Ms. Ref. Nº.: FOODCHEM-D-10-02123R1
Title: Quantitative determination of wine polysaccharides by gas chromatography-mass spectrometry (GC-MS) and size exclusion chromatography (SEC)

Highlights:

This study evaluates the suitability of GC-MS for determining the content of wine polysaccharides and compares the results with GC-FID and HRSEC-RID.

Good values of LOD, LOQ, repeatability, reproducibility and overall recoveries were achieved for the GC-MS method.

GC-MS showed to be more sensitive and selective than FID and provided unambiguous quantification of wine monosaccharides.

HRSEC-RID could serve as a rapid and simple method for estimating the global content of wine polysaccharides but it does not provide information about the concentration of specific polysaccharide families.

Quantitative determination of wine polysaccharides by gas chromatography-mass spectrometry (GC-MS) and size exclusion chromatography (SEC)

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Abstract

Wine polysaccharides play an important role on a number of technological and quality properties of wines and thus several methods have been proposed for their quantification. The present study evaluates the suitability of gas chromatography with mass spectrometry detector (GC-MS) for determining the content of wine monosaccharides and thus polysaccharide families. Factors affecting the yield of polysaccharide precipitation were firstly evaluated and the GC-MS method was characterized and compared with the previously reported GC-FID method. Repeatability and reproducibility values were similar in both methods, with values ranging from 1 to 14%. LODs obtained by MS were below 1.0 µg for all monosaccharides and LOQs were below 1.8 µg. Moreover, a recovery study of the whole method was carried out and it provided absolute recoveries between 81 and 116% for different wine samples, very good values taking into account the multi-step procedure. Both GC-MS and GC-FID were applied to determine the content of wine polysaccharide families in three wine samples and no significant differences were observed. Finally, high-resolution size exclusion chromatography with refractive index detector (HRSEC-RID) was applied to obtain the molecular weight distributions of the wine polysaccharides and to estimate their global content. The correlation observed between the polysaccharide values obtained with the GC method and the HRSEC-RID method (r = 0.746, p < 0.05) indicated that the latter could serve as a rapid and simple method to give an estimation of total wine polysaccharides although it can not be used to quantify in a precise way.

***Keywords*:** wine; monosaccharides; polysaccharides; GC-MS; GC-FID; HRSEC-RID

1. Introduction

Wine and must polysaccharides are a subject of a number of studies since they play an important role on a number of technological and quality properties of wines. Considered as *protective colloids*, they can significantly modify several colloidal phenomena during the winemaking process, such as tartrate salt crystallization (Gerbaud et al., 1996; Gerbaud, Gabas, Blouin, Pellerin & Moutounet, 1997; Lubbers, Leger, Charpentier & Feuillat, 1993; Moine-Ledoux & Dubourdieu, 2002), protein haze (Dupin, Stockdale, Williams, Jones, Markides & Waters, 2000a; Dupin et al., 2000b; González-Ramos & González, 2006; Moine-Ledoux & Dubourdieu, 1999; Waters, Pellerin & Brillouet, 1994), retention of aromatic compounds (Chalier, Angot, Delteil, Doco & Gunata, 2005; Dufour & Bayonoue, 1999; Lubbers, Voilley, Feuillat & Charpentier, 1994; Wolz, 2005), colour stabilization (Escot, Feuillat, Dulau & Charpentier, 2001; Feuillat, Escot, Charpentier & Dulau, 2001; Fuster & Escot, 2002; Saucier, Glories & Roux, 2000), or tannin aggregation and precipitation (Guadalupe, Palacios & Ayestarán, 2007a; Saucier, Glories & Roux, 2000; Vidal et al., 2004; Wolz, 2005).

Polysaccharides in wine originate mainly from grape primary cell walls and microorganisms acting during the winemaking. The main polysaccharides coming from grape berries cell walls are arabinans and arabinogalactan-proteins (AGP), homogalacturonans (HL) and rhamnogalacturonans (RG-I and RG-II) whereas those released by microorganisms are mainly mannans and mannoproteins (MP) produced by yeasts during alcoholic fermentation or aging on lees, and glucans produced by *Botrytis cinerea* on infected grapes. Exogenous polysaccharides such as arabic gum and carboxymethyl cellulose could also be present in several commercial wines as they are authorized as additives.

