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Aromatic series in sherry wines with gluconic acid subjected to different biological aging conditions by *Saccharomyces cerevisiae* var. *capensis*

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Abstract

Wines from healthy grapes supplemented with gluconic acid were subjected to biological aging under two experimental conditions. The first one was carried out under flor yeast velum as in the traditional biological aging and the second one under submerged cultures. The highest gluconic acid consumption was observed in aged wines in submerged cultures. Nine aromatic series were obtained by grouping the 48 volatile compounds quantified in wines. The aroma profile based on the aromatic series allows comparison of the changes due to the gluconic acid consumption and the changes due to the different aging conditions assayed. Only the herbaceous and fatty series showed diminished values of consequence of gluconic acid consumption. The fatty, herbaceous and roasty series show highest values, whereas the fruity, floral, solvent and medicinal series reached lower values in the submerged cultures assay. The application of the assay conditions to winemaking can reduce the gluconic acid concentration in wines obtained from rotten grapes.

Keywords: Wine; Gluconic acid; Biological aging; Volatile compounds; *Saccharomyces cerevisiae*; Aromatic series

1. Introduction

The presence of gluconic acid in must or wines from botrytized grapes causes several problems in the winemaking process such as microbiological instability, or high bindable SO_2 (Barbe, de Revel, & Bertrand, 2002; Peinado, Moreno, Ortega, & Mauricio, 2003). On the other hand, gluconic acid metabolism by lactic bacteria involves an increase in volatile acidity (Radler, 1986).

Recently, it has been reported that *Saccharomyces cerevisiae* var. *capensis* (a flor velum yeast) metabolizes gluconic acid in wines subjected to different aging conditions when the yeast has been previously adapted to glycerol consumption (Peinado et al., 2003). In this way, Sherry and Montilla-Moriles fino wines are biologically aged for several years in oak casks by the so-called flor yeast, that forms a biofilm on the wine surface growing under aerobic conditions (Medina et al., 2003). Such metabolism involves ethanol, glycerol acetic acid and ethyl acetate consumption and acetaldehyde, and its derivatives and some organic C4 acid production (Cortés, Moreno, Zea, Moyano, & Medina, 1998).

Wine aroma is the result of the volatile compounds that compose it. The aroma perceived by smelling can rarely ascribed to a specific compound. However, not all the compounds contribute with the same intensity to wine aroma. Among other factors, the contribution of a given compound depends on the odour perception threshold (OPT). The concentration-OPT ratio, known

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as "odour activity value" (OAV), allows estimation of the contribution of each compound to wine aroma (Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004).

The odour of a compound can be described by one or several descriptors (Etievant, 1991; Ferreira, Aznar, Lopez, & Cacho, 2001; Peinado, Mauricio, Medina, & Moreno, 2004). Thus, on grouping the OAVs of the aroma compounds with similar descriptors into aromatic series, an organoleptic profile of the wine can be established. Therefore, it is possible to determine the contribution of a specific compound to each series. This procedure on the one hand is possible to the quantitative information from chemical analysis to be related to sensory perception, and on the other hand, shows the influence of one or severe treatments on the wine, reducing the numbers of variables. This method has recently been used by Franco, Peinado, Medina, and Moreno (2004), Peinado et al. (2004) and Peinado, Mauricio, et al. (2004).

In this paper, the effect of gluconic acid consumption by *S. cerevisiae* var. *capensis* yeast strain on the aromatic series under several aging conditions has been studied.

2. Material and methods

2.1. Yeast strain and inocula

S. cerevisiae var. *capensis* (Gl; ATCC No.: MYA-2451) yeast strain from the Culture Collection of the Microbiology Department of the University of Cordoba (Spain) was used in this study. This flor yeast prevails, together with *Saccharomyces bayanus*, in the biofilms formed on the surface of sherry wine during biological aging in the Montilla-Moriles region (southern of Spain).

Yeast cells were cultured on YM medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, pH 6.5) containing 30 g/l of glycerol (Merck). Cells were incubated at 27 ± 2 °C with shaking for 72 h and were collected by centrifugation at 3500*g*. All wines were simultaneously inoculated with 10^6 live cells/ml.

The number of total and live cells was determined by counting under a light microscope in a Thoma chamber following staining with methylene blue (E.B.C., 1977).

2.2. Wine

Wine from healthy Pedro Ximénez grapes was used for control experiments versus the same wine supplied with 5.1 g/l of gluconic acid (Sigma–Aldrich), because this is the highest concentration of acid reported in the study area (personal communication). The wines were sterilized by passage through Supra EK filters (Seitz, Germany).

