International Journal of Food Microbiology 143 (2010) 48–53

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Antioxidant activity of lees cell surface during sparkling wine *sur lie* aging

Joan J. Gallardo-Chacón [a,b,c], Stefania Vichi [a,*], Pilar Urpí [d], Elvira López-Tamames [a], Susana Buxaderas [a]

[a] Departament de Nutrició i Bromatologia, Xarxa de Referència en Tecnologia dels Aliments (XaRTA), Facultat de Farmàcia, Universitat de Barcelona, Avda Joan XXIII, s/n, E-08028, Barcelona, Spain
[b] CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Zaragoza, Spain
[c] Centre de Recerca en Enginyeria Biomedica (CREB), Universitat Politècnica de Catalunya, c/ Pau Gargallo 5, E-08028, Barcelona, Spain
[d] Freixenet S.A. c/ Joan Sala, 2, E-08770, Sant Sadurní d' Anoia, Barcelona, Spain

ARTICLE INFO

Article history:
Received 1 March 2010
Received in revised form 9 July 2010
Accepted 17 July 2010

Keywords:
Aging
Sparkling wine
Yeast lees
Cell surface
Antioxidant
DPPH/FRAP

ABSTRACT

Given the importance of the interactions between wine and lees cell surface during sparkling wine aging, and in view of recent results proving the antioxidant potential of yeast cell wall biomolecules, the antioxidant capacity of lees cell surface was investigated to establish its possible role in the antioxidative effect of lees. The surface antioxidant activity of lees from wines with different aging periods was determined on the whole cell by two widely used methods (DPPH and FRAP assays), obtaining maximum values of 24.5 μmol Trolox/g cells (fresh weight) by the DPPH assay, and 21.3 μmol Trolox/g cells (fresh weight) by the FRAP assay. Lees surface antioxidant activity was influenced by base wine characteristics and inversely related to *sur lie* aging period. Conversely, the percentage depletion of lees surface antioxidant activity during aging was mainly determined by the length of aging, regardless of wine characteristics. To examine the influence of cell wall thiol groups and adsorbed polyphenols on lees' protective effect, their presence on cell surfaces was assessed. They accounted for 25 ± 11% and 54 ± 7% of the antioxidant activity measured by DPPH, respectively, and 0.3 ± 0.1% and 39 ± 8% measured by FRAP, respectively. Only a part of the remnant antioxidant activity of lees surface measured by FRAP could be theoretically explained by the presence of cell wall mannans.

1. Introduction

Sparkling wines obtained by the *méthode traditionnelle* are characterized by two successive fermentation processes. Yeasts for second fermentation are selected on the basis of some desirable technological attributes such as pressure tolerance, alcohol resistance, capability for growing at low temperature, low production of SO_2 and off-flavors, and flocculating ability (Suárez-Lepe, 1997; Zoecklein, 2002). Once the second fermentation has finished, cell viability decreases more than 90% in one month (Hidalgo et al., 2004) and a degradation process known as autolysis takes place (Leroy et al., 1990). Lees from the second fermentation then remain in contact with the wine during a process called *sur lie* aging (Moreno-Arribas and Polo, 2005) which lasts at least 9–12 months. The results of this practice have recently been reviewed (Caridi, 2006; Fornairon-Bonnefond et al., 2002; Pérez-Serradilla and Luque de Castro, 2008), and have indicated an increase in product structure, richness and roundness. In particular, contact with yeast lees seems to protect wine from oxidation, contributing to the prevention of browning (Caridi, 2006; Pérez-Serradilla and Luque de Castro, 2008; Palomero et al., 2009) and the development of oxidation-related volatiles (Cullere et al., 2007). Model phenolic solutions (López-Toledano et al., 2002) and sparkling wine (Bosch-Fusté et al., 2009) subjected to different accelerated oxidation tests showed a significantly lower degree of oxidative alteration when assayed in the presence of yeasts. The prevention of wine browning could be an indirect effect of the absorption of colored compounds by lees (López-Toledano et al., 2002; Razmkhab et al., 2002), but this resistance could also be ascribed to yeast-promoted protection. The protective effect of lees could be largely due to the release of intracellular compounds to the wine (Pinheiro et al., 2002; Santiago and Mori, 1993; Demasi et al., 2001), as well as to membrane lipids, which consume oxygen during wine aging, thus preventing wine oxidation (Salmon et al., 2000). Recent studies that evaluated the antioxidant activity of different cell wall fractions of spent brewer's yeast (*Saccharomyces cerevisiae*), proved the significant antioxidant activity for wall proteins and glucans (Jaehrig et al., 2007, 2008). The cell wall makes up between 25 and 50% of cell volume (Lipke and Ovalle, 1998) and consists of an inner three-dimensional network of ramified glucans and outer layer of mannoproteins (Kath and Kulicke, 1999; Gemmill and Trimble, 1999). The antioxidant activity of these wall biomolecules could also occur during *sur lie* aging contributing to prevent oxidation, and these interactions deserve to be clarified. The studies on spent brewer's yeast have demonstrated that much of yeast wall activity depends on

