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Gluconic Acid Consumption in Wines by *Schizosaccharomyces pombe* and Its Effect on the Concentrations of Major Volatile Compounds and Polyols

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Schizosaccharomyces pombe 1379 (ATCC 26760) yeast strain in wine substantially increases acetaldehyde and 1,1-diethoxyethane concentrations and to decreases *levo*-2,3-butanediol, glycerol, acetoin, and gluconic acid concentrations. In this study, *S. pombe* has been used for the first time to reduce gluconic acid in wine under aerobic conditions. Only acetaldehyde and acetoin exhibited significantly higher levels in the wines containing gluconic acid. The high in vitro specific activity of alcohol dehydrogenase observed may be directly related to the high production of acetaldehyde by the studied fission yeast.

KEYWORDS: Acetaldehyde; alcohol dehydrogenase; gluconic acid; *Schizosaccharomyces pombe*; volatile compounds

INTRODUCTION

So-called "common rot" or "gray rot" is a grape disease due to fungi such as *Botrytis cinerea*, *Penicillium*, *Aspergillus*, and *Mucor* and bacteria such as *Acetobacter* and *Gluconobacter*. The disease arises during grape ripening and is influenced by climatic factors such as moisture and rainfall, as well as by physiological factors such as vine variety and grape bunch shape (*1*). The changes it induces in the composition of white and red wines include altered color due to an increased activity of oxidase enzymes such as tyrosinase and laccase, enhanced volatile acidity, and the production of increased amounts of dry extract through the formation of glycerol, polysaccharides, uronic acids, aldonic acids, and particularly gluconic acid (*2*).

Enzyme activity and bacterial contamination can be reduced by using appropriate amounts of sulfur dioxide; also, the filtering problems arising from the presence of polysaccharides can be lessened by using a suitable technological procedure. On the other hand, the sensory properties of wines are considerably altered by the presence of gluconic acid, which decreases the wine's microbiological stability and raises long-term storage problems that can be solved only by reducing its concentration in the wine.

Deacidifying yeasts such as *Schizosaccharomyces pombe* have been used for some time to remove malic acid from musts obtained in cold regions. Thus, Sousa et al. (*3*) recommend that deacidification by *S. pombe* during wine fermentation should take place before, rather than after, the main alcoholic fermentation by *Saccharomyces cerevisiae*. However, the alcoholic fermentation of musts with this fission yeast yields wines with unpleasant odors that are probably due to the formation of large amounts of sulfur-containing compounds (*4*).

S. pombe is able to utilize D-gluconate as a growth substrate (*5*). 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 utilization is rapidly inhibited by the addition of D-glucose, as well as by exogenous cAMP (*6*).

The novelty of this work lies in the fact that, for the first time, *S. pombe* was used to remove gluconic acid from wine to be subsequently subjected to biological aging. In this way, the effect of gluconic acid consumption by this fission yeast on the composition in major volatile compounds and polyols of the resulting wine has been studied.

MATERIALS AND METHODS

Yeast Strain and Inocula. In this study, pure cultures of a *S. pombe* 1379 (ATCC 26760) strain were used. The inoculum 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 carbon source. The inocula were incubated at 27 ± 2 °C with shaking for 72 h. Yeast cells were collected by centrifugation at 3500*g* 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 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).

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Table 1. Winemaking Variables in the Control Wines and Those Supplied with Gluconic Acid (GA) Stirred and Inoculated with *S. pombe*

