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Volatile compounds produced in wine by Colombian wild *Saccharomyces cerevisiae* strains

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Abstract - Some tropical *Saccharomyces cerevisiae* strains, isolated from *Champús*, a traditional Colombian low alcoholic fermented beverage, were characterised in order to select yeasts for aroma improvement in wine. H_2S production, volatile acidity, β-glucosidase activity, higher alcoholesters and terpenes production were evaluated in this study. These tropical strains were characterised by a considerable production of ethyl hexanoate, 2-phenylethanol, 2-phenylethyl acetate, and geraniol, detected by SPME-GC-MS. Odor activity values were calculated to analyse the effects of yeasts strains on wine aroma, resulting in six distinctive wine groups, as evidenced by discriminant analysis. These results suggest that *Saccharomyces* strains isolated from *Champús* can be an important source for new tropical yeast biotypes with potential winemaking applications, producing a wide range of aroma compounds.

Key words: *Saccharomyces cerevisiae*; volatile compounds; wine; *Champús*; tropical yeasts.

INTRODUCTION

Fruits are important microhabitats for a variety of yeasts species, due to the high concentration of simple sugars, low pH and intense visitation by insect vectors (Lachance and Starmer, 1998). Tropical fruits are a natural reservoir of high biodiversity in terms of yeasts species and strains. In fact, the selective pressure exerted by the environmental conditions like temperature fluctuations between day and night, exposure to desiccation and high concentration of solutes, accounts for the consolidated dominance of particular yeasts species with a high degree of adaptability (Ribeiro *et al.,* 1999), capable of metabolizing a great variety of substrates (Ezeronye, 2004). Yeasts produce and modify important constituents of fermented beverages such as volatile organic acids, aldehydes, alcohols, and esters; terpenes, too, can be modified by β-glucosidase produced by some yeasts (Carrau *et al.,* 2005).

The addition of starter cultures in wine production has become a common practice, as a consequence of the development of methods for the isolation of pure yeasts strains, but also due to performance improvement, obtained by classical genetic methods and genetic engineering (Donalies *et al.,* 2008). Researchers and wine-makers are particularly interested in the selection and use of autochthonous selected strains of *S. cerevisiae* as starters, as these yeasts are better acclimated to specific micro-area conditions and grape must composition, and can dominate more easily on natural yeasts, emphasizing the typical sensory quality of wine (Di Maro *et al.*, 2007). Moreover, ecological studies on yeasts in products other than beer, bread and wine, may also provide the knowledge base for developing a new generation of starter cultures (Fleet, 2007). On the other hand, it has been proposed to upgrade the strategies of isolation and screening through an appropriate choice of suitable habitats, taking into account different selective pressures, favouring either biodiversity (expressed as a high number of species) or metabolic diversity (expressed as a high number of metabolic phenotypes, such as VOC phenotypes) (Buzzini *et al.,* 2003).

In this study, tropical *Saccharomyces cerevisiae* strains, isolated from *Champús*, a traditional Colombian low alcoholic fermented beverage (Osorio-Cadavid *et al.,* 2008), were characterised for the production of some secondary metabolic compounds of fermentation during their growth in grape must, to select strains producing particular aromatic notes in wine. In *Champús* (made with Colombian tropical fruits, sugar cane and corn), these strains produced high levels of esters, especially amyl acetate, 2-phenylethyl acetate and ethyl caprylate, which are mostly responsible for the flowery and fruity aromas (Osorio-Cadavid *et al.,* 2008).

MATERIALS AND METHODS

Strains source. A total of nine *Saccharomyces cerevisiae* strains isolated from Colombian *Champús*, belonging to the collection

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of Dipartimento di Science degli Alimenti of the University of Teramo, Italy, were studied. In a previous research (Osorio-Cadavid *et al.,* 2008), these strains were selected for their ability to form high percentages of esters in a medium containing sugar cane. A commercial *Saccharomyces cerevisiae* L404 strain (Oliver Oger, Verona, Italy) was used as control. The yeasts cultures were kept at -80 °C in liquid Yeast Extract Peptone Dextrose medium (YEPD, Oxoid, Basingstoke, UK), added with glycerol (25% v/v) as a cryoprotectant.

Phenotypic characterisation of yeasts. The capacity of the strains to grow at 37 and 42 °C was determined after 2 and 7 days in YEPD tubes, incubated in a thermostat bath. The ability to grow in presence of 40% glucose was evaluated according to Kurtzman and Fell (1999).

