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.

3. Results and discussion

Methanolysis and derivatization procedures of ethanol precipitated polysaccharides allowed the determination of glycosyl-residue composition of the polysaccharides present in wine aged in barrique on lees without previous raking, in wine aged in barrique on lees obtained after a previous raking, and in stainless steel containers. All the main sugars known to constitute wine polysaccharides (Vidal et al., 2000) have been detected, including arabinose, galactose, rhamnose, and galacturonic acid known to constitute arabinogalactans and rhamnogalacturonan coming from grape (Brillouet et al., 1990; Doco & Brillouet, 1993; Pellerin et al., 1996; Saulnier, Brillouet, Moutounet, Hervè du Penhoat, & Michon, 1992), mannose deriving from yeast cell during (Llauberes & Dubourdieu, 1987; Saulnier, Mercereau, & Vezinhet, 1991) and after fermentation (Babayan & Bezrukov, 1985; Leroy, Charpentier, Duteurtre, Feuillat, & Charpentier, 1990), glucose likely belonging to microbial polysaccharides (Dubourdieu, Ribèreau-Gayon, & Fournet, 1981), and small amounts of xylosyl residues arising from traces of hemicelluloses (Doco, Williams, Pauly, & O'Neil, 2003) from grape berry cell walls, and myo-inositol. This latter compound was present in the fraction analyzed, although little is reported in the literature about its provenience.

3.1. Wine sugar identification

The chromatogram reported in Fig. 1 highlights the presence of 20 peaks, whose attribution was achieved by means of standard injections and comparison of mass spectra with those reported in the literature (Table 1). Polysaccharides of all wines investigated contained the common sugars known to constitute wine polysaccharides, mentioned above.

Table 1
Summary of peaks obtained by GC–MS analysis of sugar trimethylsilyl derivatives.

Peak	Retention time (min)	Assignment
1	17.00	β-p-Arabinose
2	17.72	α-p-Arabinose
3	18.85	α-p-Rhamnose
4	23.66	f-Xylose
5	24.94	p-Xylose
6	32.93	α-f-Acid galacturonic
7	35.42	α-p-Mannose
8	35.92	β-f-Acid galacturonic
9	36.43	α-f-Galactose
10	37.35	β-p-Mannose
11	39.32	α-p-Galactose
12	39.50	β-f-Galactose
13	42.04	β-p-Galactose
14	42.89	α-p-Acid galacturonic
15	43.55	β-p-Acid galacturonic
16	44.17	α-p-Glucose
17	46.14	β-p-Glucose
18	47.40	Unknown
19	49.78	Unknown
20	64.51	Myo-inositol

Abbreviations: p, pyranoside; f, furanoside.

Methanolysis of one sugar leads to the formation of methyl glycosides of trimethylsilylated derivatives, where the OH group of the glycosidic carbon atom involved in the linkage is substituted by OCH_3 and the others are substituted by OTMS. Further, several products owing to the well-known anomerization (α and β-anomers) and ring isomerisation processes (pyranose and furanose ring forms) are formed, the number of glycoside peaks, their retention times and relative proportions being characteristics of each monosaccharide. Mass spectra are useful for attributing furanosides and pyranosides structures, although they cannot be used

