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Effect of biological ageing of wine on its nitrogen composition for producing high quality vinegar

Carmen Álvarez-Cáliz[a], Inés Mª Santos-Dueñas[a], Teresa García-Martínez[b], Ana Mª Cañete-Rodríguez[a], Mª Carmen Millán-Pérez[b], Juan C. Mauricio[b], Isidoro García-García[a,*]

[a] Departamento de Ingeniería Química, Edificio Marie Curie, Campus Universitario de Rabanales, Universidad de Córdoba, Spain
[b] Departamento de Microbiología, Edificio Severo Ochoa, Campus Universitario de Rabanales, Universidad de Córdoba, Spain

ABSTRACT

There is increasing interest in producing novel high quality vinegars. In this work, we examined the effect of biological ageing of white wine on its nitrogen composition (viz. amino acids, urea and ammonium ion, which constitute the main sources of nitrogen for acetic bacteria during the acetification process) with a view to confirming the suitability of aged wine for producing quality vinegar.

Available nitrogen contents in biologically aged wine were lower than in young (unaged) wine; this resulted in a slightly lower acetification rate and production with the former. The nitrogen composition of the two vinegars was very similar, with L-proline and L-cysteine as the major amino acids. By exception, only the vinegar from the young wine contained ammonium ion. The ageing process by flor yeast produces urea which is eliminated by the bacteria in the next stage.

Available nitrogen for use by acetic bacteria in biologically aged wine is seemingly no limiting factor for acetification. The problem posed by the formation of urea during wine ageing was suppressed by the subsequent acetification process. The study could be an example of how different microorganisms use the available substrate in serial biotransformations.

Keywords: Wine; Ageing; Wine vinegar; nitrogen composition; Amino acids; Urea

1. Introduction

Vinegar is one of the main products of the oxidative action of acetic bacteria on an alcoholic medium. Although vinegar from alcohol is the most widely available worldwide, many countries use various fruits and grains to obtain alternative vinegars with especially appreciated sensory properties (Solieri and Giudici, 2009). Especially prominent in this respect is wine vinegar. Some vine-growing areas in the Mediterranean region have traditionally produced wine vinegar of a very high quality. Unsurprisingly, the only protected designations of origin (DOs) for vinegars are either Spanish ("Vinagre del Condado de Huelva", "Vinagre de Jerez" and the forthcoming "Vinagre Montilla–Moriles") or Italian ("Aceto Balsámico Tradizionale di Módena" and "Aceto Balsámico Tradizionale di Reggio Emilia").

As in Jerez and Condado de Huelva, wines with the Montilla–Moriles origin denomination (DO) are obtained from wines subjected to biological ageing. One typical ageing method in the area is "biological ageing" under a film of flor yeasts. To this end, white wine is stored and allowed to evolve in wooden casks under the slow action of a biofilm of flor yeasts formed on its surface. This protects the wine against atmospheric oxidation and causes some changes including slight consumption of ethanol, and a substantial reduction of the proportions of glycerol and volatile acidity simultaneously to an increase in that of acetaldehyde. The resulting, high-quality product, which is called "fino wine", has a pale yellow colour and pungent flavour, and is limpid, bright and light in the glass (Peinado and Mauricio, 2009).

The wine is aged with the so-called "criaderas and solera" system, whereby wine of variable age is mixed to obtain a

* *Corresponding author*. Tel.: +34 957 218589; fax: +34 957 218589.
E-mail address: isidoro.garcia@uco.es (I. García-García).
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highly homogeneous product despite its coming from grapes from several vintages. Wines of the same age are stored in cask rows called "criaderas". A portion of the wine in each row never exceeding one-third is periodically withdrawn from each cask and replenished with an identical volume of younger wine from the previous row, the youngest wine being replenished with unaged wine ("sobretabla"). The withdrawal and replenishment operations are known as "saca" and "rocío", respectively. As a result of the successive transfers, each cask row invariably contains portions of wines from the different vintages used for replenishment. The last row, where the ageing process is completed, is called the "solera" and provides a highly homogeneous end-product containing wine spanning from the first vintage received by the "solera" to the last with which it is replenished. The average age of the end-product depends on the wine withdrawal frequency used. As stated above, the result is a high-quality product in itself; however, it may also provide an excellent substrate for obtaining quality vinegar. It is therefore interesting to determine whether wine obtained with the "criaderas and solera" system is a suitable substrate for vinegar production.

If this type of vinegar is assumed to be the end-product of a threefold biotransformation process including (a) alcoholic fermentation of grape must to a young wine under the action of yeasts, (b) biological ageing under flor yeasts – which switch to an oxidative (aerobic) metabolism and (c) acetification of the aged wine by the action of acetic bacteria, then some essential nutrients for the process can also be assumed to be depleted in the first two steps. Specifically, the presence of nitrogen is essential to ensure proper development of the third, acetification step (Callejón et al., 2008, 2010; Valero et al., 2005).

