Carbohydrate Polymers 80 (2010) 332–336

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Influence of ageing on lees on polysaccharide glycosyl-residue composition of Chardonnay wine

Sandra Pati [a,c,*], Maria Teresa Liberatore [b], Carmela Lamacchia [a,c], Ennio La Notte [a,c]

[a] Department of Food Science, University of Foggia, Via Napoli 25, 71100 Foggia, Italy
[b] Production Sciences, Engineering and Economics for Agricultural Systems Department, Agriculture Faculty, University of Foggia, Via Napoli 25, 71100 Foggia, Italy
[c] Istituto per la Ricerca e le Applicazioni Biotecnologiche per la Sicurezza e la Valorizzazione dei Prodotti Tipici e di Qualità, Università degli Studi di Foggia, Via Napoli 25, 71100 Foggia, Italy

ARTICLE INFO

Article history:
Received 11 September 2009
Received in revised form 3 November 2009
Accepted 9 November 2009
Available online 13 November 2009

Keywords:
Ageing on lees
Wine
Polysaccharide
Gas-chromatography
Mass spectrometry

ABSTRACT

The influence of different ageing conditions on lees, and in particular of lees obtained with and without a rough raking, on polysaccharide glycosyl residues of Chardonnay wine is addressed in this work. Methanolysis and derivatization procedures of ethanol precipitated polysaccharides, followed by gas-chromatography coupled to mass spectrometry, allowed the determination of all the main sugars known to constitute wine polysaccharides. The aging on lees led to an increase of the contents of all the glycosyl residues, except for both galacturonic acid, whose concentration slightly decreased, and glucose and myo-inositol, which were not affected by the ageing process. Results suggest that the use of aging on lees without a rough raking enriched wine of mannoproteins meanwhile contributing to the solubilization of grape polysaccharides more than aging on lees with a rough raking.

1. Introduction

Wine polysaccharides play an important role in wine-making technology for both their sensory characteristics and their technological implications. Indeed, polysaccharides have been demonstrated to affect the mouth-feel properties of wines conferring mellowness, fullness, roundness, through modulation of tannin astringency and stabilization of flavor (Taira & Ono, 1997). Besides, wine polysaccharides are well-known to be "protective colloids", liable to limit unstable substances aggregation (Ayestarán, Guadalupe, & León, 2004; Gerbaud, Gabas, Blounin, Pellerin, & Moutounet, 1997) thus promoting wine stabilization, but also preventing a good filterability (Belleville, Brillouet, Tarodo de la Fuente, & Moutounet, 1990).

Wine polysaccharides are complex mixtures deriving from grape (pectines residues) and yeast cell walls (Vidal, Doco, Moutounet, & Pellerin, 2000). Grape polysaccharides include type II arabinogalactan–proteins (AGPs), arabinans, and arabinogalactans (AGs) (Brillouet, Bosso, & Moutounet, 1990; Pellerin, Vidal, Williams, & Brillouet, 1995), rhamnogalacturonan type I (RG-I) (Vidal, Williams, Doco, Moutounet, & Pellerin, 2003) and rhamnogalacturonan type II (RG-II) (Doco & Brillouet, 1993; Pellerin et al., 1996), which can be released from the pectic network of berry cell walls under the action of several endogenous or exogenous enzymes during the earlier stages of wine-making. Yeast polysaccharides include mannoproteins and mannans (Doco & Brillouet, 1993), partially water-soluble components released by the action of β-1,3 glucanases during alcoholic fermentation and, above all, after that, when the autolysis process occurs, i.e., the natural and slow breakdown of yeast cell walls caused by hydrolytic enzymes. The presence of these mannoproteins in wines has many consequences (Caridi, 2006; Pèrez-Serradilla & Luque de Castro, 2008), such as the reduction of protein haze in white wine (Dupin et al., 2000; Moine-Ledoux & Dubourdieu, 1999; Waters, Pellerin, & Brillouet, 1994), the increase in color and tartrate stability (Escot, Feuillat, Dulau, & Charpentier, 2001), growth promotion of malolactic bacteria, inhibition of tannin aggregation (Riou, Vernhet, Doco, & Moutonnet, 2002), and modification in wine aroma (Dofour & Bayanove, 1999; Lubbers, Charpentier, Feuillat, & Voilley, 1994; Lubbers, Voilley, Feuillat, & Charpentier, 1994).

