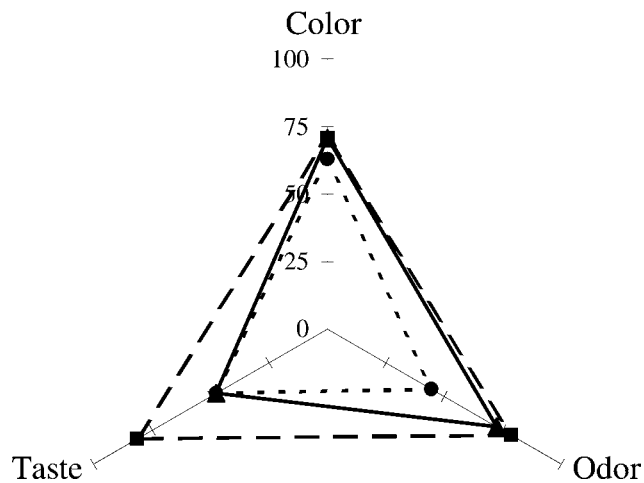


Figure 3. Color, odor, and taste scores (%), on a relative scale, in the initial control wine (●), final control wine (■), and wine supplemented with gluconic acid (▲).

effect). There were also significant differences in taste among the three wines.

Figure 3 shows the percentage of the scores given by each taster on a relative scale from 0 to 10. The three wines obtained similar scores in color and according to the paired comparison test carried out by the taster. The initial control wine had the lower odor score than the others, and the final control and supplemented wines obtained similar differences. This is in accordance with the results shown in **Table 3**, where the taster identified differences between controls at 0 and 20 days, between the initial control and supplemented wines. On the other hand, the initial control and gluconic-acid-containing wines had the lowest taste score. That low score in the gluconic-acid-containing wine may be a result of the presence of the remaining gluconic acid added at the beginning of the experiments. These contents together with the high titratable acidity of the initial wine make the taste of this wine not very pleasant. The low score obtained in the initial control wine compared with the control wine at 20 days may be explained by the *S. pombe* effect in the wine. This is because, as we reported above, *S. pombe* cause similar changes in wines to those exhibited by wines subjected to biological aging, and the initial control wine had not been subjected to this aging process.

To confirm our hypothesis, we also asked four experienced tasters from the collaborating firm Bodegas Alvear, S.A., which produces "fino" wines under flor yeasts, to describe the *S. pombe* treated wine (yeast effect). The tasters judged the odor as typical of wines obtained by biological aging and found no off-flavor; in fact, they compared the samples to wines produced by properly conducted biological aging in a cellar for 2 years. So, this yeast may be used to reduce gluconic acid contents in wines obtained from rotten grapes, and latter on, be subjected to biological aging under flor yeast. In addition, the previous use of this fission yeast in dry sherry wine may help to shorten the aging time for wines under flor yeast.

Conclusions. The use of *S. pombe* for the treatment, elimination, or reduction of gluconic acid in wines does not seem to produce off-flavor, neither does this yeast deviate largely from the organoleptic profile of the tested wines. On the other hand, the results indicate that *S. pombe* might be used, independently of the presence of gluconic acid, to shorten the biological aging process for the elaboration of the dry sherry wines, and to enrich the monoterpenes in wines. Further research will be conducted about the last subject.

