J Ind Microbiol Biotechnol (2008) 35:183–188
DOI 10.1007/s10295-007-0282-9

ORIGINAL PAPER

Influence of yeast population on characteristics of the wine obtained in spontaneous and inoculated fermentations of must from *Vitis vinifera* Lado

P. Blanco · M. Vázquez-Alén · A. Losada

Received: 27 February 2007 / Accepted: 20 November 2007 / Published online: 19 December 2007

Abstract This work describes the influence of yeast population on the chemical characteristics of wine obtained by spontaneous and inoculated fermentation of must from *Vitis vinifera* Lado, a minor white grapevine autochthonous to Galicia (NW Spain). The study was carried out for two consecutive years. The results showed that musts derived from Lado presented a high acidity though the potential alcohol level was acceptable. The genetic diversity of *S. cerevisiae* strains isolated from spontaneous fermentations was low, probably due to must characteristics, although these did not interfere with the implantation of the commercial strains used. Analyses showed that the wines subsequently produced had high alcoholic levels and very high acidities (pH 3.0) as was expected from must composition. Wines obtained from spontaneous fermentations had a lower alcohol content but higher total acidity than those from inoculated fermentations. Monovarietal wines produced from Lado were poorly evaluated in sensorial tests because of their unbalanced structure and sourness; however, when they were mixed with other autochthonous white varieties with less acidity, the resulting wines were well accepted.

Keywords Lado variety · Yeasts · *Saccharomyces cerevisiae* · Spontaneous fermentations · Inoculated fermentations · Wine chemical composition · pH

P. Blanco (✉) · M. Vázquez-Alén · A. Losada
Estación de Viticultura e Enoloxía de Galicia,
Ponte San Clodio, 32427 Leiro-Ourense, Spain
e-mail: evegalab@cesga.es

Introduction

Wine quality is influenced by several factors including grapevine variety, winemaking techniques and yeast strains employed during fermentations. The role of the yeast population on the fermentation process, final chemical composition and sensorial properties of wine has been widely reported [15, 16, 22]. Studies on yeast population succession during spontaneous fermentations have shown that several non-*Saccharomyces* yeast species are present at the early stages of the process; but, as the fermentation progresses, they are substituted by *Saccharomyces* strains which are more tolerant to alcohol and have better fermentative ability [2, 8–10, 16]. This yeast diversity during fermentation contributes to the final complexity of wine [10, 16, 18, 23, 24]; though it can also produce undesirable compounds [11, 29]. Therefore, in the wine-making industry the growth of undesirable yeasts is controlled by addition of sulphur dioxide to musts and inoculation with selected strains of *Saccharomyces cerevisiae* [20, 21, 28]. Analysis obtained from spontaneous and inoculated fermentations has shown significant differences in the chemical composition and sensory properties of wine [4, 10, 33].

Wine quality is also conditioned by grapevine variety. Although Galicia (NW Spain) has a wide range of autochthonous varieties of *Vitis vinifera*, few have been thoroughly studied from the oenological point of view. Such investigations have focused mainly on varieties like Albariño, Treixadura and Godello, which form the majority of grapevines in Galician vineyards [6, 12, 13, 32]. Nowadays, however, there is an increasing interest shown in the properties of minor cultivars that may have potential uses as complementary varieties. The study of such varieties is necessary since they could provide a good source of

diversification and, consequently, a market expansion of wines from Galicia.

Vitis vinifera cv. Lado is a white grapevine variety autochthonous to Galicia, whose growth is restricted to a limited area within the Denomination of Origin Ribeiro. Although there is some information available about the agronomic and ampelographic characteristics of Lado [14, 17], little is known about its oenological potential and the role of yeast population on the final wine composition.

This study reports the influence of yeast population on the characteristics of wine produced by spontaneous and inoculated fermentations of must from Lado for 2 years.