β-glucosidase activity was determined by replica plating onto arbutin (Sigma-Aldrich, Milan, Italy) agar after 3 and 7 days; colonies with a positive activity developed a dark brown colour. The ability to produce H_2S was evaluated by two qualitative methods: lead acetate reaction and colony appearance on Biggy agar (Oxoid) according to Vincenzini *et al.* (2005).

Microvinification. Fermentation of red grape must *Montepulciano* cultivar, containing 20% (w/v) fermentable sugars, total acidity 6 g l^{-1} as tartaric acid, pH 3.5, was carried out in 500 ml Erlenmeyer-flasks filled with 450 ml of must. Must sterilization was performed according to Gonzáles *et al.* (2007), inoculating 48 h pre-cultures grown in the same must, up to a final concentration of 10^6 cell ml^{-1}. Potassium metabisulfide was added at a concentration of 70 mg l^{-1} of SO_2 before fermentation. Grape must surface was covered with a thin layer of sterilized paraffin oil to avoid air contact. Alcoholic fermentation was carried out at 22 °C for 20 days, determining the weight loss caused by CO_2 evolution. Fermentation was considered finished when the weight remained constant. Samples were centrifuged at 5000 *x g* for 10 min and aliquots of 5 ml were transferred into 10 ml screw-capped glass vials, capped with a rubber septum and stored at -20 °C until analysed. Each fermentation was carried out in duplicate.

Reducing sugars, alcohol and volatile acidity were determined according to the methods described in the EU Official Gazette (1990).

Solid phase microextraction – Gas chromatography (SPME-GC) analysis of volatile compounds. The volatile compounds were determined by solid phase microextraction coupled with gas chromatography (SPME-GC), according to Mallouchos *et al.* (2002). Five ml of wine samples were placed in 10 ml glass vials, added with 1 g NaCl and 10 µl of 4-methyl-2-pentanol (4 mg l^{-1}) as internal standard (IS). Both equilibration and absorption were carried out under stirring conditions. The fibre used for SPME was coated with a 85 µm polyacrylate (PA) film (Supelco, Bellerofonte, PA, USA).

Quantitative determination was performed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), coupled with an Agilent 5970 mass selective detector operating in electron impact mode (ionization voltage, 70 eV). The fused silica capillary column was CP-Wax 52 CB (50 m x 0.32 mm) by Crompack (Middelburg, The Netherlands), coated with polyethyleneglycol (film thickness 1.2 µm) as a stationary phase. Injector, interface, and ion source temperatures were 250, 250, and 230 °C, respectively. Mass range was 30-400 m/z. Injection was performed in splitless mode, and helium (1 ml min^{-1}) was used as carrier gas. The temperature program was as follows: initial temperature 50 °C held for 2 min; first ramp, 1 °C min^{-1} to 65 °C (2 min hold); second ramp, 10 °C min^{-1} to 150 °C (10 min hold); third ramp 10 °C min^{-1} to 200 °C (1 min hold). The carrier gas (N_2) flow rate was 2.5 ml min^{-1}. Peaks were identified comparing their mass fragmentation with those of pure standards and those available in mass spectra databases (NIST/EPA/NIH version, 1998 and Wiley version, 1996). Quantitative determination was obtained by dividing the peak areas of the compounds by the peak area of the IS, and multiplying this ratio by the initial IS concentration (expressed as mg l^{-1}). These values were corrected using a correction factor calculated for each compound with respect to the IS in a synthetic wine solution (5 g l^{-1} of tartaric acid, dissolved in 12% ethanol solution (v/v) at pH 3.4 adjusted with NaOH). The peak areas were measured in the full scan chromatograph, using total ion current (TIC), considering that no significant co-eluting peaks were observed in the samples. Each determination was carried out in duplicate. Data presented are the means of four determinations (two determinations in two different experiments).

Odour activity values. In order to assess the influence of the single aromatic compounds produced during fermentation, Odour Activity Values (OAV) were calculated by dividing the concentration of each compound by its perception threshold, as reported by Garde-Cerdán and Ancín-Azpilicueta (2006). Theresholds were the same used by Gil *et al.* (2006) and Sánchez Palomo *et al.* (2007).

Statistical analysis. One-way analysis of variance (ANOVA) and test of comparison of means (Tukey) were used to interpret mean differences in mean values, at 95% accuracy level, and principal component analysis (PCA) was performed to examine the relationship among the variables.