2.3. Experimental conditions

Two experimental conditions were assayed. The first assay was carried out in a traditional biological aging with yeast velum formation on the wine surface in 250-ml Erlenmeyer flasks. The volume of wine used was that resulting in a surface/volume ratio of 17 cm^2/l, which is a typical value for the traditional biological aging of sherry wines in oak casks. The second condition consisted in submerged cultures with shaking, and was carried out at 150 rpm in 1-l Erlenmeyer flasks, containing 800 ml of wine. All samples were thermostatted at 25 ± 2 °C.

Changes in the concentrations of the winemaking variables and in the volatile compounds were studied in 12 flasks containing the same wine. Six flasks were supplemented with the same amount of gluconic acid. Three of these flasks were continuously shaken during 85 days (submerged cultures) and the other three remained static during 105 days (under flor velum). The remaining flasks were used as controls without gluconic acid, and were unshaken. Control flasks were analysed at 0 (initial control) and 105 days (final control). All wines were simultaneously inoculated with 10^6 viable cells/ml of *S. cerevisiae* var. *capensis* G1.

2.4. Analyses

Ethanol was quantified by dichromate oxidation (Crowell & Ough, 1979) titratable acidity, pH, residual sugars, and volatile acidity were determined by the official methods (E.E.C., 1990); gluconic acid was quantified using an enzymatic test kit from Boehringer–Mannheim (Germany) at pH 11, and the absorbances at 280, 420 and 520 nm were measured on a Beckman DU-640 UV spectrophotometer.

Major volatile compounds and polyols were determined by gas chromatography on an Agilent 6890 GC model (Palo Alto, CA), using the method reported by Peinado, Moreno, Muñoz, Medina, and Moreno (2004). A 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 4-methyl-2-pentanol, as internal standard, were injected.

Quantification was based on the response factors obtained for standard solutions of each compound. A split ratio of 30:1, a FID detector and a temperature programme involving an initial temperature of 50 °C (15 min), a ramp rate of 4 °C/min 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 (16 min) and followed by a 0.2 ml/min ramp to the final value (1.1 ml/min), which was held for 52 min.

Minor volatile compounds were determined following continuous extraction, for 24 h, of 100 ml of wine at pH 3.5, which was supplied with 5 ml of internal standard (30 mg/l of 2-octanol) and 100 ml of Freon-11. The Freon extracts containing the volatile compounds were concentrated to 0.2 ml in a Kuderna–Danish microconcentrator, and 1.5 μl aliquots were injected into an HP-6890 chromatograph equipped with an HP MS 5972 mass detector from Agilent Technologies. An HP-Innowax 60 m long × 0.32 mm i.d. capillary column (0.25 μm film thickness) was used. The oven was held at 40 °C for 10 min, which was followed by a 1 °C/min ramp to 180 °C (held for 35 min). Helium at a constant flow rate of 0.9 ml/min was used as carrier gas. The split ratio was 30:1, and the MS detector was set at 1612 V in the scan mode to sweep the mass range from 39 to 300 amu.

Retention time, Wiley mass-spectral library, and pure volatile compounds from Merck, Sigma–Aldrich, Riedel de Haën, and Fluka were used for identification, confirmation and preparation of standard solutions of volatile compounds. Quantification was based on the response factors calculated from standard solutions that were subjected to the same extraction process as the samples, using the target and qualifier ions selected for each compound by the Hewlett–Packard Chemstation.

2.5. Statistical treatment

In order to establish significant differences between the studied conditions, a one way analysis of variance (ANOVA) was carried out. This analysis allows comparison of differences due to the traditional biological aging process under flor yeast velum (comparing initial control wine against final control wine), due to the gluconic acid consumption by the flor yeast strain (comparing final control wine against final wine containing gluconic acid, both under flor velum), and the differences due to the biological aging conditions assayed (comparing wine aged under flor yeast velum against wine aged with submerged cultures, both wines containing gluconic acid).

In the same way, a single analysis of variance, to study the effect of the different conditions on the aromatic series, was carried out. The statistical software package Statgraphics Plus v. 2, from Manugistic (Rockville, MD) was used to perform the statistical analysis.

All results reported herein are the averages of all three independent experiments.

3. Results and discussion

3.1. General

Gluconic acid metabolism, in some yeasts, may be related to aerobic metabolism and to the presence of glycerol in the medium (Peinado et al., 2003; Peinado, Mauricio, Ortega, Medina, & Moreno, 2003). These two conditions occur during the biological aging of sherry type wines by means of flor yeasts (Cortés et al., 1998).