Materials and methods

Grape processing

Vitis vinifera Lado grapes were collected from the experimental vineyard of Estación de Viticultura e Enoloxía de Galicia (EVEGA) during the 2004 and 2005 vintages. The grapes were harvested manually and transported to the cellar where they were crushed and pressed for juice extraction. During grape processing SO_2 (50 mg/l) was added to avoid oxidation and for microbiological control. After 24 h of cold static clarification, the must obtained was distributed into 35-l stainless steel tanks in which fermentations were carried out.

Fermentation assays

Three fermentations were performed each year: two of them (named LD04-A and LD04-B in 2004, and LD05-A and LD05-B in 2005) were inoculated with commercial yeasts and the third one (denominated LD04-esp in 2004 and LD05-esp in 2005) was allowed to ferment spontaneously. The commercial strains of *S. cerevisiae* used were D47 from Lalvin in 2004 and Excellence B2 from Lamothe-Abiet in 2005. All microvinifications were carried out in a cold room at 18 °C and the fermentation process was monitored by the daily measurement of density and temperature. At the end of fermentation, wines were racked to a new tank and sulphited (30 mg/l). After 2 months of cold stabilization at 4 °C, they were bottled and stored for further analysis.

Microbiological control

Samples for yeast isolation and characterization were taken from must and from each vinification at initial, middle and final stages of fermentation. Each sample was adequately diluted in sterile water and spread on WL nutrient agar [26]. The plates were incubated at 28 °C until visible colonies appeared. After viable yeast counting, 20 colonies were randomly isolated on YPD [1% (w/v) yeast extract, 2% (w/v) peptone, and 2% (w/v) glucose] for further characterization. Lysine medium was used to distinguish *S. cerevisiae* from non-*Saccharomyces* yeasts [19]. *S. cerevisiae* strains were characterized by mtDNA-RFLP analysis as described by Querol et al. [27] using the restriction endonuclease *Hinf*I (Promega). Restriction fragments were separated by electrophoresis on a 0.8% (w/v) agarose gel in TAE buffer (40 mM Tris acetate, 1 mM EDTA), visualized in a transiluminator and photographed [30].

Chemical analysis of wine and must

Musts and wines were analysed according to the official analytical methods [1]. In musts, potential alcohol, total reducing sugars, pH, and total acidity were determined. In wine, the parameters analysed were alcohol content, reducing sugars, total and volatile acidity and pH.

Evaluation of Lado as complementary variety

The wine obtained from Lado in this study was mixed with monovarietal wines from Treixadura and Godello (two grapevine varieties widely employed in Denomination of Origin Ribeiro). The chemical composition of the wines used is specified in Table 4. The mixtures had the following proportions (v/v) of each monovarietal wine: mixture LD–TRX (Lado 1:Treixadura 3), mixture LD–GOD (Lado 1:Godello 3), and mixture LD–TRX–GOD (Lado 1:Treixadura 2:Godello 2).

Wines were submitted to sensorial analysis by a group of five expert testers in order to evaluate the acceptability of wine derived from Lado and its potential as a complementary variety.

Results and discussion

Must composition

The characteristics of musts obtained from Lado grapes in 2004 and 2005 vintages are summarized in Table 1. The results showed that this variety attained a very high probable alcoholic level in 2004 because then, the grapes were overripe at the harvest time. Therefore, in 2005, the grapes were collected earlier, when the potential alcohol was acceptable. However, at this stage of ripening the total acidity was high, compared to the normal values that have been described for Galician wines [31].

Table 1 Characteristics of musts from Lado in 2004 and 2005 vintages

Parameter	Vintage 2004[a]	Vintage 2005[b]
Potential alcohol (%)	14.6	12.7
Sugar content (g/l)	255.4	221.7
pH	3.12	3.14
Total acidity (g/l tartaric acid)	5.7	7.8

The data are mean values of duplicates

[a] Harvest date: 7/10/2004

[b] Harvest date: 27/9/2005

Fermentation kinetics

The progress of fermentations is shown in Fig. 1a, b. All fermentations presented a typical curve, although in 2004 the lag phase was very long (7 days in inoculated fermentations and 11 days in spontaneous fermentation). The rate of spontaneous fermentation was slower compared to the inoculated fermentations. The three fermentations stuck at a density of 995, so musts were not fermented to dryness.

