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ScienceDirect
Enzyme and Microbial Technology 40 (2006) 151–157
ENZYME AND MICROBIAL TECHNOLOGY
www.elsevier.com/locate/emt

Influence of *Saccharomyces cerevisiae* yeast strain on the major volatile compounds of wine

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Received 18 March 2005; received in revised form 19 October 2005; accepted 21 October 2005

Abstract

As must fermentation is often conducted with selected yeasts, it is important to determine the influence of the inoculated yeast strain on the wine organoleptic properties. We analysed the production of major volatile compounds (acetaldehyde, ethyl acetate and some fusel alcohols) during fermentations of musts from different grape cultivars and conducted with selected *Saccharomyces cerevisiae* yeast strains. The production of these volatile compounds was variable, and depended mostly on the must composition and the fermentation conditions. Occasionally, the amount of certain volatiles compounds in the wine depended on the yeast strain. We observed inverse correlations between acetaldehyde and isobutanol production, and, between ethyl acetate and total fusel alcohol production. Also, a direct correlation was found between the organoleptic wine quality and the amount of ethyl acetate. The most appreciated wines were made with yeast strains that did not produce high amounts of any of the analysed compounds, while the lowest quality wines were made with yeast strains that produced high amounts of acetaldehyde and fusel alcohols.

Keywords: Yeast; *Saccharomyces cerevisiae*; Wine; Fusel alcohols; Acetaldehyde; Ethyl acetate

1. Introduction

The aroma is a very important component of the organoleptic quality of wine. The total aromatic content of wine is 0.8–1.2 g/L. Most of these compounds are produced during must fermentation and are especially important in the aroma of young wines. Acetic acid, acetaldehyde, ethyl acetate, propanol, isobutanol, 2- and 3-methylbutanol account for more than half of these volatiles, the other half being distributed among 600–800 minor volatile compounds present in very low amounts (acetals, organic acids, alcohols, phenolic and heterocyclic compounds, esters, lactones, terpenes and sulfur-containing compounds). The analysis of the contribution to the aroma of these minor compounds is complicated because of their low concentrations and their interactions. The quantity and quality of the aromas and flavours originated in must fermentation depend on environmental conditions, vinification process and the participating yeasts (see the reviews by Thorhgate [1] and by Swiegers et al. [2]). It would therefore be worthwhile to select wine yeast strains that produce the most appreciated aromas and flavours [3–5].

Acetaldehyde, precursor of the acetates and ethanol, is formed from pyruvate by the glycolytic pathway enzyme pyruvate decarboxylase. Freshly made wines usually have acetaldehyde concentrations below 75 mg/L [6], although a wide range of values have been reported [7–10]. These initial values usually decline over time since acetaldehyde is a very reactive compound that combines with polyphenols and other compounds in the wine [11]. It has been found that the production of acetaldehyde during must fermentation depends on technological factors (must composition, pH, fermentation temperature, aeration and SO_2 concentration) and on the yeast strain involved [2,7,10,12,13]. With some exceptions, such as fino (pale) sherries, acetaldehyde is considered undesirable at high concentrations. Hence, it is interesting to perform must fermentation with yeast strains that produce low amounts of acetaldehyde.

Ethyl acetate is produced by the enzymatic esterification of acetic acid and ethanol. When the must fermentation is protected from aeration, the usual concentration of this compound is 30–50 mg/L, which increases to 60–110 mg/L with aeration. Ethyl acetate is the second most important component (after acetic acid) of wines volatile acidity. Concentrations below

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0141-0229/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2005.10.048

70 mg/L are considered positive for the wine aroma, but higher 150–200 mg/L they are considered negative [14]. Among the wine yeasts, *Saccharomyces cerevisiae* is one of the lowest ethyl acetate producers, while oxidative or weakly fermentative yeasts (*Candida* sp., *Debaryomyces* sp., *Pichia* sp. and *Hansenula* sp.) are the highest producers [15].

2-Methyl-1-butanol, 3-methyl-1-butanol, isobutanol (2-methyl-1-propanol), *n*-propanol, tyrosol, hexanol and 2-phenylethanol are the major fusel alcohols produced during must fermentation. In part, they are formed by transamination or disamination of the corresponding amino acid according to the Ehrlich pathway. The resulting acids are decarboxylated to aldehydes, which are finally reduced fusel alcohols. Some fusel alcohols have no possible precursor amongst the amino acids, and are formed from cetonic acids [16]. High amounts of these compounds are considered undesirable in table wines, and concentrations below 350 mg/L may be considered as positive for wine aroma [17]. The production of fusel alcohols by yeasts depends on the latters' ability to produce amino acids, and it varies according to genus, species and strain [19]. Usually, *S. cerevisiae* produces high amounts, whereas *Candida*, *Kloeckera* and *Brettanomyces* produce low amounts. Other factors such as ethanol production, fermentation temperature, pH, aeration and amount of solids in the must also influence fusel alcohol production by yeasts [6,16,18].