The consumption of gluconic acid in wines aged under flor velum and with submerged cultures is shown in Fig. 1. The uptake of acid was greater in wines aged in submerged cultures than in wines aged under flor velum (traditional biological aging process).

Table 1 shows the winemaking variables and glycerol concentrations in wines in the assayed conditions. The

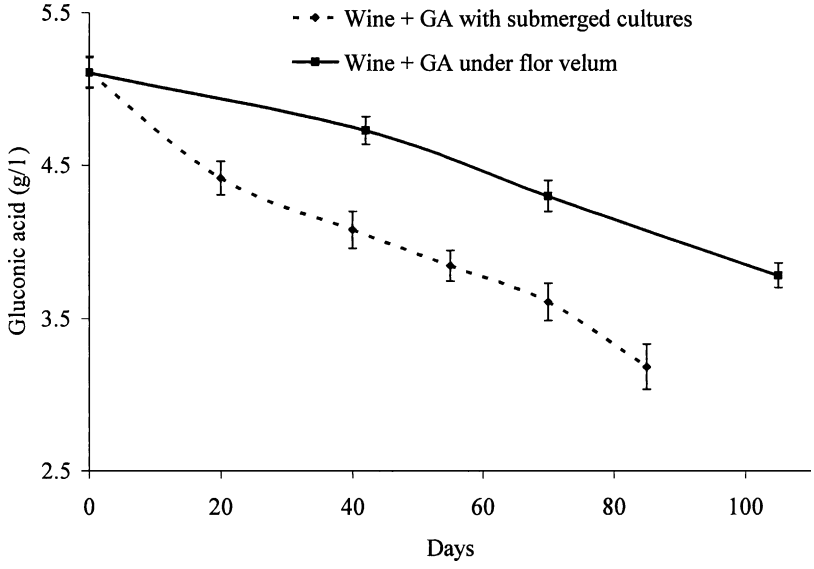

Fig. 1. Gluconic acid consumption, during the biological aging of wines, by *Saccharomyces cerevisiae* var. *capensis* G1, under flor velum or with submerged cultures.

Table 1
Winemaking variables in wines containing gluconic acid (GA) and subjected to biological aging, with *Saccharomyces cerevisiae* var. *capensis* Gl flor yeast strain, under different conditions

Winemaking variables	Control wine		Wine + GA		HG
	Initial	Final	Flor velum	Submerged cultures	
Reducing sugars (g/l)	2.2 ± 0.2	2.2 ± 0.2	2.2 ± 0.3	2.2 ± 0.2	AAAA
Ethanol percentage (v/v)	14.1 ± 0.1	8.3 ± 0.6	8.3 ± 0.1	11.6 ± 0.1	ABBC
Volatile acidity (meq/l)	4.3 ± 0.3	2.8 ± 0.5	2.0 ± 0.2	2.0 ± 0.1	
pH	3.3 ± 0.1	3.5 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	
Titratable acidity (meq/l)	41 ± 1	35 ± 1	47 ± 1	45 ± 1	
Glycerol (g/l)	9.4 ± 0.2	nd	nd	nd	ABBB

HG, homogeneous group at 95% confidence level obtained by one way variance analysis. Different letters indicate different groups. nd, not detected.

Table 2
Odour activity values (OAV) obtained for major volatile compounds quantified in wines containing gluconic acid (GA) and subjected to biological aging with *Saccharomyces cerevisiae* var. *capensis* Gl, in the assay conditions