Not all polysaccharides show the same behaviour with respect to wines and their influence on wine will depend not only on their quantity but also on the type of polysaccharide. It has been

shown that AGP have greater influence on the filtration procedures than MP (Ribéreau-Gayon, Glories, Maujean & Dubourdieu, 2002), which are more efficient at reducing protein haze in white wines (Dupin et al., 2000b; Moine-Ledoux & Dubourdieu, 1999; Waters, Pellerin & Brillouet, 1994). RG-I and II inhibit hydrogen tartrate crystallization (Gerbaud, Gabas, Blouin, Pellerin & Moutounet, M., 1997) whereas AGP do not affect this phenomenon (Ribéreau-Gayon, Glories, Maujean & Dubourdieu, 2002). Among the MP classes present in wine, some have been found to act as protective factors with regards to tartaric acid precipitation (Gerbaud, Gabas, Blouin, Pellerin & Moutounet, 1997; Moine-Ledoux & Dubourdieu, 1999) and it has also been described that the dimer RG-II/boron can form complexes with di- and trivalent cations which could reduce the level of toxic cations (e.g., Pb^{2+}) in wines (Pérez, Rodríguez-carvajal & Doco, 2003; Vidal et al., 2000a). Regarding to wine sensory properties, RG-II dimer seems to favor the self-aggregation of grape seed proanthocyanidins in wine-like solutions, whereas wine MP, acidic AGP and other ionic carbohydrates tend to inhibit tannin aggregation (Carvalho, Mateus, Plet, Pianet, Dufourc & de Freitas, 2006; de Freitas, Carvalho & Mateus, 2003; Mateus, Carvalho, Luis & de Freitas, 2004; Riou, Vernhet, Doco & Moutounet, 2002), and therefore have a different influence on wine astringency and fullness (Vidal et al., 2004).

To determine the content of grape, must or wine polysaccharides, all the methods proposed begin with an extraction step by either direct precipitation with ethanol-acid, concentration-precipitation, dialysis or ultrafiltration. After the extraction step, two alternatives can be chosen to analyze the polysaccharides in the extract: a) using rapid, simple and global methods for total polysaccharide quantification, b) using more complex and time-consuming methods to quantify specific monosaccharides present in the wine. Direct quantification of wine polysaccharides are usually based on the precipitation of total wine colloids, followed by colorimetric assays (Segarra, Lao, López-Tamames & de la Torre-Boronat, 1995) or by the

74 determination of peak areas by size-exclusion chromatography (Dubourdieu, Llauberes &
75 Ollivier, 1986; López-Barajas, López-Tamames & Buxaderas, 1998; Palomero, Morata,
76 Benito, González & Suárez-Lepe, 2007; Palomero, Morata, Benito, Calderón & Suárez-Lepe,
77 2009). However, these global methods do not allow the quantification of the different families
78 of polysaccharides present in wines, which can be estimated by assessing its monosaccharide
79 profile. Several methods have been proposed for the identification and quantification of grape
80 and wine monosaccharides: High performance anion exchange chromatography with pulsed
81 amperometric detection (HPAEC-PAD) (Arnous & Meyer, 2009), Fourier transform infrared
82 spectroscopy (FTIR) (Boulet, Williams & Doco, 2007; Coimbra, Barros, Coelho, Gonçalves,
83 Rocha & Delgadillo, 2005) and Gas chromatography (GC).
84 When GC is used two different detectors have been used: flame ionization detector (FID) and
85 mass spectrometry detector (MS). Although MS is expected to be more sensitive and selective
86 than FID, wine monosaccharide derivatives are usually quantified by GC-FID (Ayestarán,
87 Guadalupe & León, 2004; Chalier, Angot, Delteil, Doco & Gunata, 2005; Doco, Quellec,
88 Moutounet & Pellerin, 1999; Doco, Vuchot, Cheynier & Moutounet, 2003; Doco, Williams &
89 Cheynier, 2007; Dols-Lafargue, Gindreau, Le Marrec, Chambat, Heyraud & Lonvaud-Funel,
90 2007; Guadalupe & Ayestarán, 2008; Nuñez, Carrascosa, González, Polo & Martínez-
91 Rodríguez, 2005; Nuñez, Carrascosa, González, Polo & Martínez-Rodríguez, 2006; Nuñez,
92 Pueyo, Carrascosa & Martínez-Rodríguez, 2008; Vicens, Fournand, Williams, Louise,
93 Moutounet & Doco, 2009; Vidal, Doco, Pellerin & Moutounet, 2000b; Vidal, Williams,
94 O´Neill & Pellerin, 2003) while GC-MS is generally reported to confirm the identity of each
95 peak (Ayestarán, Guadalupe & León, 2004; Doco, Quellec, Moutounet & Pellerin, 1999;
96 Doco, O´Neill & Pellerin, 2001). Previous studies of our workgroup have described the
97 quantification of wine polysaccharides by GC-FID and reported the characteristics of the
98 method (Ayestarán, Guadalupe & León, 2004), but to our knowledge, the suitability of GC-