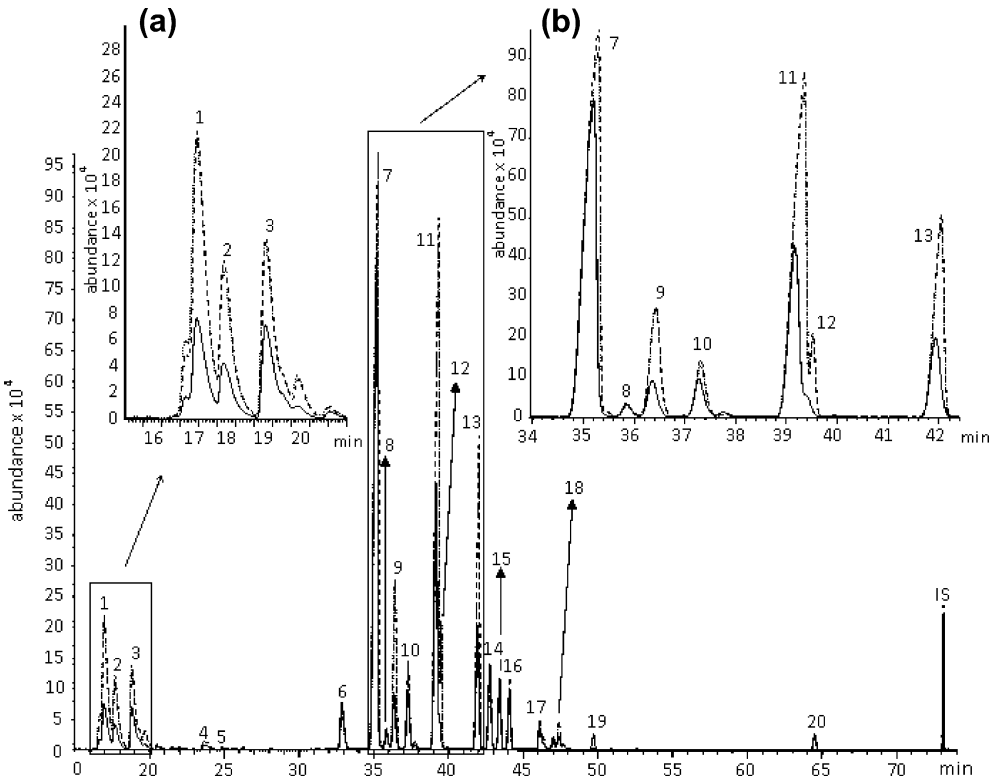

Fig. 1. Gas chromatographic profiles of the methyl glycosides of trimethylsilated sugars related to SSW (—), LW (- - -), RLW (·····). Internal standard: IS (β-phenyl glucopyranoside; 400 mg/L). Insets: (a) β-p-arabinose, α-p-arabinose, α-p-rhamnose; (b) α-p-mannose, β-f-acid galacturonic, α-f-galactose, β-p-mannose, α-p-galactose, β-f-galactose, β-p-galactose.

for the identification of α and β-anomers. Thus, assignments of these forms have been made in analogy, based on the elution order, with previous studies carried out with a chemically similar stationary phase (Doco, O'Neil, & Pellerin, 2001). Contrarily of what observed for most free sugars, methyl glycosides α-piranosides are predominant with respect to β-piranosides in all sugars except arabinose as also observed by Bleton et al. (1996), due to the strong anomeric effect of the lactol HO-group in methanolic HCl.

Mass spectra of pyranose forms (peaks 1, 2, 3, 5, 7, 10, 11, 13, 14, 15, 16, 17) were characterized by the predominant ion at m/z 204, which is known to be closely related to the ring size, thus favoured by a six-atom cyclic structure (Kennedy & Robertson, 1978); furanose forms (peaks 4, 6, 8, 9, 12) were characterized by the predominant ion at m/z 217; galacturonic acid (peaks 6, 8, 14, 15) was characterized by the ion at 234, the ion at m/z 159 of higher intensity than in the mass spectra of neutral sugars, the ion at m/z of 277 especially for the furanose forms, in accordance with other Authors (Bleton et al., 1996). Peak 18 was characterized by a high relative intensity of ion at m/z 275, peak 19 was characterized by a high relative intensity of ions at m/z 275 and 319, the major ions following the m/z 73. The presence of the odd-electron ion at m/z 234 suggests to an uronic derivative, the relatively high intensity of the ion at m/z 319 appears to be characteristic of the hexofuranosides; however the absence or low abundance of the ions at m/z 159, 230 makes the peaks not attributable to the minor species 4-*O*-methylglucuronic acid and glucurono-6,3 lactone derivatives, reported by the literature (Bleton et al., 1996; Doco, O'Neil, & Pellerin, 2001); mass spectra of these unknown species are reported in Fig. 2. No qualitative difference was observed in the gas-chromatographic profile between SSW and RLW and LW.

