LWT - Food Science and Technology 61 (2015) 503–509

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Natural sweet wine production by repeated use of yeast cells immobilized on *Penicillium chrysogenum*

Teresa García-Martínez [a], Juan Moreno [b], Juan Carlos Mauricio [a], Rafael Peinado [b, *]

[a] Department of Microbiology, University of Cordoba, Agrifood Campus of International Excellence ceiA3, Severo Ochoa Building, 14014 Cordoba, Spain
[b] Department of Agricultural Chemistry, University of Cordoba, Agrifood Campus of International Excellence ceiA3, Marie Curie, 14014 Cordoba, Spain

ARTICLE INFO

Article history:
Received 12 June 2014
Received in revised form
3 December 2014
Accepted 10 December 2014
Available online 18 December 2014

Keywords:
Sweet wine
Immobilized yeast
Biocapsules
Amino acids
Volatile compounds

Chemical compounds studied in this article:
Glucose (PubChem CID: 79025)
Fructose (PubChem CID: 5984)
Acetaldehyde (PubChem CID: 177)
Glycerol (PubChem CID: 753)
Propanol (PubChem CID: 1031)
Isobutanol (PubChem CID: 6560)
2-phenylethanol (PubChem CID: 6054)
L-serine (PubChem CID: 5951)
L-phenylalanine (PubChem CID: 6140)
L-glutamine (PubChem CID: 5961)

ABSTRACT

The production of sweet wines from sun-dried grapes is difficult because yeasts are affected by a hyperosmotic stress due to the high sugar concentration, leading to altered metabolism and growth. The aim of this study is to improve and simplify this process by means of a repeated batch fermentation system using *Saccharomyces cerevisiae* immobilized on spheres of *Penicillium chrysogenum*. Successive reuse of the immobilized yeasts revealed its gradual adaptation to the fermentation conditions and an increasingly uniform behaviour in terms of fermentation kinetics and production of metabolites. Immobilized yeasts produced higher concentration of carbonyl compounds, esters and polyols than free yeast, and the opposite was true for major alcohols. Nitrogen compounds were depending on the state of cells (free or immobilized) and also on the number of time the yeasts were used. Using this immobilization system might provide some advantages as to obtain the desired ethanol level by easier removal of yeast cells from the medium or to reduce the production costs in the *inocula* preparation. The operational stability of the immobilization system proposed might enable its use at the commercial scale for production of sweet wine.

1. Introduction

Sweet wines from sun-dried grapes are traditionally produced in regions with a Mediterranean climate including Greece, Cyprus, Turkey, Italy and Spain (Franco, Peinado, Medina, & Moreno, 2004). *Saccharomyces cerevisiae* is a saccharophilic yeast typically growing in media containing high sugar concentrations. However, fermentations in musts with high sugar content may have limitations, because yeast cells grow slowly during the initial fermentation stage, and usually exhibit a decreased growth rate in comparison with media containing small amounts of sugars (Erasmus, van der Merwe, & van Vuuren, 2003; Guidi et al., 2010). This is as a result of the high osmotic pressure borne by the yeasts, which frequently leads to very slow or even arrested fermentation (García-Mauricio & García-Martínez, 2013). In some areas, in order to suppress alcoholic fermentation and avoid these problems, the raisin must is fortified with wine ethanol up to 15% v/v obtaining the traditional Pedro Ximénez wine. However, the resulting sweet wines lack the typical fermentation aroma and usually exhibit a low acidity and a strong sweet taste (López de Lerma & Peinado, 2011). Some researchers have proposed fermenting musts with selected yeasts characterized by high osmotolerance and by support high concentration of ethanol (López de Lerma, García-Martínez, Moreno, Mauricio, & Peinado, 2012; Malacrinò, Tosi, Caramia, Prisco, & Zapparoli, 2005; Ortiz-Muñiz, Carvajal-Zarrabal, Torrestiana-Sanchez, & Aguilar-Uscanga, 2010). Besides, the use of immobilized yeasts might solve the problems associated to the fermentation of high sugar content musts as prevent stuck fermentation, sluggish

* Corresponding author. Tel.: +34 957 218534.
E-mail address: qe1peamr@uco.es (R. Peinado).

http://dx.doi.org/10.1016/j.lwt.2014.12.029
0023-6438/

fermentation delays, and microbiological contaminations (Martynenko & Gracheva, 2003).

A method for coimmobilizing strain H3 of the fungus *Penicillium chrysogenum* (generally regarded as safe, GRAS) and various strains of *S. cerevisiae* has been recently developed; the resulting structures, which we have named "yeast biocapsules", are smooth, hollow, elastic spheres consisting of fungal hyphae and trapped yeast cells, with a high resistance and tolerance to the fermentation conditions (García-Martínez, Puig-Pujol, Peinado, Moreno, & Mauricio, 2012).

It should be noted that fermentation causes the filamentous fungus to die by effect of direct contact between its hyphae and yeast cells, and to remain as a mere, but highly inert and strong, support for yeast, which can facilitate their reuse (García-Martínez, Peinado, Moreno, García-García, & Mauricio, 2011). Advantages of using biocapsules opposite to calcium alginate beads have been recently reported by Puig-Pujol et al. (2013). Previously, two osmotolerant *S. cerevisiae* strains to partially ferment must from Pedro Ximenez dried grapes have been used. The yeast strains were inoculated in free and immobilized form, and the production of volatile compounds and sensory properties of the resulting wines were examined in detail. The wines provided by immobilized yeast were the most appreciated in the sensory analysis (López de Lerma, Bellincontro, Mencarelli, Moreno, & Peinado, 2012; López de Lerma, García-Martínez, et al., 2012).

