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Potential of high pressure homogenization to induce autolysis of wine yeasts

Piergiorgio Comuzzo *, Sonia Calligaris, Lucilla Iacumin, Federica Ginaldi, Anthony Efrain Palacios Paz, Roberto Zironi

Università degli Studi di Udine, Dipartimento di Scienze degli Alimenti, via Sondrio 2/A, 33100 Udine, Italy

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ABSTRACT

High pressure homogenization (HPH) was tested for inducing autolysis in a commercial strain of *Saccharomyces bayanus* for winemaking. The effects on cell viability, the release of soluble proteins, glucidic colloids and amino acids in wine-like medium and the volatile composition of the autolysates were investigated after processing, in comparison with thermolysis. HPH seemed a promising technique for inducing autolysis of wine yeasts. One pass at 150 MPa was the best operating conditions. Soluble colloids, proteins and free amino acids were similar after HPH and thermolysis, but the former gave a more interesting volatile composition after processing, with higher concentrations of ethyl esters (fruity odors) and lower fatty acids (potential off-flavors). This might allow different winemaking applications for HPH, such as the production of yeast derivatives for wine ageing. In the conditions tested, HPH did not allow the complete inactivation of yeast cells; the treatment shall be optimized before winemaking use.

1. Introduction

Yeast autolysis is an enzymatic self-degradation of cellular constituents that begins after cell death (Charpentier & Feuillat, 1993). Autolysis is an important technological tool during the ageing of certain wine typologies, such as white wines aged on the lees (e.g. French wines from Burgundy) or sparkling wines produced by *Champenoise* method. During ageing on the lees, wine composition varies as a consequence of the release of soluble polysaccharides (Charpentier & Feuillat, 1993), proteins (Perrot, Charpentier, Charpentier, Feuillat, & Chassagne, 2002), peptides and free amino acids (Alexandre et al., 2001; Perrot et al., 2002), lipids (Pueyo, Martínez-Rodríguez, Polo, Santa-María, & Bartolomé, 2000), nucleotides and nucleosides (Charpentier et al., 2005) and these compositional modifications lead to changes in wine volatile profile (Pozo-Bayón, Pueyo, Martín-Álvarez, Martínez-Rodríguez, & Polo, 2003) and sensory characters (Carrascosa, Martínez-Rodríguez, Cebollero, & Gonzalez, 2011). Moreover, yeast lees are powerful oxygen scavengers (Salmon, Fornairon-Bonnefond, Mazauric, & Moutounet, 2000) and this allows the protection of white wines against oxygen spoilage during barrel ageing.

Despite these positive effects, the long time required for *sur lie* maturation increases the risk of microbial spoilage, such as *Brettanomyces* growth (Guilloux-Benatier, Chassagne, Alexandre, Charpentier, & Feuillat, 2001) and biogenic amine pollution (Martín-Álvarez, Marcobal, Polo, & Moreno-Arribas, 2006; González-Marco & Ancín-Azpilicueta, 2006). For these reasons, different technological strategies have been suggested for accelerating yeast autolysis and *sur lie* ageing.

The most widely proposed tool is the use of commercial preparations of β-glucanases (Rodriguez-Nogales, Fernández-Fernández, & Vila-Crespo, 2012; Torresi, Frangipane, Garzillo, Massantini, & Contini, 2014); these enzymes are able to hydrolyze β-glucans from yeast cell walls, increasing the rate of cell degradation and the release of soluble compounds. Another common practice is the addition of yeast derivatives (YD). These products are basically inactive dry yeasts, containing cell wall residues and metabolites released during production process (i.e. induced autolysis) (Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009a). YDs (extracts and autolysates) have been described, even if with some controversial results, as additives for accelerating natural autolysis (Carrascosa et al., 2011), because of their ability to release macromolecules and soluble compounds (Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009a). These substances can also modify the volatility of wine aroma, generally improving fruity and flowery characters (Rodriguez-Nogales, Fernández-Fernández, & Vila-Crespo, 2012); in a previous study (Comuzzo et al., 2012), a thermally-produced yeast autolysate was compared with a product obtained by enzyme-assisted autolysis (β-glucanase

* Corresponding author. Tel.: +39 0432 55 8166; fax: +39 0432 55 8130.
E-mail address: piergiorgio.comuzzo@uniud.it (P. Comuzzo).

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treatment) of the same *S. cerevisiae* strain: thermolysis led to lower levels of non-glycosylated proteins and higher amounts of soluble glycoproteins, and this seemed connected, respectively, with a lower capacity to retain wine aromas and with a higher ability of thermolysates to increase the volatility of certain wine compounds (e.g. esters). Unfortunately, the thermal treatments used during YD's manufacturing can lead to the formation of off-flavors that may be released into the wine, negatively affecting its sensory properties (Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009c).

Besides β-glucanases and YD supplementation, other techniques are available to accelerate natural autolysis. Recently, ultrasounds (US) have been tested from this point of view, with positive results: ultrasound-assisted autolysis reduced yeast cell viability and increased the release of proteins (García Martín, Guillemet, Feng, & Sun, 2013), total colloids and glycoproteins (Cacciola, Ferran Batllò, Ferraretto, Vincenzi, & Celotti, 2013), even if US effects seemed generally less intense than the use of β-glucanases (Cacciola et al., 2013).