Hence, the ageing on yeast lees as oenological practice for quality wine production, consisting in letting wine to stay in the presence of resting yeast cells, is of growing interest with respect to the traditional one (ageing in stainless steel tanks without lees). The ageing on lees is generally coupled with use of barrel since the wood allows oxygen exchanges limiting reduced defects eventually promoted by the presence of lees. Also the stirring, practice

* Corresponding author. Address: Department of Food Science, University of Foggia, Via Napoli 25, 71100 Foggia, Italy. Tel.: +39 0881 589240; fax: +39 0881 589217.
E-mail address: s.pati@unifg.it (S. Pati).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.11.017

known as *batonnage*, is traditionally combined with this technique. Indeed, the *batonnage* allows the homogenization of the content facilitating the exchanges between the lees and the total wine volume. In particular, Doco, Vuchot, Cheynier, and Moutounet (2003) demonstrated that the re-suspension of lees by stirring during the ageing significantly increased the amount of macromolecules extracted into the wine. Also yeast strain (Nunez, Carrascosa, Gonzàlez, Polo, & Martìnez-Rodrìguez, 2005), turbidity of fermentation medium (Guillox-Benatier, Guerreau, & Feuillat, 1995) contact time and temperature affect polysaccharide release.

Several studies in different contexts have dealt with lees polysaccharides in wine, regarding their release by commercial enzymes during the maceration–fermentation (Ayestarán et al., 2004); the impact of oxygen consumption by yeast lees on the autolysis phenomenon (Fornairon-Bonnerford & Salmon, 2003); their evolution during the aging for over 23 years (Doco, Quellec, Moutounet, & Pellerin, 1999), characterization of new polysaccharides structures (Doco et al., 2003). However, much attention has been paid to the release and role of only an individual class of polysaccharides, that is mannoproteins (Dupin et al., 2000; Waters, Wallace, Tate, & Williams, 1993).

As concerns analytical procedures for polysaccharides determination, many chromatographic methods following their previous isolation have been proposed in the literature. Undoubtly, gas-chromatography coupled to mass spectrometry (GC–MS) after hydrolysis and monosaccharide sylilation is of general acceptance (Bleton, Mejanelle, Sansoulet, Goursaud, & Tchapla, 1996), thanks to its ability to achieve efficient separation of complex mixtures and structural characterization.

Finally, the use of different aging conditions (i.e., with and without rough raking, which in turn produce fine and coarse lees, respectively) although practically experimented by wine operators, have not been systematically studied and compared. The wine-making technology requiring no raking has different implications from a technological point of view, such as easy of operating and minor spoilage by stabilization treatments.

Therefore, the aim of this work is to widely study the influence of different ageing conditions on lees on the polysaccharides glycosyl-residue composition of Chardonnay white wine, including all the main sugars involved, with respect to the traditional one by means of gas-chromatography coupled to mass spectrometry. In particular, in this context, two wine ageing technologies, with and without raking, have been investigated.

2. Experimental

2.1. Materials

All reagents were analytical-reagent grade unless otherwise stated. L-rhamnose, D-mannose and myo-inositol were obtained from Carlo Erba (Rodano, Milan, Italy); phenyl-β-D-glucopyranoside, D-glucose, D-galactose, D-galacturonic acid, DL-arabinose, and D-xylose were of HPLC gradient grade and purchased by Fluka (Buchs, Switzerland).