MS for direct quantification of wine monosaccharide derivatives has not been reported. Therefore, the aim of the present study was to evaluate the suitability of the GC-MS detection for determining the content of wine monosaccharides and polysaccharides. The proposed method was characterized in terms of linearity, detection and quantification limits and repeatability and reproducibility, comparing these results with those previously obtained with the GC-FID method. In order to evaluate the accuracy of the method a recovery study was carried out by using commercial polysaccharides. Moreover, the monosaccharide and polysaccharide composition in three red wines was determined using the GC-MS detection and the FID, and the results were compared. Finally, the potential use of size-exclusion chromatography with refractive index detector (HRSEC-RID) as a simple and rapid method for estimating total polysaccharides in wine samples was evaluated.

2. Materials and Methods

2.1. Chemicals

All reagents were analytical grade unless otherwise stated. Standards of different monosaccharides were used to perform the calibration curves. L-fucose, L-rhamnose, 2-*O*-methyl D-xylose, L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose, Kdo (3-deoxy octulosonic acid) and D-apiose solution were supplied by Sigma-Aldrich (Beerse, Belgium), and D-galacturonic acid, D-glucuronic acid and myo-inositol (internal standard) were obtained from Fluka (Buch, Switzerland).

Ethanol 96% (v/v), hexane and acetyl chloride were supplied by Scharlab (Barcelona, Spain), hydrochloric acid 37% was purchased from Carlo Erba (Rodano, Milan, Italy), and dried methanol, pyridine, hexamethyldisilazane and trimethylclorosilane were obtained by Merck (Darmstadt, Germany). Lithium nitrate of HPLC grade supplied by Sigma (Beerse, Belgium) and MilliQ deionized water (Millipore, Molsheim, France) were used. A pullulan calibration

kit (Shodex P-82) was obtained from Waters (Barcelona, Spain). All the solutions were filtered through a 0.45 µm filter before use in the HPLC.

2.2. Equipments

High-resolution size-exclusion chromatography (HRSEC) was performed using a modular 1100 Agilent liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with one G1311A quaternary pump, an on-line G1379A degasser, a G1316A column oven, a G1362 refractive index detector, a G1313A automatic injector, and controlled by the Chemstation Agilent software.

The gas chromatography (GC) system controlled by the Chemstation software and equipped with a 7653B automatic injector consisted of an Agilent 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany) coupled to a 5975C VL quadrupole mass detector (MS) and a flame ionization detector (FID).

2.3. Samples

Commercial polysaccharides were purchased from Lallemand (Lallemand-Inc., Montreal, Canada). Wine samples were elaborated in the wine cellar of Juan Carlos Sancha S.L. (Baños de Río Tobia, La Rioja, Spain) using grapes harvested on the vintage 2008. Three grape varieties from the qualified origin denomination Rioja (D.O.Ca. Rioja) were used: *Vitis Vinífera* L. Cv. red Tempranillo (Wine 1), Monastel (Wine 2) and red Maturana (Wine 3). For the red winemaking, grapes were destemmed and distributed into 500 L French oak barrels, sulphited with 3 g/HL SO_2 and inoculated with 25 g/HL *S. cerevisiae* yeast strain. The prefermentation process went on for 24 h at 12 ± 1ºC; the alcoholic fermentation–maceration process was carried out at a maximum temperature of 28 ± 2ºC and lasted 10 days. During this period, barrels were battonaged sixteen times a day. Wines were then racked and introduced in the same 500 L French oak barrels and samples were taken and analyzed. All vinifications were carried out in triplicate.