In 2005 the lag phase was only 1 day in inoculated fermentations and 3 days in the spontaneous one. However, once it started, the rate of fermentation was faster in the spontaneous vinification (Fig. 1). The analyses of yeast population showed that the number of viable yeast during the vigorous phase of fermentation (Table 2) was higher in the spontaneous process, which is in agreement with the faster rate of fermentation observed. The duration of all fermentations was about 11 days, and the sugar consumption was almost complete (residual sugars <2 g/l).

Several factors could be responsible for the delay in the onset of fermentation in 2004. It has been reported that stuck and sluggish fermentations were related to high initial concentrations of sugar [8, 16], high SO_2 content [16, 20], and the low number of viable yeast after must clarification [3]. However, it is more likely that the high content of alcohol at the end of the process (close to 15%) is the cause of the premature stopping of these fermentations [3].

Yeast community levels and composition

Yeast levels were low in the clarified must, especially in 2004 (Table 2). Strong clarification techniques, which are regularly adopted in winemaking and which decrease the number of yeasts in must as well as certain nutrients; may lengthen the lag phase of fermentation [3, 16, 25]. In this study the effect was quite drastic in 2004, when fermentations took 7 days to start. In 2005, directed fermentations started one day after inoculation, whereas the spontaneous process was delayed by 3 days until the number of yeast reached 4.2×10^6 cfu/ml. At the middle stage of fermentation, yeast density was similar in inoculated fermentations, but was higher (2.6×10^8 cfu/ml) in the spontaneous fermentation (LD05-esp) and remained stable until the end of fermentation (Table 2). The yeast population in 2004 spontaneous vinification was lower in the middle stage of fermentation and it decreased dramatically towards the end of the process, probably due to yeast settling and the toxic effect of alcohol [3, 7].