The presence of classes within the wines samples was investigated by linear discriminat analysis (LDA). A basic problem in LDA is deciding which variables should be considered. This was achieved by a stepwise LDA using Wilk's lambda as the selection criterion and F-statistic, to determine the significance of the changes of lambda when a new variable is tested. All calculations were performed using STATISTICA (statSoft, Tulsa, OK, USA) package.

RESULTS

Determination of oenological characteristics of Colombian *Saccharomyces cerevisiae* strains

Different characteristics should be considered to select yeast strains for oenological purposes, as reported by Esteve-Zarzoso *et al*. (2000). The characterisation of the strains isolated from *Champús* was based on the following criteria: fermentation kinetics in grape juice, growth at 37 and 40 °C, resistance to sulfur dioxide, low hydrogen sulfide production, β-glucosidase activity and production of volatile compounds.

Table 1 shows that all the strains were able to grow at 37 °C, whereas only 1 strain could grow at 42 °C and two strains were positive to arbutin hydrolysis. All the strains were able to consume all sugar in the medium and the fermentation kinetics were comparable with that of the commercial strain L404. As expected, all the strains produced high-alcohol wines, ranging from 10.8 to 12.6%, with a low volatile acidity (from 0.45 to 0.52 g l^{-1}, expressed as tartaric acid). Moreover, they produced very low quantities of H_2S and were able to grow in must added with 70 mg 1^{-1} of sulfur dioxide.

TABLE 1 - Some characteristics of *Saccharomyces cerevisiae* isolated from Colombian *Champús*

Strain	Fermentation of			Arbutin hydrolisis	H_2S production	Growth at			
	Glucose	Maltose	Sucrose			10% Ethanol	37 °C	40 °C	40% Glucose
CHL86	+	–	+	–	1	+	+	–	+
CHL752	+	–	+	–	2	–	+	–	+
CHL1112	+	+	+	+	3	+	+	–	+
CHL1132	+	+	+	–	2	+	+	+	+
CHL1182	+	+	+	–	1	+	+	–	+
CHL512	+	+	+	–	1	–	+	–	+
CHL732	+	+	+	+	1	–	+	–	+
CHL742	+	+	+	–	1	+	+		+
CHL772	+	+	+	–	2	–	+	–	+

* Measure of H_2S production according to Vincenzini *et al.,* 2005 (1: low, 5: high production).

Volatile secondary compounds production

The mean values of volatile compounds in the wines fermented by the *S. cerevisiae* strains from Colombian *Champús* are given in Table 2.

Significant differences were observed in the production of higher alcohols by *Champús* strains, with amounts ranging from 124 to 339 mg l^{-1}, while the commercial strain L404 produced 265.78 mg l^{-1}. In terms of higher alcohols, with possible effects on wine aroma, the ideal concentrations (140 to 210 mg l^{-1}) were produced by the following strains: CHL512, CHL742, CHL1112 and CHL86. All the strains produced low quantities of n-propanol (from 3.73 to 19.21 mg l^{-1}), except CHL752. Significant differences were observed among the wines for isobutanol, as for n-propanol, with quantities ranging from 3.83 mg l^{-1} (CHL1112) to 38.49 mg l^{-1} (CHL732).

Considering isoamyl alcohols, the highest amounts were found in the wines produced by the strains CHL732 and CHL1112 (258.66 and 177.61 mg l^{-1}, respectively), while two strains produced amounts below 80 mg l^{-1}. Five strains produced high quantities of 2-phenylethanol, with amounts ranging from 80.20 to 117 mg l^{-1}.

Esters production by these tropical strains differed from strain to strain, both in average production and in the relative proportions of each compound. In fact, significant differences ($p < 0.05$) in esters production were observed among all the strains, with the exception of diethyl malonate. The largest ester producers were the strains CHL1132 and CHL1182, not considering ethyl acetate production which exerts a different effect on wine aroma. On the other hand, a high variability ($p < 0.05$) was observed in the production of 2-phenylethyl acetate, with values ranging from 0.22 to 4.06 mg l^{-1}, whereas isoamyl acetate was produced in low concentrations, ranging from 0.10 to 2.18 mg l^{-1}. As regards the production of esters of medium chain fatty acids, most of the Colombian strains produced high quantities of ethyl hexanoate, reaching values up to 2.52 mg l^{-1}. Ethyl decanoate was only detected in the wines fermented by the strains CHL1112, CHL752 and CHL86, and was not produced by the control strain L404. Ethyl lactate and diethyl succinate were present in the wines, in amounts ranging from 0.62 to 6.07 mg l^{-1} and from 0.12 to 0.84 mg l^{-1}, respectively. Diethyl malonate was observed at similar concentrations in all the wines (about 0.30 mg l^{-1}), except those obtained from CHL1112, where a lower content was observed (0.04 mg l^{-1}), and CHL512, with the highest value of 0.51 mg l^{-1}. Ethyl acetate was produced at concentrations ranging from 8.58 to 32.78 mg l^{-1}. The ratio esters/higher alcohols is known to influence the sensory properties of fermented beverages. In particular, wines with a high content of esters possess an enhanced fruity flavour, that can be improved if the higher alcohol content decreases (Valero *et al*., 2002). Table 2 shows that the best ratio was performed by CHL1182, producing a wine with a low higher alcohol content and a high quantity of esters.