3.2. Sugar quantification

The insets in the Fig. 1 show chromatogram traces of glycosyl residues changing their concentration following the ageing on lees process: arabinose (peaks 1, 2, inset a), rhamnose (peak 3, inset a), mannose (peaks 7, 10, inset b), galacturonic acid (peak 8, inset b), and galactose (peaks 9, 11, 12, 13, inset b). The amounts of these compounds were quantified by using calibration graphs of sugar standards solutions. The correlation coefficients (R^2) obtained from the linear calibration graphs were all ⩾0.991, the linearity range was of 1–5 mg. The polysaccharides glycosyl residues undergone to concentration changes in SSW, LW, and RLW are reported in Table 2. Galacturonic acid was the main sugar detected, suggesting a significant presence of galacturonans in the investigated wines.

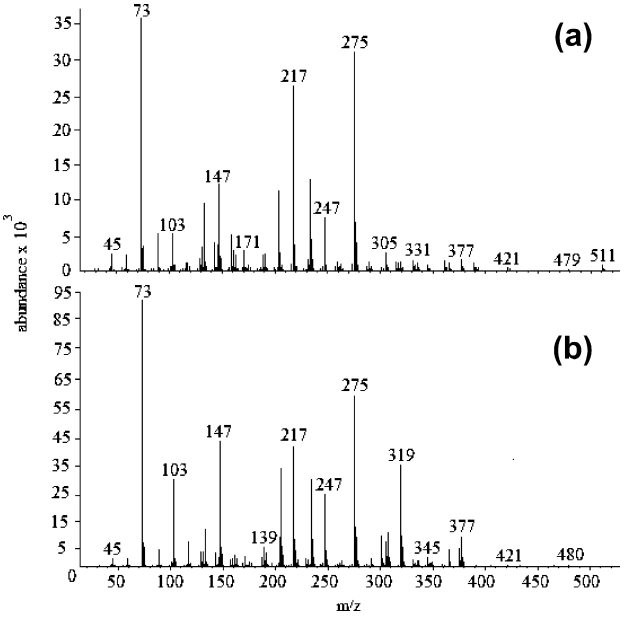

Fig. 2. Mass spectra relevant to the unknown peaks 18 and 19.

The aging on lees led to an increase of polysaccharides amount present in wine, in particular the contents of all the glycosyl residues increased, except for galacturonic acid, whose concentration slightly decreased, and both glucose and myo-inositol, which were not affected by the ageing process. Polysaccharides containing mannose can be released from yeast cell walls into the medium at the beginning of alcoholic fermentation, and by the enzymatic action producing high molecular mass mannoproteins during ageing on lees. The amount of mannoproteins released during fermentation process is similar in all wines because the yeast strain and the quantity of yeast inoculated were the same. Therefore, it is possible to calculate the amount of mannose deriving from mannoproteins released into the wine during ageing on lees. The ageing on lees produced an enrichment of mannose of about 83% and 45% for LW and RLW samples, respectively. It is expected that the removal of coarse lees by a raking operation after alcoholic fermentation reduced the release of polysaccharides. The concentration of mannose (Table 2) in the wine aged on lees was in good agreement with that found by Nunez et al. (2005).

It is worth noting that also galactose, arabinose and rhamnose increased (Table 2) following the ageing process although these monosaccharides derive from grape cell wall polysaccharides, contrarily to what observed by Doco et al. (2003), likely due to an increased solubilization of all polysaccharides, also the pectic ones. This behaviour could be due to the release into the medium of active hydrolytic enzymes following the autolysis of yeasts (Babayan & Bezrukov, 1985). The enzymatic break-down both reduces polysaccharides molecular weight and changes their composition, modifying their hydrophobic properties and consequently their solubilization. However, also the presence of "protective colloids", such as mannoproteins, can increase pectic polysaccharide solubilization. Waters et al. (1993) observed that the presence in wines of the glycoprotein termed haze-protective factor reduces the visible haziness by decreasing the particle size of the haze. According to the Authors, the improvement by the lees of the wine's thermal stability is due neither to removal of the unstable protein fractions nor to the proteolytic activities present in yeasts, but rather to the addition of yeast mannoproteins. The grape proteins responsible for the instability of white wines and containing polysaccharides moieties are thus not digested or adsorbed by the lees during ageing: they become heat-stable in the presence of a heat-stable mannoproteins (Waters et al., 1993).

As expected, LW showed the most enrichment also as concerns arabinose, rhamnose, and galactose deriving from grape.