Bacteria are known to use amino acids and ammonium ion as their main sources of nitrogen. Although these microorganisms can by themselves synthesize amino acids from ammonium ion, they require the presence of a minimum amount of these compounds to act. Also, although some studies have shown that the wines typically used as substrates usually contain large enough amounts of amino acids for acetification (Álvarez-Cáliz et al., 2012; Callejón et al., 2008, 2010; Maestre et al., 2008; Valero et al., 2005), previously aged wine may somehow hinder vinegar production by effect of flor yeasts continuing to use amino acids in the medium (Berlanga et al., 2004, 2006).

It is therefore necessary to examine the process for high-quality vinegar to ascertain whether biologically aged wines possess an adequate nitrogen load for acetic bacteria to develop their acetifying action. This led us to determine the amino acid, urea and ammonium ion contents of white wine from the Montilla–Moriles region (Córdoba, southern Spain) biologically aged under a biofilm of flor yeasts prior to conversion into high-quality vinegar. The results were compared with those for a previously studied young (unaged) wine and the respective resulting vinegars. Acetification of young wine under no biological ageing is the traditional vinegar production method not only in the Montilla–Moriles region, but also in most vinegar-making areas.

2. Materials and methods

2.1. Microorganisms

The initial inoculum was a mixture of acetic bacteria from an industrial tank (an acetator) of the firm Deóleo, S.A. (Córdoba, Spain) in full operation that is used to acetify a young wine similar to that studied here. The bacterial concentration in the inoculum was 3.5×10^8 cells mL^{-1}.

2.2. Wines

The starting substrate was a young wine from the Montilla–Moriles region containing $15 \pm 0.5\%$ (v/v) ethanol and 0.5% (w/v) acidity. A portion of the wine was diluted to an ethanol concentration of $11.5 \pm 0.5\%$ (v/v) with water for direct acetification and another subjected to biological ageing in a "criaderas and solera" system under a film of flor yeasts in the cellar of Bodegas Alvear, S.A. (Montilla, Spain). The resulting wine, with an average age of 2 years, was diluted to $11.5 \pm 0.5\%$ (v/v) ethanol with water and acetified.

2.3. Acetification conditions

Experiments were carried out in an 8 L Frings fermenter operating in a semi-batch mode. The operational variables were set as follows for all tests: temperature, 31 °C; air flow-rate, 60 L h^{-1}, loading rate, 0.06 L min^{-1}; final ethanol concentration, $0.5 \pm 0.1\%$ (v/v); final acidity, $10.0 \pm 0.2\%$ (w/v); percent unloading of the medium (75%).

The bioreactor was fully automated. Loading, unloading, control and monitoring operations were performed via a previously programmed computer. In the semi-batch operational mode used, once the reactor was partially unloaded, a new cycle was started by adding fresh wine. The experimental process was repeated at least four times in each instance.

2.4. Analytical methods

The amino acid, urea and ammonium ion concentrations were determined by previously passing the samples through Millipore filters of 0.45 mm pore size and adjusting their pH to 7.5 with NaOH, with provision for the dilution factor.

Urea and ammonium ion in the medium were quantified with an enzymatic kit from Boehringer–Mannheim/R-Biopharm (Darmstadt, Germany).

Amino acid contents were determined according to Botella et al. (1990); thus, their dansyl derivatives were separated and quantified on a Spectra-Physics P200 HPLC instrument furnished with a C18 reversed-phase column 15 cm long × 0.4 cm i.d. that was packed with Spherisorb ODS resin (particle size 5 mm) from Tracer Analytical (Barcelona, Spain). The column was thermostated at 25 °C and connected to an SP 8450UV–vis detector that was used to measure the absorbance at 254 nm. L-Norleucine (5 mM) was used as internal standard. Amino acids were identified by comparison of their relative retention times with those for standards obtained from Sigma–Aldrich (Barcelona, Spain). Data were collected and analysed by using the software Biochrom 2000 (Álvarez-Cáliz et al., 2012).

Acidity was determined by acid–base titration. An on-line connected Alcosens (Heinrich Frings GmbH and Co.) probe and a differential pressure sensor (Yokogawa Iberia S.A.) were to measure ethanol and volume, respectively (García-García et al., 2009).

The oxygen concentration was monitored by using SM31dissolved oxygen sensor and a DO402G converter, both from Yokogawa Iberia, S.A. (Spain) (Yokogawa Iberia S.A.).