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Effect of aromatic precursor addition to wine fermentations carried out with different *Saccharomyces* species and their hybrids

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ABSTRACT

This work explores the ability of different yeast strains from different species of the genus *Saccharomyces* (*S. cerevisiae*, *S. uvarum* and *S. kudriavzevii*) and hybrids between these species to release or form varietal aroma compounds from fractions of grape odourless precursors. The *de novo* synthesis by the yeasts of some of the varietal aroma compounds was also evaluated. The study has shown that *de novo* synthesis affects some lipid derivatives, shikimic derivatives and terpenes in all species and hybrids, with some remarkable differences amongst them. The release or formation of aroma compounds from precursors was found to be strongly linked to the yeast or hybrid used, and the triple hybrid *S. cerevisiae* × *S. bayanus* × *S. kudriavzevii* in particular and secondarily the hybrid *S. cerevisiae* × *S. bayanus* were highly efficient in the production of most varietal aroma compounds, including γ-lactones, benzenoids, volatile phenols, vanillin derivatives and terpenols. The presence of precursors in the fermenting media caused a surprising levelling effect on the fermentative aroma composition. Altogether, these results suggest that it is possible to modulate wine aroma by employing different yeast species in order to create new wines with different aromatic notes.

1. Introduction

From a chemical point of view, the volatile fraction of wines is mainly composed of higher alcohols and esters formed by yeast secondary metabolism, but there are many other wine aroma compound formation in which yeasts play a relevant role. The so called wine primary or grape-varietal aroma consists of lactones, benzenes, volatile phenols, vanillins, norisoprenoids, terpenes and some polyfunctional mercaptans present at low concentrations in the ng L^{-1}–μg L^{-1} range (Loscos et al., 2007; Mateo-Vivaracho et al., 2010; Tominaga et al., 1998). Most of these aroma compounds are accumulated in the grape under the form of odourless precursors (glycosides, polyhydroxylated molecules or cysteinyl-derivatives), which implies that the aroma will be effectively released only after the precursor molecule is transformed. As certain yeast strains are able to release those aroma compounds by cleavage of the precursor molecules or are even able to synthesise new aroma molecules similar to the ones present in the grape, it can be affirmed that yeast can enhance wine varietal aroma.

It should be noted that a limited number of varietal aroma compounds in an also limited number of wines, really play a predominant aroma role. This is the case of some polyfunctional mercaptans on some white wines (Tominaga et al., 1998, Mateo-Vivaracho et al., 2010), of linalool and other terpenols on Muscat wines (Ribéreau-Gayon et al., 1989) or of cis-rose oxide on wines from Gewurztraminer (Guth, 1997). In most wines, varietal aroma is formed by combinations of many grape and yeast-derived compounds, none of which play a predominant aroma role, and it is the aroma profile, i.e., the particular relative levels of aroma compounds, which is really aromatically significant and responsible for varietal and origin related difference (Escudero et al., 2007; Loscos et al., 2007, 2010). The aroma compounds derived from odourless glycosides are one of the most important constitutional parts of wine aroma and will be the subject of the present research.

The contribution of yeast to the formation of the wine varietal aroma by action on grape glycosidic precursors is well documented in the scientific literature (Darriet et al., 1988; Delcroix et al., 1994; Delfini et al., 2001; Fernández-González et al., 2003; Fernández-González and Di Stefano, 2004; Hernández et al., 2003; Hernández-Orte et al., 2008; Loscos et al., 2007; Mateo and Di Stefano, 1997; Spagna et al., 2002; Ugliano et al., 2006; Ugliano and Moio, 2008), but the role played by hybrids from different species is not well known. To the best of our knowledge, only in one work from Hernández-Orte et al. (2008) a *S. cerevisiae* × *S. bayanus* hybrid was tested.