	0 days[a]		20 days	
compound	control wine	wine + GA	control wine	wine + GA
ethanol (% v/v)	14.8 ± 0.1	14.8 ± 0.1	14.6 ± 0.1	14.6 ± 0.2
volatile acidity (g L^{-1})	0.36 ± 0.01	0.50 ± 0.02	0.32 ± 0.01	0.42 ± 0.01
pH	3.19 ± 0.01	3.10 ± 0.01	3.18 ± 0.01	3.23 ± 0.01
titratable acidity (mequiv L^{-1})	67.3 ± 0.3	85.0 ± 0.5	68.7 ± 0.3	81.3 ± 0.3
GA (g L^{-1})	0.0 ± 0.0	5.03 ± 0.1	0.0 ± 0.0	2.9 ± 0.08
malic acid (g L^{-1})	ND[b]	ND	ND	ND
SO_2 total (mg L^{-1})	102 ± 7	102 ± 7	99 ± 6	100 ± 5
residual sugar (g L^{-1})	3.2 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	3.1 ± 0.1
absorbance at 280 nm	8.1 ± 0.2	8.1 ± 0.2	8.2 ± 0.2	8.2 ± 0.2
absorbance at 420 nm	0.151 ± 0.001	0.151 ± 0.001	0.156 ± 0.006	0.155 ± 0.004
absorbance at 520 nm	0.033 ± 0.002	0.033 ± 0.002	0.032 ± 0.001	0.031 ± 0.001

[a] Data at time 0 were obtained without yeast. [b] Not detected.

Culture and Experimental Conditions. All tests were carried out in 5 L Erlenmeyer flasks containing 4 L of sterilized wine, stirred magnetically and thermostated at 23 ± 2 °C. Changes in the concentration of gluconic acid and volatile compounds were studied in six flasks. Three flasks were supplied with the same amount of gluconic acid mentioned above (wine + gluconic acid), and the other three had no gluconic acid, which were used as controls (control wine). Control wines and wines supplied with gluconic acid were examined at 0, 5, 14, and 20 days.

Analytical Methods. The number of living cells was determined by counting under a light microscope in a Thoma chamber following staining with Methylene Blue (*7*).

Ethanol was quantified using the method of Crowell and Ough (*8*), and titratable acidity, pH, volatile acidity, residual sugars, and sulfur dioxide were determined with the EEC recommended methods (*9*). Gluconic and malic acids were determined using the Boehringer-Manheim enzyme test. Absorbances at 280, 420, and 520 nm were measured on a Beckman DU-640 UV spectrophotometer. Major volatile compounds and polyols were quantified on a model 6890 gas chromatograph from Agilent Technologies (Palo Alto, CA), using the OIV method (*10*) as modified by Muñoz et al. (*11*); capillary column CP-Wax 57 CB (60 m long; 0.25 mm i.d.; 0.4 μm film thickness) was used, and 0.5 μL aliquots of 10 mL wine samples previously supplied with 1 mL of 1 g L^{-1} 4-methyl-2-pentanol as internal standard, were injected. Tartaric acid in the wine was removed by precipitation with 0.2 g of calcium carbonate, followed by centrifugation at 300*g*.

Quantification was based on the response factors obtained for standard solutions of each compound. A split ratio of 30:1, an FID, and a temperature program involving an initial temperature of 50 °C (15 min), a 4 °C min^{-1} ramp, and a final temperature of 190 °C (35 min) were used. The injector and detector temperatures were 270 and 300 °C, respectively. The flow rate of carrier gas (helium) was initially set at 0.7 mL min^{-1} (16 min) and followed by a 0.2 mL min^{-1} ramp to the final value (1.1 mL min^{-1}), which was held for 52 min.

Cell-free extracts were obtained, and the in vitro activities of alcohol and aldehyde dehydrogenases (ADH and AlDH) were determined according to the method of Mauricio et al. (*12*).

Statistical Treatment. To study the effect of the fission yeast (yeast effect) in wine, a single analysis of variance 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 single-variance analysis comparing data from the final control wines (20 days) and final wines supplied with gluconic acid (20 days). The statistical software package Statgraphics Plus v. 2, from STSC, Inc. (Rockville, MD), was used to perform the analysis of variance (ANOVA) in order to identify those compounds exhibiting significant differences.

All experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Continuously stirred wines inoculated with pure cultures of *S. pombe* previously grown on gluconic acid (5.03 g L^{-1}) exhibited a decrease in the gluconic acid concentration by 465 mg L^{-1} day^{-1} during the first 4 days. On the other hand, unstirred wines exhibited a decrease of only 166 mg L^{-1} day^{-1} over the same period (data not shown). After 5 days, the gluconic acid concentration decreased virtually negligibly, so the stirred method was chosen for the next tests.

Table 1 shows the winemaking variables and gluconic acid values of control wines and those supplemented with gluconic acid. Only pH and titratable and volatile acidity values were affected in initial wines by the addition of gluconic acid. The effect on volatile acidity was a result of the commercially available gluconic acid used containing 1% acetic acid. All other compounds determined in initial wines exhibited no detectable changes by the effect of the addition of gluconic acid; their concentrations remained at the same levels as those in the wine without gluconic acid addition (control wines).

At the end of the tests (20 days), an overall 2.13 g L^{-1} gluconic acid (42% from the total amount added to the wines) was assimilated by the fission yeast. The wines supplied with gluconic acid also exhibited significant differences at the 95% confidence level in titratable acidity and pH that might be ascribed to the effect of the gluconic acid consumption by yeast. In addition, the wines supplied with gluconic acid exhibited a more marked diminution in volatile acidity (from 0.5 to 0.42 g L^{-1}) than the control wines (from 0.36 to 0.32 g L^{-1}).

No significant changes in the ethanol concentration of the control wines or those supplied with gluconic acid were observed during the 20 days of testing; this is consistent with the results of Jong-Gubbels et al. (*13*), which found ethanol to be a poor substrate for *S. pombe*—even as the sole carbon source due to the absence of the glyoxylate cycle.

Sherry wines are characterized by a yellow pale color; therefore, the increase in the absorbance values at 420 and 520 nm gives information about browning reactions in wine. By contrast, the absorbance at 280 nm measures the total polyphenols. In all the cases of this study, no significant differences were observed in the absorbances at 280, 420, and 520 nm, which reveals that any browning reactions occurred to a negligible extent over the studied period.

As can be seen under Materials and Methods, the effect of the two factors (yeast and gluconic acid) on volatile compounds was studied by means of single ANOVA statistical analyses. The major volatile compounds, methanol, propanol, isobutanol, isoamyl alcohols, ethyl lactate, ethyl acetate, and diethyl succinate, exhibited no significant differences in their final concentration between the control wines and those supplied with gluconic acid. In the same way, no significant differences have been observed for these compounds between control wines at

Table 2. Major Volatile Compounds (mg L^{-1}) and Polyols (mg L^{-1}) in Control Wines and Those Supplied with 5.03 g L^{-1} Gluconic Acid (GA) Stirred and Inoculated with *S. pombe*[a]

	0 days[b]	20 days			
compound	control wine	control wine	wine + GA	yeast effect[c]	GA effect[c]
acetaldehyde	252 ± 11	428 ± 11	452 ± 9	***	*
ethyl acetate	59 ± 2	57.4 ± 3.8	61 ± 3		
1,1-diethoxyethane	4.7 ± 0.2	15 ± 1	14.3 ± 0.8	***	
methanol	67 ± 1	65 ± 4	66 ± 2		
propanol-1	62 ± 1	59 ± 2	59.0 ± 0.8		
isobutanol	69.5 ± 0.5	71 ± 1	69 ± 1		
isoamyl alcohols	401 ± 5	393 ± 7	396 ± 6		
acetoin	30 ± 2	25 ± 2	31 ± 2		*
ethyl lactate	453 ± 31	447 ± 26	454 ± 23		
2,3-butanediol, *levo*	990 ± 66	817 ± 48	810 ± 56	*	
2,3-butanediol, *meso*	243 ± 34	234 ± 19	234 ± 13		
diethyl succinate	34 ± 3	30 ± 2	28 ± 4		
phenethyl alcohol	70 ± 2	70 ± 6	69 ± 5		
glycerol	4000 ± 100	2900 ± 200	3130 ± 60	***	

[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 (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$).