Volatile compound	Control wine		Wine + GA		HG
	Initial	Final	Flor velum	Submerged cultures	
Acetaldehyde	1.02 ± 0.04	2.3 ± 0.2	2.0 ± 0.1	1.46 ± 0.03	ABCD
Ethyl acetate	2.9 ± 0.2	0.36 ± 0.03	0.30 ± 0.06	0.39 ± 0.04	ABBB
1,1-Diethoxyethane	nd	0.7 ± 0.5	1.0 ± 0.6	nd	ABBA
Methanol	0.10 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	ABBB
1-Propanol	0.08 ± 0.01	0.07 ± 0.00	0.08 ± 0.00	0.02 ±0.00	ABAC
Isobutanol	0.62 ± 0.02	0.40 ± 0.01	0.41 ± 0.02	0.34 ± 0.04	ABBB
Isoamyl alcohol	5.3 ± 0.3	2.6 ± 0.1	2.8 ± 0.1	2.7 ± 0.2	ABBB
Acetoin	0.14 ± 0.06	0.86 ± 0.06	0.80 ± 0.05	0.56 ± 0.05	ABBC
Ethyl lactate	0.39 ± 0.02	0.21 ± 0.01	0.23 ± 0.04	0.06 ± 0.01	ABBC
Levo 2,3-butanediol	4.1 ± 0.3	4.5 ± 0.1	5.3 ± 0.7	3.9 ± 0.2	AABA
Meso 2,3-butanediol	1.0 ± 0.1	1.8 ± 0.1	2.0 ± 0.3	1.7 ± 0.1	ABBB
Diethyl succinate	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	AAAA
2-Phenylethyl alcohol	0.37 ± 0.02	0.48 ± 0.03	0.47 ± 0.04	0.43 ± 0.01	ABBB

HG, homogeneous group at 95% confidence level obtained by one way variance analysis. Different letters indicate different groups. nd, not detected.

addition of 5.1 g/l of gluconic acid involved a diminution in pH values, and an increase in the titratable acidity and volatile acidity (data not shown) since the commercially available gluconic acid contains acetic acid as a stabilizer agent. For this reason the comparison of these variables before and after addition of the gluconic acid is not possible. The rest of the winemaking variables and compounds analysed in wines are not affected by the addition of these acids, as was reported by Peinado et al. (2003) and Peinado, Mauricio, et al. (2003).

Ethanol contents decreased during the assay conditions, but the decrease was greater in the biological aging under static conditions, with velum formation. On the other hand, reducing sugars did not change, which suggests that these sugars were nonfermentable sugars.

Table 2 lists the odour activity values (OAV) for the major volatile compounds and the homogeneous group obtained by one way analysis of variance at 95% confidence level. OAV is defined as the ratio of concentration of a volatile compound determined in wine and its odour perception threshold (OPT) value (Table 4).

The OAVs of methanol, isobutanol, isoamyl alcohols, 2-phenylethyl alcohol, *meso* 2,3-butanediol, and ethyl acetate showed no significant differences after the aging in any of the assayed conditions, although their values were significantly different from those in the initial wine.

1,1-Diethoxyethane, acetoin, and ethyl lactate showed no significant differences due to gluconic acid consumption, although their OAV depended on the different biological aging conditions assayed (under flor velum or submerged cultures). On the other hand, acetaldehyde, *levo* 2,3-butanediol and propanol depend on the gluconic acid and aging conditions. Diethyl succinate did not show significant differences in any of the assayed conditions nor with the initial wine.

Table 3 lists the OAVs obtained for 34 quantified minor volatile compounds and the homogeneous group obtained by statistical analysis. Only 4-ethylguaiacol showed no differences, in any of the assayed conditions, from the initial wine, resulting in the AAAA groups. Apparently, for the homogeneous groups ABBB, obtained for isoamyl acetate, ethyl hexanoate, 3-ethoxy-1-propanol, γ-butyrolactone, and hexanoic, octanoic

Table 3
Odour activity values (OAV) obtained for minor volatile compounds quantified in wines containing gluconic acid (GA) and subjected to biological aging with *Saccharomyces cerevisiae* var. *capensis* Gl strain in the assay conditions