The trimethylsilylation reagent (Tri-Sil®) was purchased from Pierce (Rockford, USA). Ethanol (96% v/v) and dried methanol were supplied by J.T. Baker (Phillipsburg, NJ, USA), acetyl chloride and *n*-hexane (HPLC gradient grade) by Carlo Erba (Rodano, Milan, Italy). Pure water was obtained from a Mili-Q purification system (Millipore, USA).

2.2. Wine production

The Chardonnay wine (grape harvest, 2008) was obtained from Rivera winery (Andria, Bari, Italy). Three wines have been produced, each in triplicate: a wine fermented and aged in barrique, followed by no raking operation at the end of alcoholic fermentation (LW), a wine fermented and aged in barrique, followed by a raking operation removing about 2% of lees at the end of alcoholic fermentation (RLW), and a control wine fermented and aged without lees in stainless steel containers (SSW), according to classical industrial production. Alcoholic fermentation was carried out at 12–15 °C, by adding selected *Saccharomyces cerevisiae* yeasts (20 g/hL; CM Uvaferm, Lallemand Inc.; Castel d'Azzano, VR, Italy). All wines obtained by processing in barrique were stirred every two/three days in order to re-suspend the lees and homogenize the wine. No further treatment was applied to the wine. The sampling was performed after stirring the wine, at 10 months of ageing.

2.3. Isolation, methanolysis and derivatization of polysaccharides

The fraction of soluble polysaccharides was precipitated with five volumes of ethanol (96% v/v) containing 1% HCl 1 N (Nunez et al., 2005; Segarra, Lao, Lòpez-Tamames, & Torre-Boronat de la, 1995).

After 18 h at 22 °C, the sample was centrifugated (3500 rpm for 20 min), the supernatant was discarded, the pellet washed three times with 96% ethanol and freeze-dried with a Jouan Italia centrifugal evaporator RC10-10 Thermo (Rodano Milan, Italy). Afterwards, the dried sample was added with 1 mL of dried methanol, sonicated for 5 min, and added with 35 μL of acetyl chloride (methanolysis reagent MeOH 0.5 M HCl), in order to hydrolyse monosaccharides to their corresponding methyl glycosides (Ayestarán et al., 2004). The solution was left to react for 18 h at 80 °C, then, the excess of reagent were removed by the centrifugal evaporator.

Finally, 200 μL of 1 g/L phenyl-β-D-glucopyranoside solution as internal standard and an excess of TriSil reagent (0.3 mL) were added to the dried material and left to react for 30 min at 80 °C, in order to convert the methyl glycosides forms to trimethylsilyl (TMS) derivatives. The derivatized residues were extracted with 0.5 mL of hexane and added with 0.4 mL of Mili-Q water (Millipore, USA) to obtain a phase separation. One microliter of the upper phase was injected to GC–MS according the conditions reported in Section 2.4.

Different amounts of standard carbohydrates were converted to their corresponding methyl glycosides TMS derivatives and analyzed by GC–MS (scan and SIM modes) in order to obtain fragmentation patterns and peak areas for identification and calibration.

2.4. GC–MS conditions

A 6890 N series gas chromatograph (Agilent Technologies) with an Agilent 5973 mass selective detector (MSD) and equipped with a J&W HP-5MS column (30 m, 0.25 mm i.d, 0.25 μm film thickness, Folsom, CA, USA) was used. The carrier gas was helium at a flow rate of 1.0 mL/min. The injection was made in the splitless mode, the injector temperature was 250 °C. The column oven temperature was initially held at 40 °C for 3 min, then it was programmed to 220 °C at 4 °C/min, with a final holding time of 20 min. Spectra were recorded in the electron impact mode (ionization energy, 70 eV) in a range of 30–500 amu at 3.2 scans/s; a solvent delay time of 10 min was used. Detection of analytes was accomplished by selected ion monitoring (SIM) mode using the fragment ions at 204 and 217 m/z.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) with a 95% confidence levels was carried out to test for statistically significant differences between samples by means of Statistica 6.0 software package.