The main monoterpene was geraniol, with the strain CHL752 being the highest producer (0.39 mg l^{-1}). Citronellol was detected in the experimental wines produced by CHL512 and CHL86.

The volatile compounds produced by the Colombian strains were analysed using PCA, in order to describe the data set. Firstly, the correlation matrix was computed in order to discriminate the variables, thus selecting eight parameters (isoamyl alcohol, 2-phenylethanol, ethyl lactate, ethyl octanoate, ethyl decanoate, diethyl malonate and geraniol). Two factors with eigenvalues greater than 1 were computed, accounting for 73.69% of the variance. Figures 1 and 2 show the ten wines on the plane defined by the two principal components. The first principal component (PC1) accounts for 44.91% of the total variance and correlates with 2-phenylethanol, ethyl octanoate and isoamyl alcohol, with the last two variables having the higher negative loading on this component. The second principal component (PC2) is closely related to ethyl decanoate (high positive loadings), diethyl malonate and ethyl lactate (high negative loading) (Fig. 2).

PCA showed three clearly differentiated groupings: Group I was formed by the wines produced by CHL1132, CHL1182, CHL772, CHL752, CHL742, CHL86, Group II was formed by the wines produced by CHL1112, which was the highest producer of ethyl decanoate and geraniol, and by CHL732, the highest producer of isoamyl alcohol and diethyl malonate. The wines obtained using the commercial strain gathered in Group III (Fig. 1).

Olfactory impact of volatile secondary compounds

OAVs were calculated to evaluate the influence of yeasts strains on wine aroma. Although this method has some limitations it has been found useful in practice (Frijters, 1979). On the basis of odour description and threshold, the most powerful odorants were tentatively established, and only those displaying OAVs greater than 1 were deemed to contribute to wine aroma (Mingorance-Cazorla *et al.,* 2003). In this work only the compounds at OAVs values ≥ 1 were considered. The most important active odorant compounds were: isoamyl alcohol, 2-phenyl ethanol, ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and phenyl ethyl acetate, and geraniol (Table 3).

The stepwise LDA, performed using OAV as a classification variable (Fig. 3), gave high recognition percentages for the clas-

TABLE 2 - Mean of volatile compounds (mg l^{-1}) in wines produced by *Saccharomyces cerevisiae* isolated from Colombian *Champús*