* Corresponding author. Tel.: +34 93 4024508; fax: +34 93 4035931.
E-mail address: stefaniavichi@ub.edu (S. Vichi).

0168-1605/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijfoodmicro.2010.07.027

the exposure of the reactive groups, such as protein aromatic side chains and thiols, to substrate (Jaehrig et al., 2007). At enological aging conditions, i.e. without cell disruption and fraction isolation, some of these biomolecules may hardly interact with the wine because they constitute inner layers of the cell wall or because intra- and intermolecular interactions make the reactive groups inaccessible. The role of lees surface in protecting wine from oxidation during *sur lie* aging should be then investigated on the entire cell, as it seems to maintain along the usual aging period (Martínez-Rodríguez et al., 2001b). Cell wall degradation during autolysis (Martínez-Rodríguez et al., 2001b; Piton et al., 1988) is expected to affect the antioxidant capacity of lees surface by improving the accessibility of the reactive groups and their exposition to the medium. However, the progressive loss of structural biomolecules (Pueyo et al., 2000; Martínez-Rodríguez and Polo, 2000; Martínez-Rodríguez et al., 2001a,b) could reduce the number of reactive groups. The balance between these phenomena would determine the effective participation of lees surface in preventing sparkling wine oxidation during *sur lie* aging. Moreover, as yeasts growing conditions influence the cell wall structure (Fornairon-Bonnefond et al., 2002), the antioxidant potential of cell surface could differ in model fermentation systems and real enological conditions. Therefore, to assess the role of lees cell surface, the antioxidant capacity during the long aging period should be determined on the whole cell and preferably at real enological conditions. As far as we know, the present paper represents the first study using this approach.

The objective of the present study is to determine the antioxidant capacity of cell surface in real lees samples obtained from sparkling wines produced at industrial scale by the *méthode traditionnelle*. The antioxidant activity of whole cells was determined in lees from sparkling wines with different *sur lie* aging periods. The identification of the molecules involved in the antioxidant effect was attempted by determining the presence and antioxidant activity of cell wall thiols and adsorbed wine polyphenols. In addition, the antioxidant activity of cell wall polysaccharides was assessed and applied to the amounts theoretically present in the yeast cell wall to estimate their possible influence on lees' protective effect.

2. Material and methods

2.1. Chemicals

Sodium acetate trihydrate, sodium carbonate, acetic acid glacial, hydrochloric acid, trifluoroacetic acid, $FeCl_3 \cdot 6H_2O$, 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl in free radical form (DPPH·), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), methanol, 4,4′-dithiodipyridine (DTDP), glutathione, ethylenediaminetetraacetic acid (EDTA), Folin-Ciocalteau reagent, and mannan from *Saccharomyces cerevisiae* were from Sigma-Aldrich (St. Louis, MO, USA). All reagents were prepared using Milli Q-deionized water.