0 and 20 days (**Table 2**). Only acetaldehyde and the related compounds 1,1-diethoxyethane, acetoin, and 2,3-butanediol change concentrations during the tests. 1,1-Diethoxyethane exhibited significant differences between the control wines ($p \leq 0.001$). These differences are related to acetaldehyde and ethanol levels through the formation constant (*14*); on the other hand, the differences observed in acetoin concentrations at 20 days ($p \leq 0.05$) may be related especially to the gluconic acid assimilation. The levels in acetaldehyde depend of both factors (yeast and gluconic acid), because significant differences have been observed between control wines ($p \leq 0.001$) and between control wines and those supplemented with gluconic acid in both cases at the end of the experiments ($p \leq 0.05$).

The acetaldehyde concentration increased to an even greater extent in the wines supplied with gluconic acid—probably due to the acid's being assimilated—part of which may be incorporated into the pentose phosphate cycle (*5*, *15*) and the rest into the Embden−Meyerhof glycolytic pathway, by the presence of glucose dehydrogenase in this yeast (*16*); this is also the case with other yeasts such as *Saccharomyces bulderi* (*17*). The slight increase in the acetoin concentration arose from the presence of acetaldehyde, which is a precursor for this compound (*18*).

With regard to polyols, the *meso* form of 2,3-butanediol exhibited no significant changes in their levels by gluconic acid or yeast effect, whereas its *levo* form and glycerol decreased significantly ($p \leq 0.05$ and $p \leq 0.001$, respectively) by the action of the yeast cells and exhibited no significant dependence on the gluconic acid in the wine (**Table 2**). However, it seems that glycerol uptake by *S. pombe* was less in the presence than in the absence of gluconic acid ($p \leq 0.06$). This difference can be ascribed to both being used as carbon source.

Figure 1 shows the changes of the concentrations in gluconic acid, glycerol, and the compounds related to gluconic acid in the wine (acetaldehyde and acetoin) according to **Table 2**. As can be seen, gluconic acid was partially consumed within the first 5 days, in parallel with glycerol. This was also the period during which the acetaldehyde concentration increased maximally, the wines containing gluconic acid exhibiting higher concentrations of this aldehyde than the control wines.

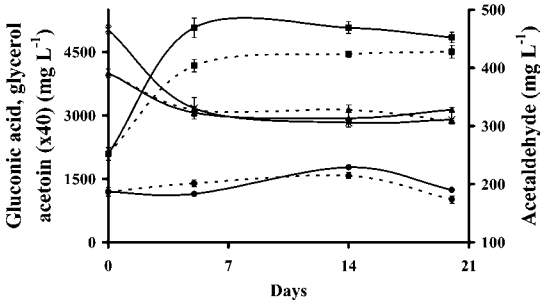

Figure 1. Temporal variation of the gluconic acid, glycerol, acetaldehyde, and acetoin concentrations in wines inoculated with *S. pombe*: wines containing (—) and not containing (- - -) gluconic acid. The right axis shows changes in the concentrations of acetaldehyde (■) and the left axis changes in glycerol (▲), acetoin (●; for better visualization the acetoin concentrations has been multiplied by 40), and gluconic acid (×) .

In addition to partial consumption of gluconic acid by the yeast, **Figure 1** reveals a high production of acetaldehyde—the main metabolite produced by the yeast cells—during the first 5 days after the inoculation. Acetaldehyde is an intermediate in the yeast metabolic pathway from the pyruvic acid, mediated by the enzymes of glycolysis, and a precursor of acetate, acetoin, and ethanol. Accumulation of acetaldehyde by yeasts has been found to depend on the balance between alcohol dehydrogenase and aldehyde dehydrogenase enzymes (*19*). Acetaldehyde is known to be an inhibitor for a wide range of metabolic activities and more toxic than ethanol (*20*−*23*). However, Stanley et al. (*24*) and other authors (*25*, *26*) have described a stimulation of yeast growth by low acetaldehyde concentrations in the presence of ethanol. By contrast, in the present study, both metabolites are present in high levels in the medium (**Tables 1** and **2**); this suggests that probably is the cause of low cellular viability observed at 5 days, and as consequence concentrations in the studied compounds of the wines varied very little throughout the remainder of the tests (20 days).