Volatile compounds	Strains									
	CHL732	CHL1112	CHL772	CHL752	CHL512	CHL742	CHL 1182	CHL1132	CHL86	Commercial strain 404
n-Propanol	12.68^{a}	7.25^{b}	19.21^{c}	66.92^{d}	3.73^{e}	5.46^{e}	9.35^{b}	6.53^{be}	12.03^{g}	26.20^{f}
Isobutanol	38.49^{a}	3.83^{b}	8.11^{c}	4.23^{b}	8.95^{c}	5.70^{b}	8.16^{c}	12.14^{d}	16.57^{f}	14.42^{f}
Isoamylic alcohol	258.66^{a}	177.61^{b}	93.14^{c}	122.43^{d}	93.^{77c}	119.35^{d}	60.65^{e}	108.42^{e}	48.10^{f}	205.02^{g}
2-Phenylethyl alcohol	29.76^{a}	15.86^{b}	117.11^{c}	99.75^{d}	80.20^{e}	26.75^{f}	46.10^{g}	105.56^{h}	102.08^{dh}	20.14^{b}
Total higher alcohols	339.59	204.55	237.57	293.33	186.65	157.26	124.26	232.65	179.05	265.78
Ethyl acetate	20.12^{a}	14.02^{ac}	11.0^{ab}	8.58^{b}	10.58^{b}	13.15^{ac}	27.30^{d}	23.63^{de}	32.78^{d}	61.27^{f}
Isoamyl acetate	0.^{44a}	0.38^{a}	0.08^{b}	0.20^{c}	0.14^{c}	0.31^{d}	2.18^{e}	0.57^{a}	tr	0.26^{c}
2-Phenylethyl acetate	0.38^{a}	1.29^{b}	1.30^{b}	0.60^{c}	0.22^{a}	1.13^{b}	1.65^{b}	4.06^{d}	0.32^{a}	0.04^{i}
Ethyl hexanoate	1.73^{a}	0.56^{b}	2.07^{c}	0.33^{d}	0.64^{b}	1.57^{a}	2.52^{c}	0.58^{b}	0.95^{d}	1.12^{e}
Ethyl octanoate	0.22^{a}	1.21^{b}	0.37^{c}	0.42^{c}	0.05^{e}	0.21^{a}	0.21^{a}	0.11^{d}	0.02^{e}	1.18^{b}
Ethyl decanoate	tr	1.46^{a}	n.d	0.53^{b}	tr	tr	n.d	n.d	0.03^{c}	n.d
n-Amyl isovalerate	n.d	n.d	0.34	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Ethyl lactate	2.35^{a}	1.13^{b}	2.20^{a}	1.15^{b}	1.11^{b}	5.70^{c}	2.87^{a}	3.72^{d}	0.91^{b}	8.17^{e}
Diethyl succinate	0.33^{a}	0.28^{a}	0.16^{b}	0.48^{c}	0.14^{b}	0.12^{b}	0.18^{b}	0.21^{b}	0.84^{d}	0.56
Diethyl malonate	0.25^{a}	tr	0.27^{a}	0.28^{a}	0.51^{c}	0.30^{a}	0.35^{a}	0.25^{a}	0.23^{a}	0.99^{d}
Total esters	25.82	20.33	17.19	12.57	13.39	22.49	40.26	33.13	36.26	73.59
ΣEsters/ΣHigher alcohols	7.6	9.93	7.2	4.2	7.4	14.30	32.46	32.39	20,25	28.65
Geraniol	0.26^{a}	0.20	0.11	0.39	0.27	n.d	0.05	n.d	n.d	n.d
Citronellol	n.d	0.19	n.d	n.d	0.21	n.d	n.d	n.d	n.d	n.d

* Mean value ± standard deviation. Different letters in the same row mean significant differences at $p > 0.05$ (T-test). n.d: not detectable with the used method; tr: traces.

TABLE 3 - Perception threshold and Odour Activity Values (OAVs) for volatile compounds in wines obtained from strains of *Saccharomyces cerevisiae* isolated from Colombian *Champús*

Volatile compounds	Perception threshold (mg l^{-1})*	Odour description	Strains									
			CHL732	CHL1112	CHL772	CHL752	CHL512	CHL742	CHL 1182	CHL1132	CHL86	Commercial strain 404
Isoamylic alcohol	30^{a}	Solvent	8.62	5.92	3.10	4.08	3.13	3.98	2.02	1.95	1.60	6.83
2-Phenylethanol	14^{a}	Rose	2.13	1.13	8.36	7.13	5.73	1.91	3.29	7.54	7.29	1.44
Ethyl acetate	12.26^{a}	Fruity, solvent, balsamic	1.64	1.64	1.14	0.90	0.70	0.86	1.07	2.23	1.93	2.68
Isoamyl acetate	0.030^{a}	Fruity, banana, sweet	123.21	40	147.50	23.57	45.71	111.79	179.64	41.07	67.86	67.25
Ethyl hexanoate	0.014^{a}	Fruity,green apple	43	254	74	83	9	42	42	21	4.14	236.19
Ethyl octanoate	0.005^{a}	Fruity, pineapple, peach,pear	–	4.90	–	–	–	–	–	–	0.17	–
Ethyl decaonoate	0.200^{a}	Sweet, fruity, dry fruits	14.67	12.50	2.50	6.67	4.50	9.33	72.50	19.00	–	8.59
Phenyl ethyl acetate	0.250^{a}	Pleasant, flowery with a honey note	1.50	5.14	5.08	2.38	0.88	4.50	6.60	16.24	1.28	0.18
Geraniol	0.030^{b}	Citric, Orange flowers	6.39	4.98	2.63	9.76	6.79	–	1.13	–	–	–

* Thresholds from ^{a} Gil *et al*., 2006, ^{b} Sánchez Palomo *et al*., 2007.