Volatile compound	Control wine		Wine + GA		HG
	Initial	Final	Flor velum	Submerged cultures	
Ethyl propanoate	0.091 ± 0.01	0.72 ± 0.05	0.53 ± 0.05	0.146 ± 0.01	ABCD
Ethyl isobutanoate	nd	nd	nd	0.010 ± 0.001	AAAB
2,3-Butanedione	0.07 ± 0.01	0.022 ± 0.003	0.039 ± 0.005	0.039 ± 0.002	ABCC
Isobutyl acetate	0.030 ± 0.002	0.012 ± 0.001	0.009 ± 0.002	0.051 ± 0.002	ABBC
2-Butanol	0.039 ± 0.004	0.023 ± 0.006	0.021 ± 0.002	0.011 ± 0.001	ABBC
Ethyl butanoate	0.38 ± 0.02	0.22 ± 0.05	0.13 ± 0.01	0.30 ± 0.02	ABCD
2,3-Pentanedione	nd	3.1 ± 0.7	2.2 ± 0.2	1.10 ± 0.07	ABCD
Isoamyl acetate	3.7 ± 0.3	0.29 ± 0.07	0.16 ± 0.02	0.1 6 ± 0.02	ABBB
1-Butanol	0.018 ± 0.001	0.015 ± 0.002	0.013 ± 0.001	0.005 ± 0.001	AAAB
Ethyl hexanoate	1.45 ± 0.04	nd	nd	nd	ABBB
3-Methyl-1-pentanol	0.18 ± 0.01	0.15 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	ABCD
1-Hexanol	0.70 ± 0.01	0.19 ± 0.02	0.24 ± 0.02	0.062 ± 0.005	ABBC
3-Ethoxy-1 -propanol	nd	5.1 ± 0.5	5.7 ± 0.5	4.3 ± 0.3	ABBB
Ethyl octanoate	0.019 ± 0.003	0.16 ± 0.01	0.21 ± 0.02	0.50 ± 0.03	ABBC
Ethyl 3-hydroxybutanoate	0.69 ± 0.04	1.30 ± 0.07	1.2 ± 0.1	1.17 ± 0.04	ABBC
Linalool	nd	1.7 ± 0.3	1.9 ± 0.2	0.98 ± 0.06	ABBC
1-Octanol	0.66 ± 0.01	0.27 ± 0.01	0.28 ± 0.03	nd	ABBC
Isobutanoic acid	0.053 ± 0.002	0.34 ± 0.02	0.23 ± 0.02	0.61 ± 0.02	ABCD
γ-Butyrolactone	0.97 ± 0.07	1.4 ± 0.2	1.6 ± 0.2	1.5 ± 0.1	ABBB
Butanoic acid	0.79 ± 0.04	2.0 ± 0.3	1.72 ± 0.04	2.35 ± 0.04	ABCD
2&3-Methylbutanoic acids	0.78 ± 0.03	4.4 ± 0.2	2.4 ± 0.3	9.8 ± 0.3	ABCD
Furanmethanol	0.016 ± 0.001	0.03 ± 0.01	0.03 ± 0.01	0.009 ± 0.001	ABBC
Neral	0.11 ± 0.02	0.29 ± 0.02	0.19 ± 0.02	0.21 ± 0.01	ABCC
Methionol	3.2 ± 0.3	5.6 ± 0.7	2.6 ± 0.2	6.7 ± 0.3	ABAC
β-Citronellol	0.37 ± 0.03	nd	nd	0.029 ± 0.019	ABBC
2-Phenylethyl acetate	0.193 ± 0.006	0.12 ± 0.01	0.031 ± 0.006	0.045 ± 0.004	ABCC
Hexanoic acid	0.47 ± 0.02	nd	nd	nd	ABBB
Benzyl alcohol	nd	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	ABBC
4-Ethylguaiacol	4.5 ± 0.2	4.4 ± 0.5	4.4 ± 0.5	4.7 ± 0.5	AAAA
Pantolactone	0.20 ± 0.01	1.0 ± 0.2	0.84 ± 0.03	2.07 ± 0.07	ABBC
Z-Nerolidol	0.027 ± 0. 000	0.064 ± 0.002	0.08 ± 0.01	0.042 ± 0.003	ABCD
Diethyl malate	0.052 ± 0.002	0.049 ± 0.005	0.041 ± 0.004	0.031 ± 0.002	AABC
Octanoic acid	0.26 ± 0.01	nd	nd	nd	ABBB
Decanoic acid	0.036 ± 0.004	nd	nd	nd	ABBB

HG, homogeneous group at 95% confidence level obtained by one way analysis of variance. Different letters indicate different groups. nd, not detected.

and decanoic acids, the OAV values only depend on the biological aging when initial and final control wines are compared. The OAV values of such compounds were independent of the gluconic acid consumption and of the biological aging conditions assayed (under flor velum or submerged cultures) Isobutyl acetate, 2-butanol, 1-hexanol, ethyl octanoate, ethyl-3-hydroxy-butanoate, linalool, 1-octanol, furanmethanol, β-citronellol, benzyl alcohol and pantolactone showed the ABBC distribution for their respective homogeneous group, which indicates a significant difference due to the biological aging and due to the use of submerged cultures; nevertheless, these compounds are independent of gluconic acid consumption.

The homogeneous groups ABCC, obtained for 2,3-butanedione, neral and 2-phenylethyl acetate, indicate significant differences due to gluconic acid consumption during the biological aging, as well as significant differences of wines subjected to biological aging in relation to the initial wine.