2.2. Lees samples

Lees were obtained from industrial sparkling wines produced by Freixenet S.A. winery during *sur lie* aging. Yeasts were from the same strain of *Saccharomyces cerevisiae* belonging to the private collection of the winery and selected for its second fermentation and aging abilities.

Samples were from two distinct *coupages* of base wines (A and B). Lees were collected at the same time from wines which had aged *sur lie* for 2, 10, 18 and 40 months. Lees from two bottles were analyzed for each sample (total 8 × 2 bottles), making a total of 16 independent samples analyzed in triplicate.

Sampling points corresponded to the aging periods of Cava sparkling wine categories: Cava (9 months), Reserva (18 months) and Gran Reserva (30 months) (BOE no. 50, February, 2007), plus one sampling point in the initial stages of *sur lie* aging (2 months).

2.3. Base wines analytical determinations

The following parameters were determined in accordance with Commission Regulation (EC) 1990/2004 regarding analytical methods in oenology: L-malic acid, lactic acid, citric acid, glucose-fructose (enzymatic kits from Boehringer Mannheim, GmbH, Germany), tartaric acid, polyphenols (colorimetric methods), acidity (potentiometric method). The remaining general parameters were determined as follows: glycerol was analyzed by enzymatic kits from Boehringer Mannheim; protein was determined by the Bradford Method (Bio-Rad Laboratories, Munchen/Germany).

2.4. Lees isolation

Lees were prepared as follows: the content of 1 bottle (750 mL) was centrifuged for 15 min at 1410 × *g* at 4 °C (Rotina 48CR); the pellet was washed in 10 mL of acetate buffer (pH 3.6, 0.3 M) and resuspended in 5 mL of the buffer. The lees were maintained refrigerated under a nitrogen atmosphere. All the determinations were performed in the dark.

2.5. Cell number and dry weight determination

Each lees suspension in acetate buffer (pH 3.6, 0.3 M) was tested using a Multisizer TM Coulter Counter with a 70 μm aperture. The number of cells/mL was established by dividing the number of events by the volume of the loop (500 μL). For each suspension the measure was made in triplicate, and the mean values presented RSD < 5%.

In order to estimate the approximate mannan amount in cell wall, lees' dry weight was determined as follows: 1 mL of lees suspension in the acetate buffer was dried at 105 °C for 1 h in glass vials (tare weight known). The samples were cooled in a desiccator then weighed (the weight of 1 mL of acetate buffer was taken into account). The dry matter is expressed as mg/million cells.

2.6. Extraction of adsorbed polyphenols

Yeast polyphenols were extracted using the method described by Mazauric and Salmon (2006) modified as follows: 100 μL of the isolated lees suspension was added to a mixture of methanol/water/trifluoroacetic acid (80/20/0.05) with a final volume of 2 mL and placed in an ultrasound bath with ice for 5 min. Supernatant was isolated by centrifugation for 15 min at 1410 × *g* at 4 °C (Rotina 48CR).

2.7. Folin–Ciocalteau reagent (FCR) total phenol assay

The FCR reducing capacity of polyphenol extracts was determined by modifying the official method CEE, 2676/90. Each sample (80 μL) was diluted with 630 μL of water in a 1 mL spectrophotometer cuvette. After adding 40 μL of FCR and 100 μL of sodium carbonate 20%, the solution was mixed and incubated at room temperature for 60 min. The absorbance was read at 750 nm. The calibration curve was calculated by analyzing gallic acid solutions in the concentration range of 10–50 nmol/mL, and the results were expressed as nmol of gallic acid equivalents/million cells.

2.8. Antioxidant activity of lees surface, polyphenol extracts and reference compounds

The antioxidant activity of lees surface, polyphenol extracts and some reference compounds was evaluated using two common methodologies: the free radical scavenging activity assay using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and the reducing