Notwithstanding the fact that *S. cerevisiae* is the predominant species responsible for alcoholic fermentation, other species of the genus *Saccharomyces* seem to have an important role during fermentation processes (for a revision see Blondin et al., 2009). *S. bayanus* var. *uvarum* (or simply *S. uvarum*) has been described as adapted to low temperature fermentations during winemaking

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whereas the other four species included in the genus, *S. arboriculus*, *S. cariocanus*, *S. mikatae*, and *S. kudriavzevii*, have only been isolated from natural environments. In addition, some hybrids between the species of the genus *Saccharomyces* can participate in fermentation processes. The first and best known examples are *S. pastorianus* strains involved in ale beer production. In the case of wine, other hybrids potentially involved in the alcoholic fermentation are the commercial wine strain *S. cerevisiae* × *S. uvarum* S6U (Masneuf et al., 1998) and several hybrids *S. cerevisiae* × *S. kudriavzevii* (González et al., 2006). These strains are better adapted to the present winemakers' tendency to decrease the wine fermentation temperature which strongly affects yeasts' metabolism, but has been empirically shown to improve the aroma, taste and flavour density of the wine. Some basic research about the oenological characterization of the different species from *Saccharomyces* and hybrids between them have recently been published (Gangl et al., 2009; González et al., 2007; Masneuf-Pomarède et al., 2010), but none of them addressed the question of varietal aroma formation.

Despite the important role of the *Saccharomyces* genus in wine fermentation, this genus is not known as a good enzyme producer for releasing varietal aroma compounds from glycosidic precursors (glycosidases), although there are some scientific publications about certain *Saccharomyces* strains which are capable of doing this action in wine. Mateo and Di Stefano (1997) analysed β-glucosidase activity in three *S. cerevisiae* and three *S. bayanus* strains; Fia et al. (2005) analysed the production of this enzyme by *S. cerevisiae* and non-*Saccharomyces* strains and Gamero et al. (2011) studied the activity of this enzyme in different *Saccharomyces* species and hybrids.

In the present work we have studied the ability of different yeast strains from the different species from the genus *Saccharomyces* (*S. cerevisiae*, *S. uvarum* and *S. kudriavzevii*) and hybrids between these species to release or form varietal aroma compounds from glycosidic precursors or from other sources during fermentation (*de novo* synthesis).

2. Materials and methods

2.1. Reagents and standards

Dichloromethane and methanol (LiChrosolv quality) were purchased from Merck (Darmstadt, Germany), pentane from Fluka (Buchs, Switzerland), and ethyl acetate, absolute ethanol, sodium hydroxide, sodium fluoride, L(+)-ascorbic acid, ammonium sulphate, sodium dihydrogenphosphate1-hydrate, and disodium hydrogen phosphate 12-hydrate were supplied by Panreac (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, U.S.). LiChrolut EN resins were purchased from Merck. The chemical standards were supplied by Aldrich (Gillingham, UK), Sigma (St. Louis, MO), ChemService (West Chester, PA), PolyScience (Niles, IL), Firmenich (Geneva, Switzerland), Panreac, Merck, Fluka, and Lancaster (Strasbourg, France) as shown in Table 1.

2.2. Samples

Grapes from *Vitis vinifera* vars. Macabeo, Muscat, Verdejo, Tempranillo, Parellada and Parraleta cultivated in different regions of Spain in 2007, were harvested by hand and they were stored frozen at −30 °C.

2.3. Precursor extract preparation

Precursors were extracted from six different floral and non-floral grape varieties (Macabeo, Muscat, Verdejo, Tempranillo, Parellada and Parraleta) to obtain a complex "multivarietal" pool of precursors. The procedure is based on that described in Ibarz et al. (2006). Grapes were treated in batches of 500 g of a single variety, and they were

Table 1
Retention indexes and chemical standards used for identification and quantification of volatile compounds.