Ethyl propanoate, ethyl butanoate, 2,3-pentanedione, 3-methyl-1-pentanol, isobutanoic acid, butanoic acid, 2&3 methylbutanoic acids and *Z*-nerolidol showed the ABCD homogeneous group distribution, indicating their dependence on all the assayed conditions. Finally, four compounds (ethyl isobutanoate, 1-butanol, methionol, and diethyl malate) showed homogeneous groups, namely AAAB, ABAC, AABC, respectively, indicating dependence on some of the studied factors.

3.2. Aromatic series

The odour perception threshold has previously been defined by Cutzach, Chatonnet, and Dubourdieu (2000), Kotseridis and Baumes (2000) and Peinado, Mauricio, et al. (2004) as the lowest concentration

capable of producing a sensation. That sensation has to be detected by at least 50% of the judges in a taste panel. On the other hand, an aromatic series could be defined as a group of volatile compounds with similar odour descriptors.

Table 4 lists the odour descriptor, odour perception threshold and aromatic series in which the volatile compounds determined were grouped. Acetaldehyde, isoamyl alcohols, *levo* 2,3-butanediol, *meso* 2,3-butanediol 4-ethylguaiacol, and methionol were the compounds most markedly contributing to wine aroma, all with OAVs greater than unity in all the assay conditions (Tables 2 and 3). By contrast, 2,3-pentanedione, 3-ethoxy-l-propanol, ethyl-3-hydroxybutanoate, linalool, ϒ-butyrolactone, butanoic acid, and 2&3-methyl butanoic acid, that showed OAVs below 1 in the initial wine, increased their OAV value above 1 by the aging effect in all the assay conditions (Table 3). On the other hand, ethyl acetate, isoamyl acetate and ethyl hexanoate, that showed OAV values above 1 in the initial wine,

Table 4
Odour perception threshold (OPT) in mg/l, odour descriptor, and assignment to aromatic series (AS) of volatile compounds quantified in wines

Volatile compound	OPT[a]	Odour descriptor	AS[b]
Acetaldehyde	110	Pungent, ripe apple	1
1,1-Diethoxyethane	1	Liquorice, green fruit	1,2
Acetoin	150	Buttery, fatty	4
Levo 2,3-butanediol	150	Fruity	1
Meso 2,3-butanediol	150	Fruity	1
Ethyl acetate	12	Pineapple, varnish, balsamic	1,2,3
Methanol	500	Alcohol	3
1-Propanol	306	Ripe fruit, alcohol	3
Isobutanol	75	Alcohol, solvent	3
Isoamyl alcohol	60	Nail polish, alcohol	3
Ethyl lactate	150	Buttery, cream, sweet, fruity	1,4
Diethyl succinate	1200	Fruity, wine	1
2-Phenylethanol	200	Rose	5
1-Butanol	150	Medicinal, phenolic	6
2-Butanol	50	Alcohol, solvent	3
1-Hexanol	1.1	Herbaceous, wood	7
1-Octanol	0.8	Jasmine, lemon	5
3-Methyl-1-pentanol	1	Pungent, solvent, green	3,7
3-Ethoxy-1-propanol	0.1	Fruity	1
Benzyl alcohol	900	Roasted, toasted	8
Methionol	1.5	Cooked potato, garlic	7
Furanmethanol	15	Solvent	3
Ethyl propanoate	1.8	Banana, apple	1
Ethyl isobutanoate	5	Fruity	1
Ethyl butanoate	0.4	Strawberry, apple, banana	1
Ethyl-3-hidroxybutanoate	1	Grape	1
Ethyl hexanoate	0.08	Fruity, green apple, banana, wine-like	1
Ethyl octanoate	0.58	Sweet, floral, fruity, banana, pear, brandy	1,5
Diethyl malate	10	Fruity	1
2,3-Butanedione	4.74	Celery	7
Isobutyl acetate	1.6	Sweet, fruity, apple, banana	1
Isoamyl acetate	0.16	Banana, fruity, sweet	1
2-Phenylethyl acetate	1.8	Fruity	1
Butanoic acid	2.2	Cheese, rancid	4
Isobutanoic acid	30	Fatty, rancid	4
2&3-Methylbutanoic acids	1.5	Rancid	4
Hexanoic acid	3	Rancid, cheese, fatty	4
Octanoic acid	10	Rancid, cheese, fatty	4
Decanoic acid	6	Fatty, rancid	4
γ-Butyrolactone	20	Sweet, cake, caramel, fruity	1
Pantolactone	2	Licorice, smoky, toasted bread	2,8
Linalool	0.015	Citrus, floral, sweet, grape-like	1,5
Neral	0.5	Fruity	1
β-Citronellol	0,1	Rose	5
Z-Nerolidol	1	Rose, apple, green, citrus, woody	1,5,6
4-Ethylguaiacol	0.02	Spicy, clove	9
2,3-Pentanedione	0.9	Buttery	4