To carefully examine this interesting finding, as is the high increase of acetaldehyde released by this fission yeast, the in vitro specific activities of the two enzymes influencing the acetaldehyde level, namely, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AlDH), were determined. The results were as follows: the specific activity of ADH remained at high levels (3.2 ± 0.3 U mg^{-1} of protein) during the first 72 h and decayed subsequently, whereas that of AlDH was very low or even negligible over the same period of time in the stirred wine. According to Tsai et al. (*27*), ethanol stimulates ADH and exposes the possible presence of alcohol dehydrogenase isoenzymes in the fission yeast, even though only one ADH has so far been reported for *S. pombe*. For this reason, and on the basis of the fact that the isoenzymes ADH I and ADH II in *S. cerevisiae* can be differentiated by thermal denaturation of the former (61 °C for 15 min) from crude extracts (*28*, *29*), the same procedure was used for yeast cells in the present study without the presence of glucose (dry wine), and 100% of thermostable ADH activity was always obtained in *S. pombe*. The thermostable isoenzyme in *S. cerevisiae* is the ADH II isoenzyme, which is regulated by catabolite repression (*30*). Therefore, the in vitro specific activity of ADH in *S. pombe* was analyzed during the first 24 h of a fermentation on YM medium containing 85 g L^{-1} glucose obtaining also an activity 100% thermostable ADH (1.9 ± 0.2 U mg^{-1} of protein).

The obtained findings are consistent with the slightly increased sequence identity (percent) between the ADH II isoenzyme in *S. cerevisiae* and the ADH enzyme in *S. pombe*

(*31*) suggest that the ancestor cytoplasmic protein shared by both yeasts (*32*) must be more similar to the thermostable isoenzyme and that ADH II must be the oldest ADH isoenzyme among the seven identified to date in *S. cerevisiae* (*33*). Accordingly, the accumulation of acetaldehyde in the wine must result from the high ADH activity under the working conditions used, where *S. pombe* may convert part of the ethanol into acetaldehyde and NADH. Such an accumulation is also consistent with the little or no activity of AlDH under these conditions and with the results of Jong-Gubbels et al. (*13*) for *S. pombe* grown in a chemostat: they found that activities of gluconeogenic and glyoxylate-cycle enzymes were not detected in cell-free extracts of any of the cultures and that activities of acetaldehyde dehydrogenase and acetyl-CoA synthetase were low and of the same order of magnitude as the in vivo rates of acetate activation to acetyl-CoA.

The results show that ethanol is a poor substrate for *S. pombe*, even as an auxiliary energy source. For this reason, the yeast's inability to convert acetaldehyde into acetic acid at a high rate leads to the release of excess acetaldehyde as a detoxification mechanism.

Acetaldehyde, 1,1-diethoxyethane, and acetoin are typically encountered in wines under biological aging with flor yeasts; their concentrations are measures of the aging time of Sherry wines (*34*). These compounds are major contributors to the flavor of such wines (*35*).