148 *2.4. Precipitation of total soluble wine polysaccharides*

149 Wine polysaccharides were recovered by precipitation after ethanolic dehydration. Samples
150 were centrifuged (14,000 rpm for 5 min) using a RC-5B Sorvall refrigerated centrifuge (Du
151 Pont, BH, Germany) and 2.5 mL of the supernatants were taken and introduced into 15 mL
152 falcon-tubes. Three assays were then carried out: a) polysaccharides were directly precipitated
153 by adding 10 mL of cold acidified ethanol (ethanol of 96% containing HCl 0.3 M) and kept
154 for 18h at 4 ºC (non-concentrated samples); b) the supernatants were concentrated to dryness
155 in a Jouan RC10-10 centrifugal evaporator (Fisher Scientific, Madrid, Spain), the residues
156 were dissolved in 0.5 mL of water to obtain wine concentrated five times and then 2.5 mL of
157 cold acidified ethanol was added and kept for 18h at 4 ºC (samples concentrated five times);
158 c) the supernatants were concentrated to dryness in a centrifugal evaporator and the residues
159 directly precipitated with 2.5 mL of cold acidified ethanol and kept for 18h at 4 ºC (samples
160 concentrated to dryness). Thereafter, all the samples were centrifuged (14,000 rpm for 20
161 min), the supernatants discarded, and the pellets washed several times with 96% ethanol to
162 remove the interference materials. The precipitates were finally dissolved in ultrapure water
163 and freeze-dried using a Virtis freeze drying (New York, USA). The freeze-dried precipitates
164 obtained contained the total soluble polysaccharides (TSP).

165 This polysaccharide extraction was performed in triplicate in each sample.

166 *2.5. Identification and quantification of monosaccharides by GC-MS and GC-FID*

167 The monosaccharide composition of the TSP precipitates was determined by GC-MS and GC-
168 FID of their trimethylsilyl-ester *O*-methyl glycolsyl-derivades (TMS) obtained after acidic
169 methanolyisis and derivatization.

170 TSP fractions were treated with 1.5 mL of the methanolysis reagent (MeOH containing HCl
171 0.5 M) in order to hydrolyze neutral and acidic monosaccharides to their corresponding
172 methyl glycosides. The reaction was conducted in nitrogen atmosphere at 80 ºC for 16 hours

and thereafter the excess of reagent was removed using a stream of nitrogen gas. The conversion of the methyl glycosides to their trimethylsilyl (TMS) derivatives was performed by adding 0.5 mL of a mix pyridine: hexamethyldisilazane: trimethylclorosilane (10:2:1 v/v) to the dried material. The reaction was carried out at 80 ºC for 30 minutes and the reagent removed using a stream of nitrogen gas. A solution (25 µL) of derivatized myo-inositol was then added as internal standard and the derivatized residues were extracted with 1 mL of hexane. GC-MS and GC-FID was performed with 1 µL of these solutions and samples were injected in duplicate. Different standard carbohydrates were also converted to their corresponding TMS derivatives and analyzed by GC-MS and GC-FID in order to obtain patterns for identification and the standard calibration curves.

The chromatographic column was a Teknokroma fused silica capillary column (30 m × 0.25 mm × 0.25 µm) of phase 5% phenyl - 95% methylpolysiloxane. The oven program started at an initial temperature of 120 °C which was increased at a rate of 1 ºC min^{-1} to 145 °C and then to 180 ºC at a rate of 0.9 °C min^{-1} and finally to 230 ºC at 40 °C min^{-1}. The GC injectors were equipped with a 3.4 mm I.D. and were maintained at 250 ºC with a 1:20 split ratio. The carrier gas was helium (99.996%) at a flow rate of 1 mL min^{-1}. Ionisation was performed by electron impact (EI) mode at 70 eV. The temperatures used were 150 °C for the MS Quad, 230 ºC for the MS Source, and 250 °C for the transfer line.

2.6. Analysis of polysaccharides by HRSEC-RID

The high-resolution size-exclusion chromatography (HRSEC) system with a refractive index detector was used to obtain the molecular weights and molecular weight distributions of the wine polysaccharides. Two serial Shodex OHpack KB-803 and KB-805 columns (0.8 x 30 cm, Showa Denko, Japan) were used. TSP precipitates were dissolved in 2.5 mL of $LiNO_3$, filtered through a membrane with a 0.45 µm pore size, and 100 µL was injected and eluted with a 0.1 M solution of $LiNO_3$ at a flow rate of 1 mL min^{-1}. Calibration was performed with

198 narrow pullulan molecular weight standards (Shodex P-82, Waters, Barcelona, Spain): P-5,
199 Mw = 5.9 KDa; P-10, Mw = 11.8 KDa; P-20, Mw = 22.8 KDa; P-50, Mw = 47.3 KD; P-100,
200 Mw = 112 KDa; P-200, Mw = 212 KDa; P-400, Mw = 404 KDa.