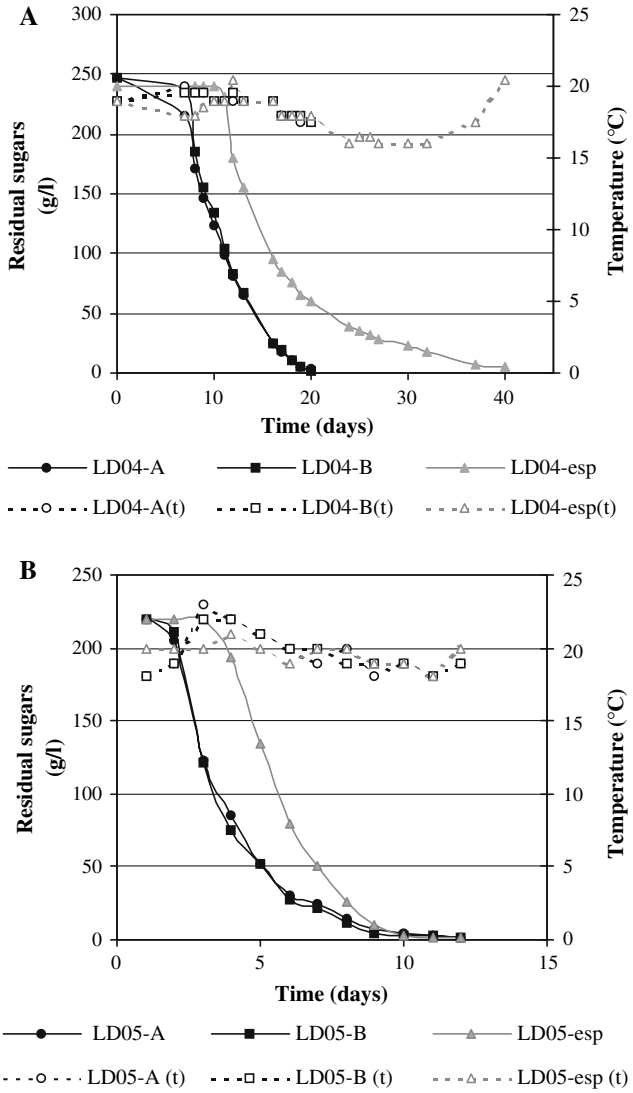

Fig. 1 Evolution of fermentations from Lado in 2004 (**a**) and 2005 (**b**). *Unbroken lines* indicate the variation of density. *Broken lines* represent temperature variation

The implantation ability of commercial yeasts was evaluated by genetic analysis of several yeast colonies isolated at the middle stage of fermentation. The results showed that all strains tested showed identical mtDNA restriction patterns (Fig. 2); therefore, inoculated strains had led those fermentations. Furthermore, it was observed that the genetic profile of commercial strains D47 and B2 was identical with this technique.

Table 2 Yeast population (cfu/ml) in must and at different stages of fermentation

Fermentation	Clarified must	Initial fermentation	Middle fermentation	Final fermentation
LD04-A	2.50×10^2	4.2×10^7	7.1×10^7	
LD04-B	2.50×10^2	1.1×10^7	6.9×10^7	
LD04-esp	2.50×10^2	4.4×10^6	3.3×10^7	1.1×10^5
LD05-A	5.80×10^3		6.2×10^7	
LD05-B	5.80×10^3		8.8×10^7	
LD05-esp	5.80×10^3	4.2×10^6	2.6×10^8	1.9×10^8

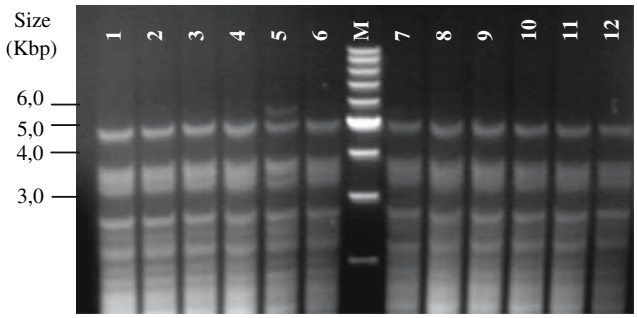

Fig. 2 Genetic characterization of *S. cerevisiae* strains isolated from inoculated fermentations. *M* 1-kb molecular marker (Promega). *Line 1* control, *lines 2–6* and *lines 7–12* yeast strains isolated from fermentations LD05-A and LD05-B, respectively