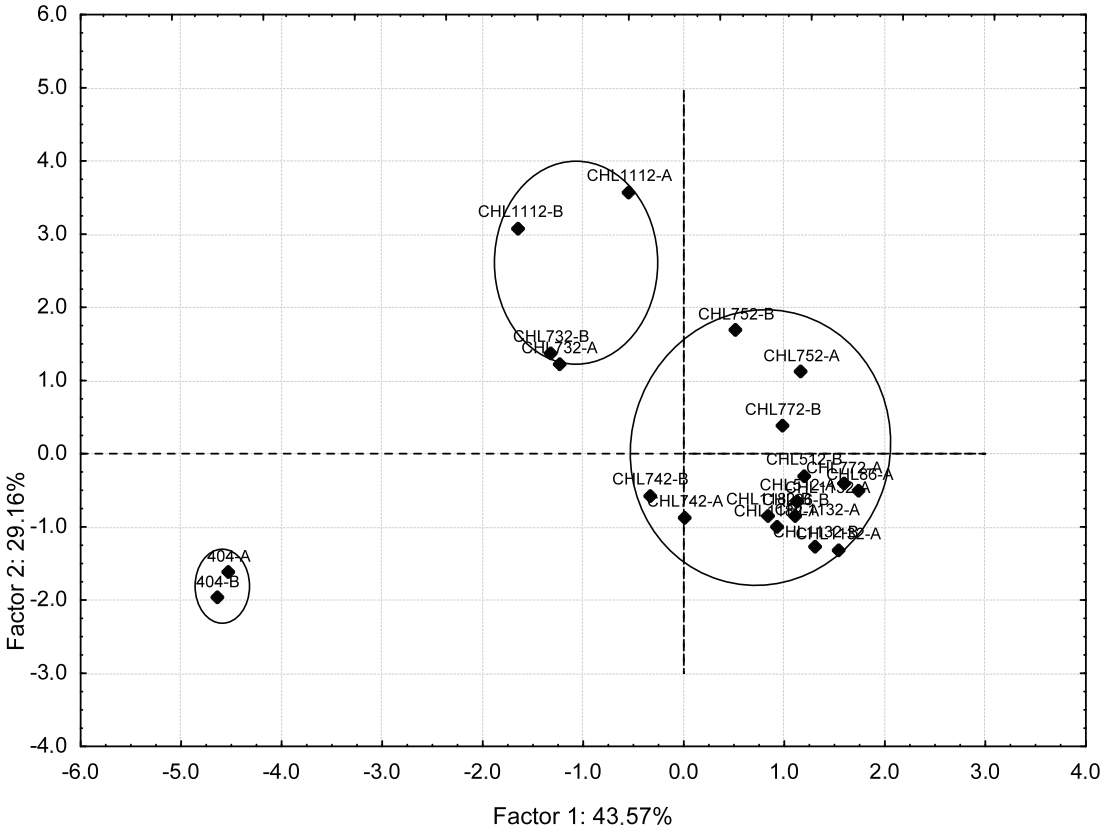

FIG. 1 - Principal Component Analysis scores for *Montepulciano* must fermented with different tropical yeast strains.