LITERATURE CITED

- (1) Reynier, A. Leaf and clusters diseases. In *Viticulture Manual*; Mundi-Prensa: Madrid, Spain, 2002; pp 405–464.
- (2) Ribéreau-Gayon, P.; Dubourdiue, D.; Donèche, B.; Lonvaud, A. *Handbook of Enology*; Wiley: New York, 2000; Vol. I.
- (3) Pérez, L.; Valcarcel, M. J.; González, P.; Domecq, B. Influence of Botrytis infection of the grapes on the biological aging process of fino sherry. *Am. J. Enol. Vitic.* **1991**, *42*, 58–62.
- (4) Couto, J. A.; Graça, A. R.; Soares-Franco, J. M.; Hogg, T. The use of gluconic acid level as an indicator of the activity of acetic acid bacteria in grapes. Presented at the VII Symposium International d'Oenologie, Bordeaux, France, 2003.
- (5) Berlanga, T. M.; Peinado, R.; Millán, C.; Mauricio, J. C.; Ortega, J. M. Influence of blending on the content of different compounds in the biological aging of sherry dry wines. *J. Agric. Food Chem.* **2004**, *52*, 2577–2581.
- (6) Peinado, R. A.; Moreno, J. J.; Ortega, J. M.; Mauricio, J. C. Effect of gluconic acid consumption during simulation of biological aging of sherry wines by a flor yeast strain on the final volatile compounds. *J. Agric. Food Chem.* **2003**, *51*, 6198–6203.
- (7) Peinado, R. A.; Mauricio, J. C.; Ortega, J. M.; Medina, M.; Moreno, J. J. Changes in gluconic acid, polyols and major volatile compounds in sherry wine during aging with submerged flor yeast cultures. *Biotechnol. Lett.* **2003**, *25*, 1887–1891.
- (8) Sousa, M. J.; Mota, M.; Leao C. Effects of ethanol and acetic acid on the transport of malic acid and glucose in the yeast *Schizosaccharomyces pombe*: implications in wine deacidification. *FEMS Microbiol. Lett.* **1995**, *126*, 197–202.
- (9) Dharmadhikari, M. R.; Wilker, K. L. Deacidification of high malate must with *Schizosaccharomyces pombe*. *Am. J. Enol. Vitic.* **1998**, *49*, 408–412.
- (10) Silva, S.; Ramon-Portuga, F.; Andrade, P.; Abreu, S. Texeira, M.; Strehaino, P. Malic acid consumption by dry immobilized cells of *Schizosaccharomyces pombe*. *Am. J. Enol. Vitic.* **2003**, *54*, 50–55.
- (11) Rankine, B. C. Decomposition of l-malic acid by wine yeast. *J. Sci. Food Agric.* **1966**, *17*, 312–316.
- (12) Gallander, J. F. Deacidification of eastern table wine with *Schizosaccharomyces pombe*. *Am. J. Enol. Vitic.* **1977**, *13*, 65–68.
- (13) Hoever, M.; Milbradt, B.; Höfer, M. D-Gluconate is an alternative growth substrate for cultivation of *Schizosaccharomyces pombe* mutants. *Arch. Microbiol.* **1992**, *157*, 191–193.
- (14) Tsai, C. S.; Ye, H. G.; Shi, J. L. Carbon-13 NMR studies and purification of gluconate pathway enzymes from *Schizosaccharomyces pombe*. *Arch. Biochem. Biophys.* **1995**, *316*, 155–162.
- (15) Caspari, T.; Urlinger, S. The activity of the gluconate-H⁺ symporter of *Schizosaccharomyces pombe* cells is down-regulated by D-glucose and exogenous cAMP. *FEBS Lett.* **1996**, *395*, 272–276.
- (16) Peinado, R. A.; Moreno, J. J.; Maestre, O.; Ortega, J. M.; Medina, M.; Mauricio, J. C. Gluconic acid consumption in wines by *Schizosaccharomyces pombe* and its effect on the concentrations of major volatile compounds and polyols. *J. Agric. Food Chem.* **2004**, *52*, 493–497.
- (17) Moyano, L.; Zea, L.; Moreno, J.; Medina, M. Analytical study of aromatic series in sherry wines subjected to biological aging. *J. Agric. Food Chem.* **2002**, *50*, 7356–7361.
- (18) Peinado, R. A.; Moreno, J. J.; Bueno, J. E.; Moreno, J. A.; Mauricio, J. C. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chem.* **2004**, *84*, 585–590.
- (19) Peinado, R. A.; Moreno, J.; Medina, M.; Mauricio, J. C. Changes in volatile compounds and aromatic series in sherry wine with high gluconic acid levels subjected to aging by submerged flor yeast cultures. *Biotechnol. Lett.* **2004**, *26*, 757–762.
- (20) Cutzach, I.; Chatonnet, P.; Dubourdiue, D. Influence of storage conditions on the formation of some volatile compounds in white fortified wines (Vins doux naturels) during the aging process. *J. Agric. Food Chem.* **2000**, *48*, 2340–2345.
- (21) Kotseridis, Y.; Baumes, R. Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation, and in the produced wine. *J. Agric. Food Chem.* **2000**, *48*, 400–406.
- (22) Spanish Association for Normalization and Certification (AENOR). Sensory analysis. Paired comparison test. In *Sensory Analysis*; AENOR: Madrid, Spain, 1997; pp 75–82.
- (23) Voirin, S. G.; Baumes, R. L.; Sapis, J. C.; Bayonove, C. L. Analytical methods for monoterpene glycosides in grape and wine. II. Qualitative and quantitative determination of monoterpene glycosides in grape. *J. Chromatogr.* **1992**, *595*, 269–281.
- (24) Cortés, M.; Moreno, J.; Zea, L.; Moyano, L.; Medina, M. Changes in aroma compounds of sherry wines during their biological aging carried out by *Saccharomyces cerevisiae* races *bayanus* and *capensis*. *J. Agric. Food Chem.* **1998**, *46*, 2398–2394.
- (25) Cortés, M. B.; Moreno, J.; Zea, L.; Moyano, L.; Medina, M. Response of the aroma fraction in Sherry wines subjected to accelerated biological aging. *J. Agric. Food Chem.* **1999**, *47*, 3297–3302.
- (26) Cortés M. B. Biological aging of sherry wines inoculated with pure cultures of *Saccharomyces* yeast strain. Thesis, University of Cordoba, 2002.

Received for review January 28, 2004. Revised manuscript received May 3, 2004. Accepted May 8, 2004. This research was supported by a grant from the Spanish government (Project VIN 00-039-C2-01).

JF049853R