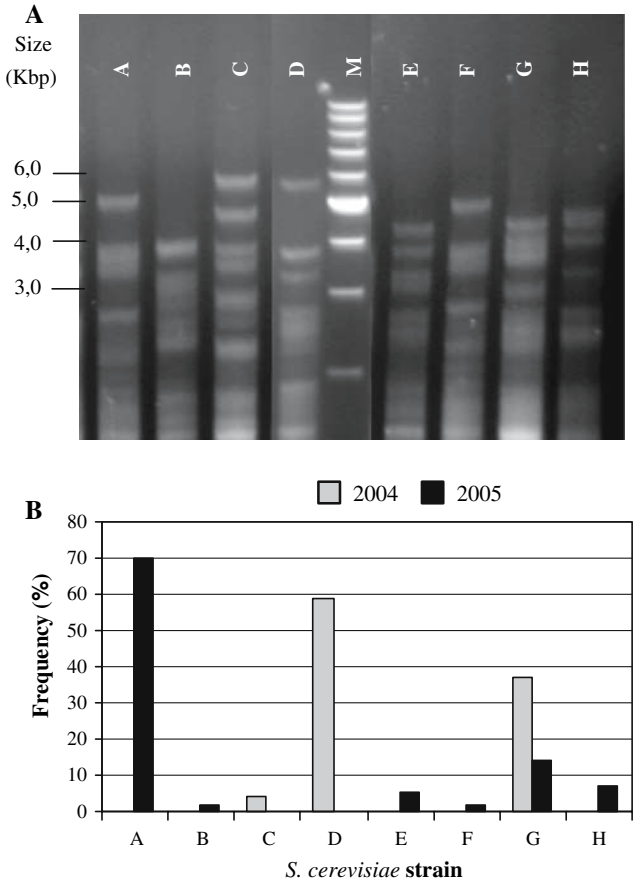

Fig. 3 **a** Genetic profiles (mtDNA-RFLP) of *S. cerevisiae* strains isolated from spontaneous fermentations with Lado must. *M* 1-kb molecular weight marker (Promega). *Lanes A–H* are different strains. **b** Frequency of each strain in 2004 and 2005 fermentations

In addition, yeast strains isolated during spontaneous fermentations were genetically characterized. This approach allowed the identification of only three different strains of *S. cerevisiae* in 2004 and six different strains in 2005. The genetic profile of these strains and the frequency found for each strain are represented in Fig. 3.

In 2004 strain D was the major yeast component, appearing with a frequency of 58%, although strain G reached 37%. The latter could be a strain well adapted to the cellar environment, since it also appeared at 15% in 2005 fermentation, being the only common strain for both years. This strain, mainly isolated from must samples, was substituted by strain D during fermentation.

Spontaneous fermentation carried out in 2005 was clearly dominated by strain A, whose genetic profile was similar to the commercial strains used. In this case, five other secondary strains were identified at frequencies lower than 10%.

The genetic diversity found was low when compared to that reported earlier for the same experimental cellar [5]. Although several factors can affect yeast population, the characteristics of must from Lado (high sugar content and low pH), in conjunction with excessive clarification, are likely to be the main causes for the low diversity observed in our study [16, 25].

Chemical composition of wine

The chemical composition of wines derived from Lado is summarized in Table 3. In general, the wines showed a very high alcohol content and total acidity, whereas the pH (3.0) was low when compared to the standard values described for white wines from Galicia [31]. The wines obtained in 2004 reached an extremely high degree of alcohol (14.8%), although this was in agreement with the amount of sugars and the expected degree recorded for the must (Table 1). In addition, these wines contained residual sugars, probably due to the premature stopping caused by excessive alcohol concentration at the end of fermentation. Wines from 2005 were better balanced. The degree of alcohol was lower than in 2004, and the amount of sugars confirmed that the wines were fermented to dryness. Despite

Table 3 Chemical composition of wines obtained from Lado

Wine	Alcohol content (%)	Reducing sugars (g/l)	Total acidity (g/l)[a]	Volatile acidity (g/l)[b]	pH
LD04-A	14.8	5.2	7.0	0.34	3.09
LD04-B	14.8	5.3	6.9	0.34	3.09
LD04-esp	13.0	6.4	8.1	0.32	2.92
LD05-A	13.7	1.3	7.0	0.20	3.07
LD05-B	13.6	1.2	7.1	0.15	3.05
LD05-esp	13.1	1.2	7.9	0.26	2.90

The data are mean values of duplicates

[a] Expressed as tartaric acid

[b] Expressed as acetic acid

Table 4 Characteristics of monovarietal and mixture wines used in this study

Wines	Alcohol content (%)	Residual sugars (g/l)	Total acidity (g/l)[a]	Volatile acidity (g/l)[b]	pH
LD[c]	13.6	1.2	7.1	0.15	3.05
TRX[d]	11.1	1.0	3.6	0.60	3.51
GOD[e]	13.6	1.6	5.7	0.27	3.23
LD–TRX	11.9	1.0	4.5	0.62	3.43
LD–GOD	13.6	1.5	5.9	0.26	3.21
LD–TRX–GOD	12.7	1.2	5.1	0.47	3.33

The data are mean values of duplicates

[a] Expressed as tartaric acid

[b] Expressed as acetic acid

[c] Lado

[d] Treixadura

[e] Godello

the degree of alcohol reached, the total acidity was high (7.0 g/l) for all wines analysed.