Table 2
Concentrations of polysaccharides glycosyl residues in stainless steel aged wine, SSW, lees aged wine, LW, and lees aged wine after raking, RLW.

	SSW (mg/L)	LW (mg/L)	RLW (mg/L)
Arabinose	$25.2^{a} \pm 0.3$	$83^{b} \pm 4$	$66^{c} \pm 4$
Rhamnose	$32^{a} \pm 7$	$83^{b} \pm 9$	$63^{c} \pm 5$
Mannose	$65^{a} \pm 7$	$121^{b} \pm 11$	$92^{c} \pm 2$
Galactose	$66^{a} \pm 2$	$165^{b} \pm 5$	$158^{b} \pm 2$
Galacturonic acid	$348^{a} \pm 8$	$296^{b} \pm 6$	$304^{b} \pm 3$
Peak 18*	$0.32^{a} \pm 0.04$	$0.69^{b} \pm 0.04$	$0.712^{b} \pm 0.007$
Peak 19*	$0.48^{a} \pm 0.04$	$0.04^{b} \pm 0.03$	$0.063^{b} \pm 0.003$
Total proteins	$2.1^{a} \pm 0.3$	$7.6^{b} \pm 0.8$	$7.1^{b} \pm 0.6$

Data are expressed as means of 3 replicates ± standard deviation. SSW, stainless steel produced wine; LW, lees aged wine; RLW, lees aged wine after raking.
[a,b,c]Means in the same row followed by different letters differ significantly (one-way variance analysis, $P < 0.05$).
* Data are expressed as relative peak area obtained as area normalized to the peak area of internal standard (β-phenyl glucopyranoside, 400 mg/L).

Galacturonic acid slightly decreased following the ageing on lees. This could be due to a minor solubility of galacturonans with respect to heteropolysaccharides deriving from the enzymatic break-down of the original pectic polysaccharides and/or to a minor interaction with mannoproteins which are known to protect mostly proteins, likely also the ones containing sugar moieties. Interestingly, the area of the unknown peak 18 increased following the ageing on lees and that one of peak 19 decreased (Table 2), the two peaks being probably correlated since structurally similar, as resulted from mass spectra (Fig. 2). No statistically significant difference was observed between RLW and LW.

Dupin, McKinnon et al. (2000) showed that wine aged on yeast lees has lower haze potential and consequently lower bentonite requirements for stability than wine aged without lees but containing the same level of proteins. Both a mixture of arabinogalactan–proteins and mannoproteins and rhamnogalacturonan-II have been demonstrated to increase the fullness sensation above that of the base wine (Vidal et al., 2004). Besides, rhamnogalacturonan-II fraction has been shown to significantly decrease the astringency of the base wine (Riou et al., 2002), suggesting thus that several classes of polysaccharides have similar important roles. Therefore, it is reasonable hypothesizing that all polysaccharides have a key role in improving wine quality.

In conclusion, this work demonstrated that the ageing on lees process enriches wine of several polysaccharides, also the ones deriving from pectines. The enzymatic break-down of original polysaccharides changing their hydrophobic properties together with the presence of the protective colloids mannoproteins likely improve polysaccharide solubilization. Furthermore, the wine technology using no partial removal of lees enriched wine more than when rough raking is applied. As no raking is required, this wine-making technology offers other undoubted advantages, such as easy of operating and minor spoilage by stabilization treatments.

Acknowledgements

We thank Leonardo Palumbo, oenologist at “Rivera” winery and President of the Oenologist Association of Apulia, Basilicata and Calabria, for his expertise and the supply of Chardonnay white wine samples.

References

Ayestarán, B., Guadalupe, Z., & León, D. (2004). Quantification of major grape polysaccharides (Tempranillo v.) released by maceration enzymes during the fermentation process. *Analytica Chimica Acta, 513*, 29–39.

Babayan, T. L., & Bezrukov, M. G. (1985). Autolysis in yeast. *Acta Biotechnologica, 2*, 129–136.

Belleville, M. P., Brillouet, L. M., Tarodo de la Fuente, B., & Moutounet, M. (1990). Polysaccharide effects on cross-flow microfiltration of two red wines with a microporous alumina membrane. *Journal of Food Science, 55*, 1598–1602.