201 *2.7. Statistical analysis*

202 Performance of the method and significant differences between samples were evaluated by an
203 analysis of variance (ANOVA). Statistical evaluations were performed using the SPSS 15.0
204 program for Microsoft Windows (SPSS Inc., Chicago, IL).

205 **3. Results and Discussion**

206 *3.1. Extraction of wine polysaccharides*

207 Wine samples were first centrifuged to remove insoluble material and three assays were then
208 carried out: a) direct precipitation of polysaccharides in non-concentrated samples, b)
209 precipitation of polysaccharides in samples concentrated five times, and c) precipitation of
210 polysaccharides in samples concentrated to dryness. Previous studies had proven the
211 concentration step to be critical for the quantitative precipitation of all soluble
212 polysaccharides since some polysaccharide families seemed to be precipitated only partially
213 in non-concentrated wines (Ayestarán, Guadalupe & León, 2004; Doco, Quellec, Moutounet
214 & Pellerin, 1999). Concentration by filtration could cause the loss of material on microfilters
215 and heating was avoided to prevent from degradations or losses. Samples were thus
216 concentrated in a centrifugal evaporator with controlled temperature being less than 35 ºC.
217 Concentrated samples showed higher concentrations in all monosaccharides than non-
218 concentrated samples indicating that non-concentration could lead to an underestimation.
219 Besides, HRSEC-RID of non-concentrated samples provided very small areas in all the peaks
220 corresponding to high and low molecular weight polysaccharides, to the point that some peaks
221 were even lost (data not shown). When comparing samples concentrated to dryness with
222 samples concentrated five times, the former showed higher concentrations of

monosaccharides, which means higher signal/noise ratio in the GC-MS chromatogram and thus higher sensitivity for the lower concentrated monosaccharides. When analyzed by HRSEC-RID, both sample preparations showed identical areas in the peaks corresponding to high molecular weight polysaccharides but signals corresponding to low molecular weight compounds were significantly lower in samples concentrated five times, indicating that the precipitation of oligosaccharides and small fragments of wine polysaccharides had been affected by the concentration step. In conclusion, concentration of samples is recommended in order to achieve the quantitative determination of all wine polysaccharides. Concentration to dryness was chosen in order to simplify the method and obtain higher monosaccharide responses, although five-times concentrated samples also ensured the quantitative precipitation of wine polysaccharides. Anyway, and regardless the concentration-step chosen, all the samples must be treated in the same way for comparative purposes.

3.2. Identification and quantification of glycosyl residues by GC-MS detection

The monosaccharide composition of the TPS fractions was determined by GC-MS of their trimethylsilyl (TMS) residues. The identification of the peaks was carried out by comparing retention times and mass spectra with those obtained by injections of pure standards. Typical GC-MS chromatogram for a wine polysaccharide extract is shown in Figure 1.

In order to be able to quantify the monosaccharides in the GC-MS chromatograms, calibration curves of monosaccharide standards were needed. Standard curves of L-fucose, L-rhamnose, 2-*O*-methyl D-xylose, L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose, Kdo (3-deoxy octulosonic acid), D-galacturonic acid and D-glucuronic acid were used for monosaccharide quantification and myo-inositol was used as internal standard. MS fragmentation patterns reported in Refs. (Doco, O´Neill & Pellerin, 2001) were used to identify those monosaccharides for which no commercial standards were available. 2-*O*-methyl fucose, aceric acid and Dha (3-deoxy-D-*lyxo*-heptulosaric acid) and apiose were

quantified using the 2-*O*-methyl xylose calibration curve. The different monosaccharides were quantified in selected ion monitoring (SIM) mode, selecting the appropriate number of ions for each compound (*m*/*z*) in one segment from 3 to 65 minutes. D-galacturonic acid, L-rhamnose, L-fucose, D-galactose, D-glucose, D-mannose and D-xylose with 204 ion and D-glucuronic acid, L-arabinose, Kdo, 2-O-methyl-L-fucose, Dha and aceric acid with 217 ion, 2-O-methyl D-xylose with 146 ion, apiose with 191 and myo-inositol (internal standard) with 305 ion. For all the spectra, these ions showed the highest signal/noise ratio and were selected for recording SIM mode chromatograms.