Inoculated fermentations always yielded a higher amount of alcohol than spontaneous ones, indicating that the efficiency of commercial strains, in that the amount of sugar necessary to increase the alcohol content in 1° was less than that required by native yeasts. Conversly, total acidity was higher in spontaneous fermentations. These data indicated that although yeasts with a good capacity for alcohol production are desirable in winemaking, when the grape variety used has a high potential alcohol, strains with lower alcohol yields might be more suitable. Such strains would allow a total consumption of sugar without producing excessive alcohol levels.

Sensory analysis proved that the wines from Lado were not well accepted by testers because of their unbalanced structure and sourness. The chemical properties of wines, joined to the minority character of Lado, make this variety as a good candidate to be used in mixtures with other main grapevine cultivars from Galicia to counteract its acidity.

To evaluate the potential of Lado as a complement, wine from this variety was mixed with monovarietal wines of Treixadura and Godello as indicated in "Materials and methods". The chemical characteristics of wines used and the resulting wines are listed in Table 4. Godello wine presented the same alcohol content as Lado, but the acidity was lower. Therefore, the blend of these varieties yielded a wine with a relatively high alcohol level but with acceptable acidity. With respect to Treixadura, this wine showed an alcohol degree lower than the two previous ones, and a very low acidity. Both parameters increased to more acceptable values when it was mixed with Lado. Finally, a better-balanced wine was obtained when the three varieties were blended, this being also the wine that had better acceptance in sensory analysis.

Acknowledgements This work was supported by the Spanish Government grant VIN03-029 from Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and by FEDER research funds.

References

1. Anonymous (1993) Métodos oficiales de análisis, Tomo II. Ministerio de Agricultura, Pesca y Alimentación, Madrid, Spain
2. Beltran G, Torija MJ, Novo M, Ferrer N, Poblet M, Guillamón JM, Rozes N, Mas A (2002) Analysis of yeast populations during alcoholic fermentation: a six year follow-up study. Syst Appl Microbiol 25:287–293
3. Bisson LF (1999) Stuck and sluggish fermentations. Am J Enol Vitic 50:107–119
4. Blanco P, Orriols I, Losada A, Cortés S (2006) Estudio de la capacidad fermentativa de distintas levaduras autóctonas (*S. cerevisiae*) con mosto Albariño y su influencia en la composición del vino. Tecnol Vino 33:37–43
5. Blanco P, Ramilo A, Cerdeira M, Orriols I (2006) Genetic diversity of wine *Saccharomyces cerevisiae* strain in an experimental winery from Galicia (NW Spain). Antonie Leeuwenhoek 89:351–357
6. Carballeira Lois L, Cortés Diéguez S, Gil de la Peña ML, Fernández Gómez E (2001) SPE-GC determination of aromatic compounds in two varieties of white grape during ripening. Chromatographia 53:350–355
7. Casey GP, Ingledew WM (1986) Ethanol tolerance in yeasts. CRC Crit Rev Microbiol 13:219–280
8. Charoenchai C, Fleet GH, Henschke PA (1998) Effects of temperature, pH, and sugar concentration on the growth rates and cell biomass of wine yeasts. Am J Enol Vitic 49:283–288
9. Constantí M, Poblet M, Arola L, Mas A, Guillamón JM (1997) Analysis of yeast populations during alcoholic fermentation in a newly established winery. Am J Enol Vitic 48:339–344
10. Egli CM, Edinger WD, Mitrakul CM, Henick-Kling T (1998) Dynamics of indigenous and inoculated yeast populations and their effects on the sensory character of Riesling and Chardonnay wines. J Appl Microbiol 85:779–789
11. Esteve-Zarzoso B, Manzanares P, Ramón D, Querol A (1998) The role of non-*Saccharomyces* yeasts in industrial winemaking. Int Microbiol 1:143–148