[a] Determined in 10% (v/v) ethanol–water solution, adjusted to pH 3.5 with tartaric acid.
[b] 1, fruity; 2, balsamic; 3, solvent; 4, fatty; 5, floral; 6, medicinal; 7, herbaceous; 8, roasty; 9, spicy.

decreased their OAVs below 1 by the aging effect in all the assay conditions. Methanol, 1-propanol, diethyl succinate, 2,3-butanedione, ethyl isobutanoate, isobutyl acetate, butanol-2, butanol-1, furanmethanol, benzyl alcohol, *Z*-nerolidol, diethyl malate, and decanoic acid were compounds with OAVs 10 times lower than their odour perception thresholds in all the assay conditions (Tables 2 and 3).

An odour profile for the wines was obtained by grouping the volatile compounds with similar descriptors in nine aromatic series (see Table 4). The value of each aromatic series was obtained adding the OAVs of the compounds that form such a series. Therefore, it is possible to determine the contribution of a specific compound to each series. This procedure makes it possible to relate quantitative information obtained by chemical analysis to sensory perception, providing a single aroma profile based on an objective. It has recently been used by Franco et al. (2004), Peinado et al. (2004) and Peinado, Mauricio, et al. (2004).

The aroma profiles for the wines are shown in Figs. 2–4. An ANOVA was performed to identify differences between the aromatic series. In this way the influence of traditional biological aging was studied by comparing initial and final control wines (Fig. 2). The gluconic acid consumption effect was studied by comparing the final control wines with wines containing gluconic acid, both under flor velum formation (Fig. 3). Lastly, the effect of the biological aging condition assayed (velum formation or submerged cultures) was studied by comparing wine aged under flor velum with wine aged in submerged cultures, both supplemented with gluconic acid (Fig. 4).

Fig. 2 shows the aromatic profile of the initial and final control wines. All the aromatic series, except the spicy series, changed their values significantly ($p \leqslant 0.05$) by

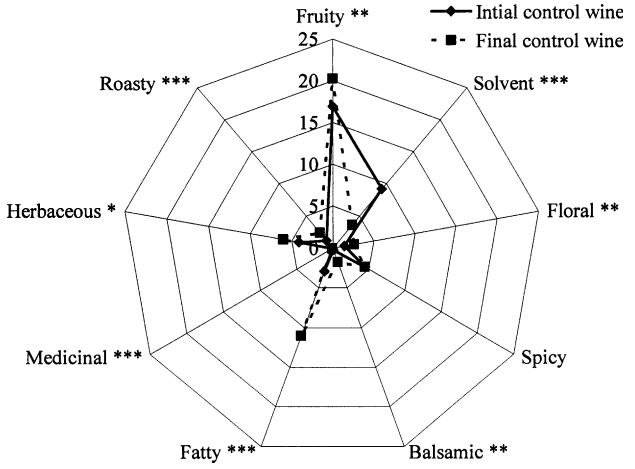

Fig. 2. Aromatic series in initial and final control wines aged under flor velum with *Saccharomyces cerevisae* var. *capensis* G1. Significance levels obtained by one way analysis of variance: ***, $p \leqslant 0.001$; **, $p \leqslant 0.01$; *, $p \leqslant 0.05$.

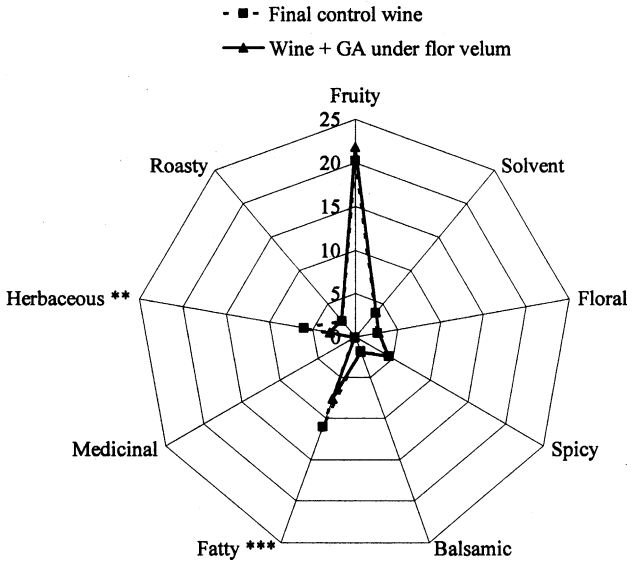

Fig. 3. Aromatic series in final control wine and in wine supplemented with gluconic acid (GA), both aged under flor velum with *Saccharomyces cerevisae* var. *capensis* Gl. Significance levels obtained by one way analysis of variance: ***, $p \leqslant 0.001$; **, $p \leqslant 0.01$.