Recently, we have put in evidence (*36*) that a flor yeast strain assimilates part of gluconic acid during the biological aging process of sherry wines and exerts significant changes on the final volatile compounds of wines. In the present work, *S. pombe* was used for the first time to remove gluconic acid from wines to be subsequently subjected to biological aging. *S. pombe* is known to produce off-flavor during must fermentation (*4*); however, in the present study no evident off-flavor in the wine inoculated with this fission yeast was detected. In principle, the effects observed may be beneficial for the subsequent biological aging of wines with a high concentration in gluconic acid because the major compounds produced by *S. pombe*, in wines, are essentially identical with those produced by flor yeasts during the biological aging of white dry sherry wines (*35*−*37*). This fission yeast might therefore be used to remove gluconic acid from wines obtained from rotten grapes and to increase their acetaldehyde level; this may help shorten the aging time for wine under flor yeasts (*35*). To confirm the result obtained in the previous study, the minor volatile compounds are being analyzed and also organoleptic characterizations by means of odorant series are being carried out.

LITERATURE CITED

(1) Reynier, A. Leaf and clusters diseases. In *Viticulture Manual*; Mundi-Prensa: Madrid, Spain, 2002; pp 405−464.

(2) Ribéreau-Gayon, P.; Dubourdieu, D.; Donèche, B.; Lonvaud, A. *Handbook of Enology*; Wiley: New York, 2000.

(3) 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 Micobiol. Lett*. **1995**, *126*, 197−202.

(4) Carre, E.; Lafon-Lafourcade, S.; Bertrand, A. Biological deacidification of white dry wines for malic acid fermentation by yeasts. *Connaiss. Vigne Vin*. **1983**, *17*, 43−53.

(5) 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.

(6) 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.

(7) EBC (European Brewery Convention). Analytical microbiology. *J. Inst. Brew.* **1977**, *83*, 115−117.

(8) Crowell, E.; Ough, C. A modified procedure for alcohol determination by dichromate oxidation. *Am J. Enol. Vitic*. **1979**, *30*, 61−63.

(9) EEC. *Official Report of the European Community*; Mundi-Prensa: Madrid, Spain, 1990; L-272.

(10) OIV. Compendium of international methods of analysis of spirituous drinks, alcohols and aroma compounds of drink. *Off. Int. Vigne Vin Paris* **1994**, 123−124.

(11) Muñoz, D.; Peinado, R. A.; Moreno, J.; Medina, M. Determination of major volatile compounds and polyols in wine by GC in capillary column and direct injection. Presented at the XXIV Conferences of Viticulture and Enology, Tierra de Barros, Almendralejo (Badajoz), Spain, 2002.

(12) Mauricio, J. C.; Moreno, J. J.; Ortega, J. M. *In vitro* specific activities of alcohol and aldehyde dehydrogenases from two flor yeasts during controlled wine aging. *J. Agric. Food Chem*. **1997**, *45*, 1967−1971.

(13) de Jong-Gubbels, P.; van Dijken, J. P.; Pronk, J. T. Metabolic fluxes in chemostat cultures of *Schizosaccharomyces pombe* grown on mixtures of glucose and ethanol. *Microbiology* **1996**, *142*, 1399−1407.

(14) Misselhorn, K. Formation of acetals in rum: a kinetic study. *Ann. Technol. Agric*. **1975**, *24*, 371−378.

(15) Tsai, C. S.; Shi, J. L.; Ye, H. G. Kinetic studies of gluconate pathway from *Schizosaccharomyces pombe*. *Arch. Biochem. Biophys*. **1995**, *316*, 163−168.

(16) Petit, T.; Blázquez, M. A.; Gancedo, C. *Schizosaccharomyces pombe* possesses an unusual and a conventional hexokinase: biochemical and molecular characterization of both hexokinases. *FEBS Lett.* **1996**, *378*, 185−189.

(17) Van Dijken, J. P.; van Tuijl, A.; Luttik, M. A. H.; Middelhoven, W. J.; Pronk, J. T. Novel pathway for alcoholic fermentation of δ-gluconolactone in the yeast *Saccharomyces bulderi*. *J. Bacteriol.* **2002**, *184*, 672−678.

(18) Romano, P.; Suzzi, G. Origin and production of acetoin during wine yeast fermentation. *Appl. Environ. Microbiol.* **1996**, *62*, 309−315.