Although there have been demonstrated differences in the production of volatiles between yeast strains, these differences have not been unambiguously shown to be reproducible. After exhaustively reviewing the subject, Thorhgate [1] concluded that, since extrinsic factors can greatly affect a wine's volatile profile, it is too easy to reach erroneous conclusions regarding yeast strain effects. It therefore seems necessary to conduct comprehensive studies of strain variability, so that winemakers may know what possible flavour effects to expect for a specific yeast strain. In previous work [20], we isolated and selected several wine yeasts for industrial winemaking. In this paper, we report the influence of must inoculation with some of these wine yeasts (and two commercial yeasts as reference standards) on the amount of the major volatile compounds in white and red wines elaborated from different grape varieties and under different fermentation conditions.

2. Material and methods

2.1. Yeast strains

JP1, JP33, JP34, JP47, JP73, JP85, JP88, JP93 and JP98 are *S. cerevisiae* wine yeast strains from a number of wineries located in different areas of Spain and tested for industrial use [20]. Two commercial yeasts were used as reference standards: *S. cerevisiae* SM102 (sold as FERMIBLANC AROM®, Gist-Brocades, BP239, 59427 Seclin Cedex, France) and *Saccharomyces bayanus* 67J (sold as FERMICHAMP®, Gist-Brocades, BP239, 59427 Seclin Cedex, France). These yeasts are being used with good results in some Spanish wineries.

2.2. Vinifications

We prepared five fermentation groups (labeled G1–G5; Table 1) in Erlenmeyer-flasks with 5 L of white must (from white grape Parellada and Pardina cultivars) or crushed grapes (from red grape Garnacha Tintorera, Cabernet Sauvignon and 50% Cabernet Sauvignon–50% Merlot cultivars). After harvesting, the grapes were immediately destemmed, crushed and thereafter sulfur dioxide (as potassium metabisulfite) was added sulfited to a final concentration of 40–60 mg/L. The white musts were clarified by cold-settling (18 h at 10 °C) to remove most suspended solids. Parellada juice of G1 was thermovinified by heating to 70 °C for 20 min. The rest of the white must (G2) or crushed red grapes (G3, G4 and G5) were sterilized by the addition of 200 mg/L dimethyl dicarbonate [21], and then homogenised by strong shaking before dispensing into the different flasks so as to get the same amount of suspended solids in each fermentation group. Yeast strains were cultured in YEPD broth (at 28 °C with vigorous aeration), washed twice (by centrifugation) with sterile water and suspended in the must or crushed grapes at a concentration of 2–5 × 10^6 cells/mL. The implantation of the inoculated yeast strain during fermentation was monitored by mitochondrial DNA restriction analysis [22]. For all vinifications groups except G1, a parallel non-inoculated vinification control (spontaneous fermentation) was performed with 5 L of non-inoculated fresh juice or crushed grapes without any addition of dimethyl dicarbonate. White must fermentation was conducted at 18 °C and red grape fermentation at 22 °C. The Brix° and density were monitored. Flasks were capped hermetically when reducing sugars reached around 1% to avoid oxidation problems. At the end of fermentation, the settled solids were discarded and a sample of each wine (500 mL) was centrifuged for the analytical assays. The uncentrifuged wines were stored at 4 °C. Settled solids were discarded again at 35 and 85 days following the end of fermentation, and thereafter the wines were bottled. Each fermentation was carried out in duplicate, and the mean of the two determinations for each parameter is presented. After 105 days following the end of fermentation, the organoleptic characteristics of the wines were tested according to Regodón et al. [20].

2.3. Analytical methods

Ethyl acetate and fusel alcohols (propanol, isobutanol and 2- and 3-methylbutanol) were analysed by direct injection gas chromatography (Perkin-Elmer 8600 gas chromatograph equipped with a flame-ionization detector) on a C-432 (SGL-20) capillary column (25 m × 0.25 mm × 0.25 μm film) with a temperature programming (initial temperature 33 °C, 5 min at 33 °C, from 33 to 107 °C increasing 10 °C per minute, 5 min at 107 °C, from 107 to 145 °C increasing 30 °C per minute and 5 min at 145 °C). 4-Methyl-2-pentanol (340 mg/L) was added as an internal standard to the filtered samples (0.45 μm Millipore filter). 2- and 3-methylbutanol were not separated by the GC and these parameters are shown toghether. Acetaldehyde was analysed by the spectrophotometric method described by Di Stefano and Ciolfi [23]. This method was used instead of gas chromatography because of its better reproducibility. Each assay was made twice, and the results represent the mean of the two determinations. The other physicochemical parameters of wine and must were determined according to the method of Ribéreau-Gayon et al. [24].

Table 1
Characteristics of musts and crashed grapes used in each vinification group

Vinification group	Grape cultivar	Total SO_2 (mg/L)	Brix°	pH	Total acidity[a] (g TH_2/L)
G1	Parellada (thermovinified)	40	19.5	3.47	4.77
G2	Pardina	51	21.0	3.56	4.64
G3	Garnacha Tintorera	60	21.5	3.52	5.56
G4	Cabernet Sauvignon	45	19.5	3.71	4.97
G5	50% Cabernet Sauvignon–50% Merlot	56	21.5	3.62	4.34

[a] TH_2: tartaric acid.