The aim of the present work was to assess the reusability of *S. cerevisiae* immobilized on *P. chrysogenum* in the fermentation of raisin must from Pedro Ximenez variety with a view to improve the process of sweet winemaking and to obtain a natural sweet wine.

2. Materials and methods

2.1. Microorganisms and biocapsules production

Biocapsules were obtained from *S. cerevisiae* strain G1 (ATCC: MYA-2451) and *P. chrysogenum* strain H3. Strain G1 is a flor yeast isolated from wine under biological ageing by the members of the Microbiology Department of the University of Córdoba (Spain). This specific strain was chosen on the grounds of its tolerance of high glucose and ethanol concentrations (Aguilera, Peinado, Millán, Ortega, & Mauricio, 2006). *P. chrysogenum* strain H3 was isolated by the same research group and identified by the Spanish Collection of Standard Cultures (CECT). This strain was selected in terms of the ease with which it forms strong biocapsules that are highly resistant to fermentation conditions (García-Martínez et al., 2011).

Production of biocapsules was carried out according to García-Martínez et al. (2011), under special conditions in a formation medium consisting of yeast nitrogen base without amino acids (YNB, Difco, Becton Dickinson and Company, Sparks, MD) containing a 5 g/L concentration of gluconic acid (Sigma–Aldrich, St. Louis, MO) as carbon source. The medium was buffered at pH 7 with sodium and potassium phosphate. The free cells used in the fermentation experiments were processed identically but in the absence of the filamentous fungus.

2.2. Must from dried grapes and fermentation conditions

The must used was obtained from sun-dried grapes of the Pedro Ximenez variety grown in the Montilla–Moriles Protected Designation of Origin (Cordoba, Spain). The sugar content of the must was 545 g/L. The must was aseptically centrifuged at 5000 × *g* for 5 min to withdraw part of the microorganisms and solid particles. The initial pH, 4.3, was lowered to 3.8 by addition of tartaric acid, and was also supplied with a 100 mg/L concentration of SO_2. Fermentation batches were conducted in 500 mL Erlenmeyer flasks filled with 400 mL of must each. Those using biocapsules were performed as follows: before biocapsules were used, they were easily removed from the production medium using a sterilized strainer and then were washed twice with sterile distilled water at 4 °C and then with fresh must at the same temperature. Next, they were confined and packed in stainless steel cylinders 62 cm^3 in volume having holes 3 mm in diameter, all under aseptic conditions. The cylinders were hanged on Nylon wires and immersed in the Erlenmeyer flasks (Fig. 1). This system facilitated easy withdrawal of the immobilized yeasts and their reuse. A control fermentation batch with free yeast cells was also performed; the free cells were centrifuged at 5000 × *g* and washed twice with sterile distilled water at 4 °C before their use in fermentation. 500-mL Erlenmeyer flasks filled with 400 mL of raisin juice were inoculated with 5 × 10^6 free viable cells of the yeast per millilitre or with biocapsules reaching the same concentration of yeast cells. The number of yeast cells in the biocapsule and fermentation broth was measured according to García-Martínez et al. (2012).

Once the desired alcohol content (13% v/v) was reached, the metal cylinders containing the biocapsules were withdrawn from the fermentation medium and placed in another Erlenmeyer flask containing fresh raisin must for subsequent reuse. Raisin grape must and fermentation batches were stopped by adding wine alcohol up to a final ethanol content of 15% v/v and refrigerating at 2 °C. The obtained wines for this study were traditional Pedro Ximénez wine without fermentation, which was obtained by fortification of the raisin must with 15% (v/v) wine ethanol (T), sweet wine from free yeast cells (F) and sweet wines from repeated batch fermentations with immobilized yeast cells (B1–B5). All fermentation batches were performed at 24 °C in triplicate.

2.3. Analytical methods

Viable cells in the culture medium were counted by using classical microbiological techniques. Following serial dilution of the must, samples were plated on YPD agar (1% yeast extract, 2% peptone, 2% glucose and 2% agar) and counted after incubation at 28 °C for two days. The fermentation process was monitored via the amount of CO_2 released as a measure of weight loss (Bely, Sablayrolles, & Barre, 1990). Enological parameters were determined according with the European Union Official Methods (CEE, 1990). Sugars (glucose and fructose), succinic acid, ammonia and urea were quantified with specific enzymatic kits from Boehringer–Mannheim (Germany). The major volatile compounds and polyols were quantified in a gas chromatograph HP 6890 Series II equipped with a capillary column with molten silica CP-WAX 57 CB (50 m in length, 0.25 mm in internal diameter and 0.4 μm in coating thickness) and a Flame Ionization Detector. The chromatographic conditions and sample preparation were described by Peinado, Moreno, Muñoz, Medina, and Moreno (2004). The identification and quantification of the major volatile compounds performed by means of the standards were submitted to the same treatment as the analysed samples. Amino acids were identified and quantified by previously passing the samples through a Millipore filter of 0.22 μm pore diameter and then using the method of Botella, Pérez-Rodríguez, Domecq, and Valpuesta (1990) for separation and quantitation of their dansyl derivatives (Tapuhi, Schmidt, Lindner, & Karger, 1981) on a Spectra-Physics P200 HPLC instrument. The chromatograph used a 15 × 0.4 cm reversed C18 column packed with Spherisorb ODS2 resin of 5 μm particle size from Tracer Analítica (Barcelona, Spain) that was thermostated at 25 °C and coupled to an Sp 8450 UV–Vis detector for measurement of the absorbance at 254 nm, using 5 mM L-norleucine as an internal standard. Amino acids were identified by comparing their relative retention times to those of standards from Sigma–Aldrich (Germany). Data were acquired and processed by using the software