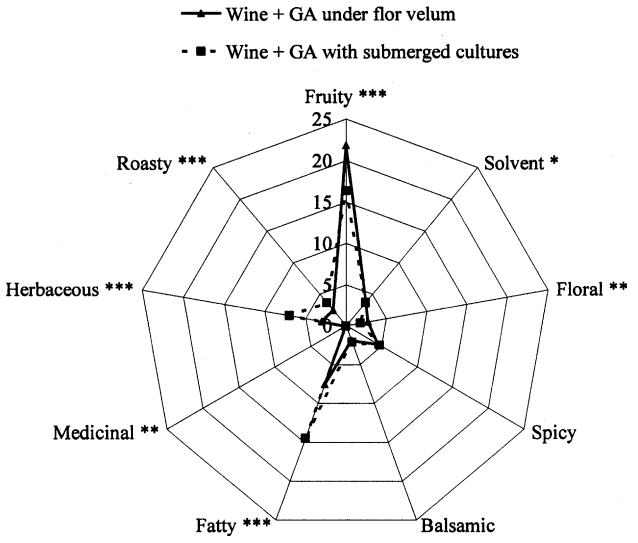

Fig. 4. Aromatic series in wines supplemented with gluconic acid (GA) and subjected to aging under flor velum or in submerged cultures with *Saccharomyces cerevisae* var. *capensis* Gl. Significance levels obtained by one way analysis of variance: ***, $p \leqslant 0.001$; **, $p \leqslant 0.01$; *, $p \leqslant 0.05$.

effect of the biological aging. The spicy series contains only 4-ethyguaiacol, a volatile phenol extracted from the wood during industrial biological aging. The observed changes are typical of the biological aging process of sherry wines.

Fig. 3 shows the OAV values of the aromatic series obtained in the final control and in gluconic acid-containing wines, both aged under flor velum. Only the herbaceous ($p \leqslant 0.01$) and fatty ($p \leqslant 0.001$) series change

significantly, the final control wines having the highest values. The changes observed in herbaceous series can be related to the methionol OAV (Table 3), whereas changes in the fatty series are due to acids with four carbon atoms (butanoic, isobutanoic and 2&3-methylbutanoic acids). These acids are synthesized by flor yeast during biological aging Cortés et al. (1998), and are associated with cheese, butter and sweat odours (Table 4), so this decrease can be considered to be favourable effect due to gluconic acid consumption.

Fig. 4 shows the aromatic series of the gluconic acid-containing wine, aged under flor velum or with submerged cultures. All the aromatic series, except the spicy and balsamic, change their values significantly. The herbaceous and fatty series show highest values $p \leqslant 0.001$, in the wine aged with submerged cultures. The changes are related to increases in the OAVs of methionol and C4 acid, respectively (Table 3). Also the roasty series increase their values ($p \leqslant 0.001$) in wine aged with submerged cultures, which can be related to increase of pantolactone (Table 3). The fruity ($p \leqslant 0.001$), floral ($p \leqslant 0.01$), medicinal ($p \leqslant 0.01$) and solvent ($p \leqslant 0.05$) series showed lower values in wine aged with submerged cultures. The changes produced in these series cannot be explained by the changes observed in one or several compounds, but rather the changes are due to the contribution of all the compounds that form them.

In conclusion, gluconic acid concentration in wine diminishes during the biological aging, more with submerged cultures than with flor velum formation. Gluconic acid consumption only diminished the herbaceous and fatty aromatic series with respect to control wine, which may be considered as a favourable effect. The use of submerged cultures causes changes in volatile compounds similar to those obtained during traditional biological aging. Nevertheless, significantly higher values for the OAVs of fatty, herbaceous, and roasty series and minor values for the fruity, floral, solvent, and medicinal series were obtained in the shaken condition. Experiments in semi-industrial conditions, together with sensory analysis, will be carried out to study the ability of the assay conditions to reduce gluconic acid concentration in wines obtained from rotten grapes.

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