Bleton, J., Mejanelle, P., Sansoulet, J., Goursaud, S., & Tchapla, A. (1996). Characterization of neutral sugars and uronic acids after methanolysis and trimethylsilylation for recognition of plant gums. *Journal of Chromatography A, 720*, 27–49.

Brillouet, J. M., Bosso, C., & Moutounet, M. (1990). Isolation, purification and characterization of an arabinogalactan from red wine. *American Journal of Enology and Viticulture, 41*, 29–36.

Caridi, A. (2006). Enological functions of parietal yeast mannoproteins. *Antonie van Leeuwenhoek, 89*, 417–422.

Doco, T., & Brillouet, J. M. (1993). Isolation and characterization of a rhamnogalacturonan II from red wine. *Carbohydrate Research, 243*, 333–343.

Doco, T., O'Neil, M. A., & Pellerin, P. (2001). Determination of the neutral and acid glycosyl-residue compositions of plant polysaccharides by GC–EI–MS analysis of trimethylsilyl methyl glucoside derivatives. *Carbohydrate Polymers, 46*, 249–259.

Doco, T., Quellec, N., Moutounet, M., & Pellerin, P. (1999). Polysaccharide patterns during the ageing of Carignan noir red wines. *American Journal of Enology and Viticulture, 50*, 26–32.

Doco, T., Vuchot, P., Cheynier, V., & Moutounet, M. (2003). Structural modification of arabinogalactan–proteins during aging of red wines on lees. *American Journal of Enology and Viticulture, 54*, 150–157.

Doco, T., Williams, P., Pauly, M., & O'Neil, M. A. (2003). Polysaccharides from grape berry cell walls. Part II: Structural characterization of the xyloglucan polysaccharides. *Carbohydrate Polymers, 53*, 253–261.

Dofour, C., & Bayanove, C. L. (1999). Influence of wine structurally different polysaccharides on the volatility of aroma substances in a model system. *Journal of Agricultural and Food Chemistry, 47*, 671–677.

Dubourdieu, D., Ribèreau-Gayon, P., & Fournet, B. (1981). Structure of the exocellular β-D-glucan from *Botritis cinerea*. *Carbohydrate Research, 93*, 294–299.

Dupin, I., McKinnon, B. M., Ryan, C., Boulay, M., Markides, A., Jones, G., et al. (2000). *Saccharomyces cerevisiae* mannoproteins that protect wine from protein haze: Their release during fermentation and lees contact and a proposal for their mechanism of action. *Journal of Agricultural and Food Chemistry, 48*, 3098–3105.

Dupin, I., Stockdale, V., Williams, P., Jones, G., Markides, A., & Waters, E. (2000). *Saccharomyces cerevisiae* mannoproteins that protect wine from protein haze: Evaluation of extraction methods and immunolocalization. *Journal of Agricultural and Food Chemistry, 48*, 1086–1095.

Escot, S., Feuillat, M., Dulau, L., & Charpentier, C. (2001). Release of polysaccharides by yeast and the influence of released polysaccharides on colour stability and wine astringency. *Australian Journal of Grape and Wine Research, 7*, 153–159.

Fornairon-Bonnerford, C., & Salmon, J. M. (2003). Impact of oxygen consumption by yeast lees on the autolysis phenomenon during simulation of wine aging on lees. *Journal of Agricultural and Food Chemistry, 51*, 2584–2590.

Gerbaud, V., Gabas, N., Blounin, J., Pellerin, P., & Moutounet, M. (1997). Influence of wine polysaccharides and polyphenols on the crystallization of potassium hydrogen tartrate. *Journal of International Science Vigne Vin, 31*, 65–83.

Guillox-Benatier, M., Guerreau, J., & Feuillat, M. (1995). Influence of initial colloid content on yeast macromolecule production and on the metabolism of wine microorganisms. *American Journal of Enology and Viticulture, 46*, 486–492.

Kennedy, J. F., & Robertson, S. M. (1978). Mass-spectrometric fragmentation pathways of the *O*-trimethylsilyl derivatives of hexuronic acids and their lactones. *Carbohydrate Research, 67*, 1–15.