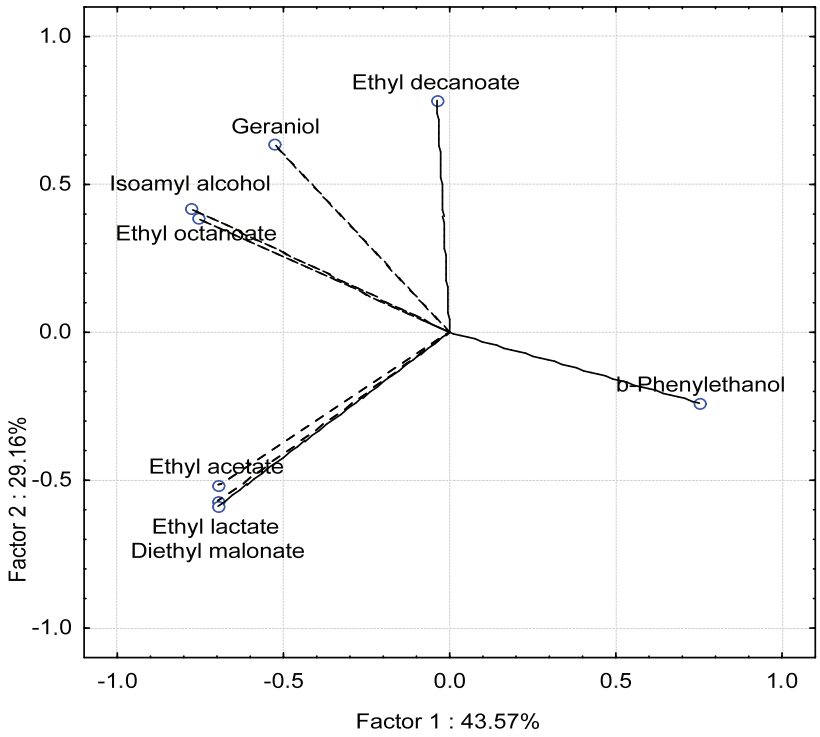

FIG. 2 - Plot of the samples of *Montepulciano* wines, produced using tropical strains of *Saccharomyces cerevisiae*, on the plane defined by the two first principal components from data of aroma compounds.

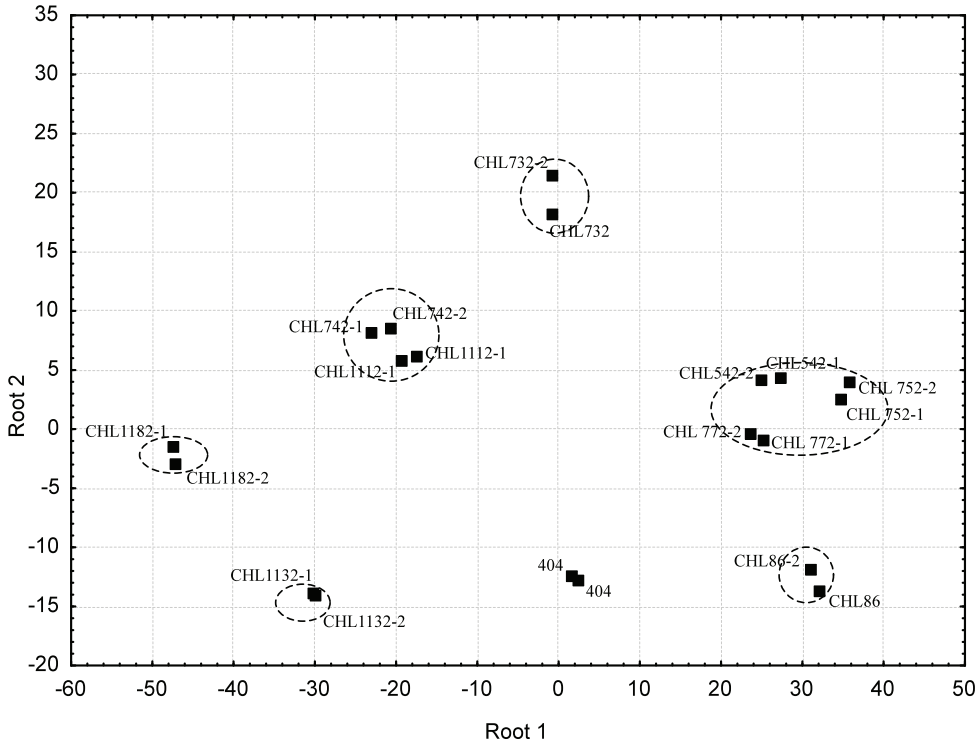

FIG. 3 - Stepwise Discriminant Analysis of the Odour Activity Values (OAVs) obtained from the wines produced by the different tropical *Saccharomyces cerevisiae* strains.

sification of the wine samples according to the strains, reaching recognition levels of 100%, although a tendency to grouping was observed for some strains, such as CHL772, CHL752 and CHL542.

According to standard coefficients, the best discriminating attributes were rose notes, apple notes, flowery notes, fruity notes and banana notes. The rose notes (from 2-phenylethanol), apple notes (from ethyl hexanote) and flowery notes (from phenylethyl acetate) showed the highest SCC (Standard Canonical Coefficients) with the first Canonical Variable (CV1). As a consequence, these volatiles can be considered important for the discrimination between the strains along CV1. On the other hand, fruity notes (from ethyl acetate isoamyl alcohol) showed a significant contribution to CV2.

DISCUSSION

Very little information is available on the volatile profiles of tropical microbiota, as previous research focussed mainly on strains identification. In this study, the production of esters, higher alcohols and terpenes by Colombian *Saccharomyces* strains during *Montepulciano* must fermentation was investigated.