12. Falqué E, Fernández E, Dubourdieu D (2001) Differentiation of white wines by their aromatic index. Talanta 54:271–281
13. Falqué E, Fernández E, Dubourdieu D (2002) Volatile components of Loureira, Dona Branca and Treixadura wines. J Agric Food Chem 50:538–543
14. Fernández B, Iglesias I, Rego F, Cid N (2005) Caracterización ampelográfica de diferentes variedades de vid cultivadas en Galicia. Tecnol Vino 27:39–47
15. Fleet GH (2003) Yeast interactions and wine flavour. Int J Food Microbiol 86:169–180
16. Fleet GH, Heard GM (1993) Yeasts-growth during fermentation. In: Fleet GM (ed) Wine microbiology and biotechnology. Harwood Academic Publishers, Chur, pp 27–54
17. Freijanes J, Alonso MP (1997) Variedade Lado. In: Xunta de Galicia, Videiras galegas (eds) Catálogo de variedades autóctonas. Santiago de Compostela, pp 37–39
18. Gil JV, Mateo JJ, Jimenez M, Pastor A, Huerta T (1996) Aroma compounds in wine as influenced by apiculate yeasts. J Food Sci 61:1247–1249
19. Heard GM, Fleet GH (1986) Evaluation of selective media for enumeration of yeasts during wine fermentation. J Appl Bacteriol 60:477–481
20. Henick-Kling T, Edinger W, Daniel P, Monk P (1998) Selective effects of sulfur dioxide and yeast starter culture addition on indigenous yeast populations and sensory characteristics of wine. J Appl Microbiol 84:865–876
21. Herraiz T, Reglero G, Herraiz M,Martin-Alvarez P, Cabezudo M (1990) The influence of the yeast and type of culture on the volatile composition of wines fermented without sulfur dioxide. Am J Enol Vitic 41:313–318
22. Lambrechts MG, Pretorius IS (2000) Yeast and its importance to wine aroma—a review. S Afr J Enol Vitic 21:97–129
23. Lema C, García-Jares C, Orriols I, Angulo L (1996) Contribution of *Saccharomyces* and non-*Saccharomyces* populations to the production of some components of Albariño wine aroma. Am J Enol Vitic 47:206–216
24. Mateo JJ, Jimenez M, Huerta T, Pastor A (1991) Contribution of different yeasts isolated from musts of Monastrell grapes to the aroma of wine. Int J Food Microbiol 14:153–160
25. Mora J, Mulet A (1991) Effects of some treatments of grape juice on the population and growth of yeast species during fermentation. Am J Enol Vitic 42:133–136
26. Pallmann CL, Brown JA, Olineka TL, Cocolin L, Mills DA, Bisson LF (2001) Use of WL medium to profile native flora fermentations. Am J Enol Vitic 52:198–203
27. Querol A, Barrio E, Ramón D (1992) A comparative study of different methods of yeast strain characterization. Syst Appl Microbiol 15:439–446
28. Romano P, Fiore C, Paraggio M, Caruso M, Capace A (2003) Function of yeast species and strains in wine flavour. Int J Food Microbiol 86:169–180
29. Romano P, Suzzi G, Comi G, Zironi R (1992) Higher alcohol and acetic acid production by apiculate wine yeasts. J Appl Bacteriol 73:126–130
30. Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbour Laboratory Press, New York
31. Vázquez I, Soto E, Rodríguez JM, Losada A, Orriols I (2006) Aproximación a las características de los vinos de las DO Gallegas. Tecnol Vino 29:46–51
32. Versini G, Orriols I, Dalla Serra A (1994) Aroma components of Galician Albariño, Loureira and Godello wines. Vitis 33:165–170
33. Vilanova M, Sieiro C (2006) Contribution by *Saccharomyces cerevisiae* yeast to fermentative flavour compounds in wines from cv. Albariño. J Ind Microbiol Biotechnol 33:929–933