3.3. Performance of the GC-MS detection

The features of the GC-MS detection including equation, the slope with its standard deviation, the correlation coefficients (r^2), the linear range and the limits of detection (LOD) and quantification (LOQ), for the carbohydrate standards are listed in Table 1. The features of the GC-MS method were established after a linearity study using solutions of standard carbohydrates. The analyte to internal standard peak area ratio was used as analytical signal for constructing the calibration graphs. The limit of detection was calculated as the concentration of a signal to noise of three and the limit of quantification from the signal to noise of ten.

The correlation coefficients obtained from the calibration curves were all higher than 0.96 ($p < 0.001$). These curves were, therefore, considered to be linear for the range of concentrations studied (to 1500 µg for majority monosaccharides, i.e., arabinose, rhamnose, mannose galactose, galacturonic acid and glucose, around 500 µg for fucose and glucuronic acid, and 125 to 270 µg for the rest of monosaccharides). The limits of detection and quantification were good and in all the cases they were below the values obtained for the monosaccharides present in the wine samples.

A validation of the proposed method was carried out by analyzing real wine samples. The precision of the GC-MS method was checked in terms of repeatability and reproducibility (Table 1) by means of an analysis of variance (ANOVA). Repeatability was evaluated by the analysis of 6 aliquots of the same wine under normal operating conditions and it was expressed as relative standard deviation values (inter-wine standard deviation). The reproducibility of the method was expressed as the relative standard deviation values obtained for the three different wines (intra-wine standard deviation). As described in the experimental section, samples were centrifuged and the supernatants concentrated to dryness and precipitated. The residues obtained were freeze-dried, methylated, derivatizated and submitted to GC-MS analysis to calculate the amount of carbohydrates. Both repeatability and reproducibility values were good taking into account that a multi-step procedure was performed with values ranging between 2-12% and 1-14%, respectively.

In order to test the accuracy of the whole method, a recovery study was also carried out (Table 2). Recovery studies were accomplished with the three wine samples by using a commercial wine polysaccharide composed of galactose, mannose, glucose and glucuronic acid. Each sample was divided in two fractions, one of them was spiked with the commercial polysaccharide at the 400 mg L^{-1} level, and the other one considered as a blank. Samples were then treated as previously described and recoveries were calculated for monosaccharides which form wine polysaccharides. The obtained recoveries ranged from 81 to 116% (Table 2), again good values for this complex analysis. In contrast to previous studies, this is the first time to study the recovery of the whole method and not just of the chromatographic determination.

3.4. Comparison of the GC-MS detection with the GC-FID detection

Figure 1 shows the chromatogram obtained for the glycosyl residues of a wine sample using the GC-MS detection. All the chromatograms obtained showed good chromatographic peaks

297 and revealed no evidences of interferences or overlaps between compounds. Although good
298 chromatographic peaks were also obtained for FID (chromatogram not shown), the presence
299 of interferences or overlaps was difficult to detect with this type of detector. Taking into
300 account that the co-elution of compounds is frequent in real wine samples, MS detector was
301 preferred to solve this difficulty.
302 Features of the GC-MS method were compared with those previously reported for the GC-
303 FID method (Ayestarán, Guadalupe & León, 2004). Although the studied linear range differed
304 between MS and FID, the correlation coefficients of the calibration curves were similar for
305 both methods. In principle, both detectors provided similar precisions, with values of
306 repeatability and reproducibility ranging from 1 to 14%; however, detection and
307 quantification limits of the MS method were lower than those from the FID in all cases. LOQ
308 values were even around ten times lower 10 times for the majority of monosaccharides. Thus,
309 the LOQ of some monosaccharides which are present in wines in very low amounts but
310 whose concentrations are essential for RG-II quantification, i.e. 2-*O*-methyl xylose and Kdo,
311 were reduced from 7 to 1.3 µg and from 9 to 1.7 µg, respectively, and the LOQ for glucose,
312 one of the major monosaccharides of wine and must samples, was reduced from 21 to 1.5 µg.
313 In conclusion, GC-MS quantification was preferred to provide unambiguous identification
314 and quantification of the complex mixtures of monosaccharides present in grape derived
315 beverages. Besides, MS detector showed to be more sensitive and selective than FID,
316 allowing lower detection limits which may be useful when quantifying rare RG-II
317 polysaccharides as they are present in must and wines in very low concentrations.
318 *3.5. Method application: analysis of wine polysaccharides by GC-MS and GC-FID*
319 The described method was applied to analyze polysaccharide families in the three wine
320 samples. Table 3 shows the glycosyl residue composition of the three wines determined by
321 the GC-MS method proposed. Without any exception, all monosaccharides were detected in