Tropical yeasts usually grow at high temperatures; daily temperature fluctuations exert a selective pressure on many metabolic features, such as thermotolerance and formation of volatile and non volatile compounds (Morais *et al.,* 1992). Some *S. cerevisiae* strains isolated from Colombian *Champús* were characterized by the following characteristics: growth at 37 °C, low production of ethyl acetate, high production of 2-phenylethyl acetate and 2-phenylethanol. The latter compound, possessing a delicate fragrance of rose petals, is commonly formed in the early fermentation steps (Mallouchos *et al.,* 2002) and is deemed one of the most important aromatic alcohols contributing to wine flavour. Four of the tropical strains considered in this study produced this compound in quantities ranging from 71 mg l^{-1} (CHL512) to 113.91 mg l^{-1} (CHL772), which were four times higher than the quantity produced by the commercial strain L404 in wine. The ability to produce high levels of 2-phenylethyl alcohol may be related to a higher capacity to utilise the assimilable nitrogen in must (Torrea *et al.*, 2003). In a previous study (Clemente-Jiménez *et al.,* 2005), an unusual production of 2-phenylethanol (189 mg l^{-1}) was reported for a *S. cerevisiae* strain isolated from a particular ecosystem, characterized by a low rain fall, many hours of sunlight, humidity, and cold air.

As regards n-propanol, some authors (Giudici and Kunkee, 1994) related its content in wine to a high SO_2 production by yeasts, suggesting that the capacity to form *n*-propanol might be closely linked to the inability to produce H_2S; on the contrary, it has been reported that the largest producers of H_2S formed the highest quantities of *n*-propanol (Regodón Mateos *et al.,* 2006). In our tropical strains, the differences in *n*-propanol production could not be related to H_2S production.

The major flavour-active esters in wine are acetate esters such as ethyl acetate, isoamyl acetate, hexyl acetate and 2-phenylethyl acetate. Moreover, C:6-C:10 medium-chain fatty acid ethyl esters, such as ethyl hexanoate (ethyl caproate) and ethyl octanoate (ethyl caprylate), are also important for the overall bouquet. In the experimental *Montepulciano* wines, the amounts of 2-phenylethyl acetate (flowery flavour with a honey note) produced by the tropical strains were higher with respect to the commercial strain 404 and to amounts reported by other authors (from 0.12 to 1.51 mg l^{-1}) (Rojas *et al.,* 2003; Torrea *et al.,* 2003; Garde-Cerdán and Ancín-Azpilicueta, 2006; González *et al.,* 2007). In addition, the tropical strains produced significant quantities of ethyl hexanoate (apple-like aroma). Our data

indicate that the strains CHL772 and CHL86 might be useful to obtain new aromatic profiles in wine.

The other interesting characteristic of these tropical *S. cerevisiae* strains was the presence of extracellular β-glucosidase. Glucosidases exert an important role in must fermentation, due to their capacity to hydrolyse a considerable portion of monoterpenes that occur in bound forms, and particularly glucosides. These compounds do not seem to contribute to aroma, unless they are hydrolysed and released. The glucosidically-bound forms can be hydrolysed by glucosidases to free odourous forms (linalool, nerol, geraniol, α-terpineol and citronellol). For this reason, the two arbutine-positive strains were expected to produce high levels of free terpenols; actually, five strains increased the quantity of free terpenols, such as geraniol (orange flowers), in the fermented must. The differences observed in geraniol concentration might be related to the different glucosidase activity, as well as to the different consumption of free geraniol by yeasts. In fact, this compound is drastically reduced during alcoholic fermentation, due to its transformation into linalool, β-terpenol, and citronellol (Zoecklein *et al*., 1997; Vaudano *et al.*, 2004). However, the very small quantities of citronellol detected in the different wine samples might be due to the anaerobic conditions, exerting a negative influence on the production of this compound (Vaudano *et al.,* 2004).

Although it is well known that the sensory characteristics of wines generally derive from many chemical compounds acting in concert, *Champús* strains showed an interesting ability to influence the aromatic profile of *Montepulciano*.

Most of these strains were characterized by a high ratio esters/higher alcohols, as well as by the production of high quantities of 2-phenylethanol and phenylethyl acetate; the latter characteristic may be considered responsible for an increase of flowery aroma in wine, based on OAV data. The analysis of the olfactory impact of the secondary volatile compounds produced during wine fermentation was useful to screen the aromatic traits of the tropical *S. cerevisiae* from *Champús*, although a series of interactive phenomena, such as additive, synergistic or suppressive effects, may occur among the different compounds detected in wine.

This study suggests the possibility of selecting new starters from the tropical environment, with the aim of producing wines with distinct flowery or fruit aroma, lower H_2S production and low acidity. Further research is needed to assess the impact of technological conditions on the expression of such aromatic traits during wine manufacturing.

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