High pressure homogenization (HPH) could also be a good alternative to accelerate *sur lie* ageing. In fact, HPH has already been reported as effective tool for promoting the disruption of *Saccharomyces* cells and the recovery of yeast intracellular components, such as enzymes and proteins (Follows, Hetherington, Dunnill, & Lilly, 1971; Middelberg, 1995; Hetherington, Follows, Dunnill, & Lilly, 1971). The microbial cell disruption during HPH has been associated to the occurrence of phenomena, such as cavitation, shear and turbulence that occur when the fluid is forced to pass through the narrow gap in the homogenizer valve (Popper & Knorr, 1990). Recently, the ability of HPH in destroying yeast cells has also been tested for the microbial stabilization of fruit juices (Campos & Cristianini, 2007; Maresca, Donsi, & Ferrari, 2011; Patrignani, Vannini, Kamdem, Lanciotti, & Guerzoni, 2009) and beer (Franchi, Tribst, & Cristianini, 2013). Beside these results, HPH has been poorly considered in winemaking and the few applications reported are limited to the reduction of the indigenous flora in grape musts (Puig, Olmos, Quevedo, Guamis, & Minguez, 2008), or the modulation of autolysis in yeast starter *tirage* cultures during sparkling wine production (Patrignani et al., 2013). Based on literature results, HPH could be particularly interesting as a system to improve natural autolysis, because of the possibility to use it for promoting a non-thermal inactivation of yeasts during the manufacture of YDs, or to replace expensive or noisy technologies (e.g. enzymes or ultrasounds respectively) for processing the lees before wine ageing.

The aim of this work was to study the performances of HPH treatments, in promoting autolysis of a commercial strain of *Saccharomyces bayanus* for winemaking use, in comparison with a thermally-induced cell lysis. The effects of different HPH treatments, as well as thermolysis, were studied by measuring cell viability and the release of free amino acids, proteins and glucidic colloids in wine-like medium. Finally, the impact of the different processes on the development of volatile compounds was also investigated.

2. Materials and methods

2.1. Chemicals

Sodium chloride, tartaric acid, sodium hydroxide and ethanol (96% v/v) were purchased from Carlo Erba Reagents (Milan, Italy); *o*-phthaldialdehyde (OPA), bovine serum albumin (BSA) fraction V and HPLC grade isoleucine (Ile) were from Sigma–Aldrich (St. Louis, MO, USA); bacteriological peptone and Malt Extract Agar were from Oxoid (Basingstoke, UK).

2.2. Yeast and lysis treatments

A commercial *S. bayanus* active dry yeast (ADY) preparation (Mycoferm Cru-05, from EverIntec – Pramaggiore, VE, Italy) was used for the experimental trials. 20 g of ADY was suspended in 200 ml of distilled water and the suspension was immediately subjected to high pressure homogenization and thermolysis, as reported below.

HPH was carried out by using a two stage high pressure homogenizer (Panda PLUS 2000, Gea Niro Soavi, Parma, Italy) provided with cylindrical tungsten carbide homogenizing valves. The first valve, which is the actual homogenization stage, was set at 0, 50, 100 and 150 MPa; whereas the second valve was fixed at 5 MPa. Samples (200 ml) were homogenized *via* one single pass at 10.8 l h^{-1} flow rate. The homogenizer inlet and outlet were connected to a heat exchanger (Julabo F70, Julabo GmbH, Seelbach, Germany) set at 4 °C. The sample temperature was measured just before and immediately after homogenization by a copper-constantan thermocouple probe (Ellab, Hillerød, Denmark) connected to a portable data logger (mod. 502A1, Tersid, Milan, Italy): sample temperature at the homogenizer inlet was 19 ± 0.5 °C. As control, thermolysates (200 ml) were prepared by heating the yeast suspension at 121 °C for 2 h in autoclave.

The HPH treated samples as well as the thermolysates were stored overnight at 0/+4 °C and then analyzed for microbial viability. The remaining samples were immediately after treatments arranged in food-grade aluminum trays (approx. in a 1 cm layer), frozen at −18 °C, and freeze-dried by using a pilot plant model Mini Fast 1700 (Edwards Alto Vuoto, Milan, Italy). At the end of the process, the samples were finally ground in a ceramic mortar and stored in sealed glass containers (0/+4 °C), until chemical and GC–MS analyses. The active dry yeast preparation used for the experiments, was also subjected to all the analytical determinations reported below, as a reference sample.

2.3. Soluble proteins and free amino acids

The amounts of proteins and free amino acids soluble in wine-like solution, were determined on freeze-dried samples, respectively by Lowry method and *o*-phthaldialdehyde (OPA) derivatization. Aliquots of 1.00 g of powder were suspended in 100 ml of a hydroalcoholic-tartaric buffer (12% v/v ethanol, in 0.03 M tartaric acid, buffered at pH 3.20 with 4 M sodium hydroxide); after 10 min, the suspensions were centrifuged (5000 rpm for 10 min) and the supernatant was analyzed as reported below.

Concerning soluble proteins, 400 µl of limpid solution was subjected to the Lowry assay, as reported by Regenstein and Regenstein (1984); results were given in mg g^{-1} of dried powder, according to a calibration line prepared with bovine serum albumin (BSA).

Free amino acids were determined on the supernatant, by OPA derivatization, according to the method published by Dukes and Butzke (1998); the results were expressed in mg g^{-1} of dried powder, on the basis of a calibration line obtained with isoleucine (Ile).

2.4. Glucidic colloids

The amount of glucidic colloids soluble in wine-like medium were determined by ethanol precipitation, modifying the method reported by Usseglio-Tomasset and Castino (1975); aliquots of 1.00 g of freeze-dried powder were suspended in 10 ml of