(19) Romano, P.; Suzzi, G.; Turbanti, L.; Polsinelli, M. Acetaldehyde production in *Saccharomyces cerevisiae* wine yeasts. *FEMS Microbiol. Lett.* **1994**, *118*, 213-218.

(20) Jones, R. P. Intracellular ethanol accumulation and exit from yeast and other cells. *FEMS Microbiol. Rev*. **1988**, *4*, 239−258.

(21) Jones, R. P. Roles for replicates deactivation in yeast-ethanol fermentation. *CRC Crit. Rev. Biotechnol*. **1990**, *10*, 205−222.

(22) Stanley, G. A.; Pamment, N. B. Transport and intracellular accumulation of acetaldehyde in *Saccharomyces cerevisiae*. *Biotechnol. Bioeng*. **1993**, *42*, 24−29.

(23) Aranda, A.; Querol, A.; Olmo, M. Correlation between acetaldehyde and ethanol resistance and expression of *HSP* genes in yeast strains isolated during the biological aging of sherry wines. *Arch. Microbiol*. **2002**, *177*, 304−312.

(24) Stanley, G. A.; Douglas, N. G.; Every, E. J.; Tzanatos, T.; Pamment, N. B. Inhibition and stimulation of yeast growth by acetaldehyde. *Biotechnol. Lett.* **1993**, *15*, 1199−1204.

(25) Walker-Caprioglio, H. M.; Parks, L. W. Autoconditioning factor relieves ethanol-induced growth inhibition of *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **1987**, *53*, 33−35.

(26) Roustan, J. L.; Sablayrolles, J. M. Modification of the acetaldehyde concentration during alcoholic fermentation and effects on fermentation kinetics. *J. Biosci. Bioeng*. **2002**, *93*, 367−375.

(27) Tsai, C. S.; Shi, J. L.; Beehler, B. W.; Beck, B. Enzyme activities of D-glucose metabolism in the fission yeast *Schizosaccharomyces pombe*. *Can. J. Microbiol.* **1992**, *38*, 1313−1319.

(28) Schimpfessel, L. Presence and regulation of the synthesis of two alcohol dehydrogenases in *Saccharomyces cerevisiae* yeast. *Biochim. Biophys. Acta* **1968**, *151*, 317−329.
(29) Fernández, M. J.; Gómez-Moreno, C.; Ruiz-Armil, M. Induction of isoenzymes of alcohol dehydrogenase in flor yeast. *Arch. Mikrobiol*. **1972**, *84*, 153−160.
(30) Beier, D. R.; Sledziewski, A.; Young, E. T. Deletion analysis identifies a region upstream of the ADH-2 gene of *S. cerevisiae* which is required for ADR 1-mediated derepression. *Mol. Cell. Biol.* **1985**, *5*, 1743−1749.
(31) Ladrière, J. M.; Georis, I.; Guérineau, M.; Vandenhaute, J. *Kluyveromyces marxianus* exhibits an ancestral *Saccharomyces cerevisiae* genome organization downstream of *ADH2*. *Gene* **2000**, *255*, 83−91.
(32) Shain, D. H.; Salvadore, C.; Denis, C. L. Evolution of the alcohol dehydrogenase ADH genes in yeast: characterization of a fourth ADH in *Kluyveromyces lactis*. *Mol. Gen. Genet*. **1992**, *232*, 479−488.
(33) Larroy, C.; Pares, X.; Biosca, J. A. Characterization of a *Saccharomyces cerevisiae* NADP(H)-dependent alcohol dehydrogenase (ADHVII), a member of the cinnamyl alcohol dehydrogenase family. *Eur. J. Biochem*. **2002**, *269*, 5738−5745.
(34) Schreier, P. Flavor composition of wines: a review. *CRC Crit. Rev. Food Sci. Nutr*. **1979**, *12*, 59−111.
(35) Cortés, M. B.; Moreno, J. 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.
(36) 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.
(37) Cortés, M. B.; 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*, 2389−2394.

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