Leroy, M. J., Charpentier, M., Duteurtre, B., Feuillat, M., & Charpentier, C. (1990). Yeast autolysis during Champagne aging. *American Journal of Enology and Viticulture, 41*, 21–28.

Llauberes, R. M., & Dubourdieu, D. (1987). Exocellular polysaccharides from *Saccharomyces cerevisiae*. *Journal of the Science of Food and Agriculture, 41*, 277–286.

Lubbers, S., Charpentier, C., Feuillat, M., & Voilley, A. (1994). Influence of yeast walls on the behaviour of aroma compounds in a model wine. *American Journal of Enology and Viticulture, 45*, 29–33.

Lubbers, S., Voilley, A., Feuillat, M., & Charpentier, C. (1994). Influence of mannoproteins from yeast on the aroma intensitè of a model wine. *Lebensmittel-Wissenschaft und Technologie, 27*, 108–114.

Moine-Ledoux, V., & Dubourdieu, D. (1999). An invertase fragment responsible for improving the protein stability of dry white wines. *Journal of the Science of Food and Agriculture, 79*, 537–543.

Nunez, Y. P., Carrascosa, A. V., Gonzàlez, R., Polo, M. C., & Martìnez-Rodrìguez, A. J. (2005). Effect of accelerated autolysis of yeast on the composition and foaming properties of sparkling wines elaborated by a Champenoise method. *Journal of Agricultural and Food Chemistry, 53*, 7232–7237.

Pellerin, P., Doco, T., Vidal, S., Williams, P., Brillouet, J. M., & O'Neil, M. A. (1996). Structural characterization of red wine rhamno-galacturonan II. *Carbohydrate Research, 190*, 183–197.

Pellerin, P., Vidal, S., Williams, P., & Brillouet, J. M. (1995). Characterization of five type II arabinogalactan–protein complexes from red wine with increasing uronic acid content. *Carbohydrate Research, 277*, 135–143.

Pèrez-Serradilla, J. A., & Luque de Castro, M. D. (2008). Role of lees in wine production: A review. *Food Chemistry, 111*, 447–456.

Riou, V., Vernhet, A., Doco, T., & Moutonnet, M. (2002). Aggregation of grape seed tannins in model wine-effect of wine polysaccharides. *Food Hydrocolloids, 16*, 17–23.

Saulnier, L., Brillouet, J. M., Moutounet, M., Hervè du Penhoat, C., & Michon, V. (1992). New investigation of the structure of grape arabinogalactan–protein. *Carbohydrate Research, 224*, 219–235.

Saulnier, L., Mercereau, T., & Vezinhet, F. (1991). Mannoproteins from flocculating and non-flocculating *Saccharomyces cerevisiae* yeast. *Journal of the Science of Food and Agriculture, 54*, 275–286.

Segarra, I., Lao, C., Lòpez-Tamames, E., & Torre-Boronat de la, M. C. (1995). Spectrophotometric method for the analysis of polysaccharides levels in winemaking products. *American Journal of Enology and Viticulture, 46*, 564–570.

Taira, S., & Ono, M. (1997). Reduction of astringency in persimmon caused by adhesion oif tannins to cell wall fragments. *Acta Horticultura, 436*, 235–241.

Vidal, S., Doco, T., Moutounet, M., & Pellerin, P. (2000). Soluble polysaccharide content at initial time of experimental must preparation. *American Journal of Enology and Viticulture, 51*, 115–121.

Vidal, S., Francis, L., Williams, P., Kwiatkowski, M., Gawel, R., Cheynier, V., et al. (2004). The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chemistry, 85*, 519–525.

Vidal, S., Williams, P., Doco, T., Moutounet, M., & Pellerin, P. (2003). The polysaccharides of red wine: Total fractionation and characterization. *Carbohydrate Polymers, 54*, 439–447.

Waters, E., Wallace, W., Tate, M., & Williams, P. (1993). Isolation and partial characterization of a natural haze protective factor from wine. *Journal of Agricultural and Food Chemistry, 41*, 724–730.

Waters, E. J., Pellerin, P., & Brillouet, J. M. (1994). A Saccharomices mannoprotein that protects wines from protein haze. *Carbohydrate Polymers, 23*, 185–191.