	RI[a]	Source, purity	Compounds
Terpenes			
1	1447	Tentatively identified	(Z)-linalool oxide
2	1476	Tentatively identified	(E)-linalool oxide
3	1556	Fluka, 98.5%	Linalool
4	1565	Tentatively identified	Linalyl acetate
5	1608	Tentatively identified	Terpinen-4-ol
6	1613	Tentatively identified	2,6-Dimethyl-1,7-octadiene-3,6-diol
7	1664	Tentatively identified	δ-Terpineol
8	1705	Fluka, 97%	α-Terpineol
9	1775	Fluka, 90–95%	β-Citronellol
10	1858	Fluka, 99.5%	Geraniol
11	2366	Tentatively identified	Neric acid
Nor-isoprenoids			
12	1526	Tentatively identified	Vitispirane A[b]
13	1529	Tentatively identified	Vitispirane B[b]
14	1637	Tentatively identified	Riesling acetal[b]
15	1748	Tentatively identified	1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)
16	1832	Tentatively identified	t-1-(2,3,6-trimethylphenyl)but-1,3-diene (TPB)
17	1829	Firmenich, 90%	β-Damascenone
18	1939	Tentatively identified	3-Oxo-β-ionone
19	1950	Sigma, 98%	β-Ionone
20	1952	Tentatively identified	Actinidols[b]
21	2657	Tentatively identified	3-Oxo-α-ionol
Volatile phenols			
22	1876	Aldrich, 98%	Guaiacol
23	2068	Lancaster, 98%	4-Ethylguaiacol
24	2237	Aldrich, 99%	Eugenol
25	2244	Aldrich, 99%	4-Ethylphenol
26	2262	Aldrich, 98%	4-Vinylguaiacol
27	2317	Aldrich, 99%	2,6-Dimethoxyphenol
28	2279	Lancaster, 97%	(E)-Isoeugenol
29	2404	Lancaster, 10% soln.	4-Vinylphenol
30	2563	Aldrich 90%	4-Allyl-2,6-dimethoxyphenol
Vanillin derivatives			
31	2592	Panreac, 99%	Vanillin
32	2629	Aldrich, 99%	Methyl vanillate
33	2654	Lancaster, 97%	Ethyl vanillate
34	2664	Aldrich, 98%	Acetovanillone
35	2829	Aldrich, 96%	Zingerone
36	2892	Aldrich, 99%	Homovanillyl alcohol
37	3040	Aldrich, 98%	Syringaldehyde
38	3099	Tentatively identified	Homovanillic acid
39	3123	Aldrich, 97%	Acetosyringone
Benzenes			
40	1520	Fluka, 99%	Benzaldehyde
41	1659	Aldrich, 90%	Phenylacetaldehyde
42	1891	Aldrich, 99%	Benzyl alcohol
43	1908	Aldrich, 99%	Ethyl dihydrocinnamate
44	1926	Fluka, 99%	β-Phenylethanol
45	2081	Aldrich, 99%	Ethyl cinnamate
46	2219	Fluka, 98%	2-Phenoxyethanol
47	2725	Tentatively identified	1,2-Dimethoxy-4-propylbenzene
Lactones			
48	1988	Lancaster, 98%	δ-Octalactone
49	2068	Aldrich, 97%	γ-Nonalactone
50	2141	Aldrich, 98%	γ-Decalactone
51	2260	Lancaster, 98%	δ-Decalactone
Miscellaneous			
52	1390	Aldrich, 98%	(Z)-3-hexen-1-ol
53	1672	Lancaster, 98%	3-Methylbutyric acid
54	1677	Aldrich, 98%	2-Methylbutyric acid

[a] Retention index calculated in a DBWAXetr column.
[b] Actinidols: 2,2,6-trimethyl-8-(1-hydroxy)ethyl-7-oxabicyclo[4.3.0]nona-4,9-dienes; Riesling acetal: 2,2,6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.0(1,6)]undec-4-ene; Vitispirane: 2,10,10-trimethyl-6-methylen-1-oxaspiro-[4,5]dec-7-ene.