the wines at the levels above the quantification limits of the method, and all the concentrations were always within the linear range. Wine samples contained all the monosaccharides that form wine polysaccharides and they were present in similar proportions than those found in other studies (Ayestarán, Guadalupe & León, 2004; Doco, Quellec, Moutounet & Pellerin, 1999; Doco, Williams & Cheynier, 2007; Guadalupe & Ayestarán, 2007b). Aceric acid, 2-*O*-methyl xylose, 2-*O*-methyl fucose, apiose, Dha and Kdo, the rare monosaccharides markers of the rhamnogalacturonan II molecule (RG-II), were present in all the samples in lower amounts. On the opposite, rhamnose and galacturonic acid, principal components of rhamnogalacturonans; mannose, the main component of yeast mannoproteins (MP); and arabinose and galactose, the glycosyl residues found in arabinogalactans and arabinogalactan-proteins (AGP), were present in high concentrations. The high quantities of glucose were attributed to the presence of grape and yeast polysaccharides but also to anthocyanins; the presence of xylosyl residues indicated that traces of hemicelluloses (arabinoxylans or xyloglucans) were solubilized from grape cell walls. The content of each polysaccharide family in the wine samples was estimated from their concentration of individual glycosyl residues which are characteristic of structurally-identified wine polysaccharides (Ayestarán, Guadalupe & León, 2004; Doco, Quellec, Moutounet & Pellerin, 1999). AGP were therefore estimated from the sum of galactosyl, arabinosyl, rhamnosyl and glucuronosyl residues; all the mannose content was attributed to yeast mannoproteins; the RG-II content was calculated from the sum of its diagnostic monosaccharides, which represent approximately 25% of the RG-II molecule. Taking into account the molar ratios of the RG-II (1 residue of 2-*O*-methyl fucose, 3.5 rhamnose, 2 arabinose, 2 galactose, 1 glucuronic acid and 9 galacturonic acid), the remaining part was attributed to the presence of AGP in the case of rhamnose, arabinose and galactose, and the remaining galacturonosyl residues was used to estimate the content of oligomers of homo- and rhamnogalacturonans

(GL). The results of the analysis are shown in Table 4, which shows that except for RG-II, the concentrations of the different polysaccharide families were significantly different depending on the wine sample ($p < 0.05$). The content of total polysaccharides was estimated from the sum of AGP, MP, RG-II and GL.

In order to compare the results of the method using the MS detection and the FID, the monosaccharide (data not shown) and polysaccharide composition of the three wine samples was also analyzed by CG-FID. Table 4 shows the results for polysaccharide concentrations analysed by both methods. The comparison of the data obtained with the two methods was carried out by an analysis of variance (ANOVA) as all the data met random and normality assumptions. No significant differences were found in the content of monosaccharide residues and thus in any polysaccharide family ($p < 0.05$), indicating that both methods provided similar results when determining wine polysaccharides.

3.6. Analysis of wine polysaccharides by HRSEC-RID

HRSEC of wine samples on two serial Shodex columns was performed in order to obtain the molecular weights and molecular weight distributions of the wine polysaccharides. Moreover, we tried to evaluate if this method, quite frequent for wine polysaccharide evaluation, could serve as a rapid and simple method to estimate the content of total wine polysaccharides.

The sizes and content of the polysaccharides were compared to those of known pullulan standards. The apparent molecular weights were deduced from the calibration equation log $M_w = 11.188 – 0.403\ t_R$ (t_R = column retention time at peak maximum, and $r^2 = 0.999$) with a correlation coefficient r^2 of 0.998. Polysaccharide contents were estimated using calibration curves constructed from the pullulan standards; the pullulan P-10 of 11.8 KDa, P-50 of 47.3 KDa, P-100 of 112 KDa, and P-200 of 212 KDa were chosen to obtain the calibration curves because their peaks properly matched with those obtained for the wine samples. The four

calibration curves showed a correlation coefficient r^2 higher than 0.998 and they were linear for all the range of concentrations detected (0-1000 mg L^{-1}).

In the wine samples analyzed, the HRSEC fractionation allowed the separation of soluble polysaccharides in different peaks (Figure 2). According to previous work (Guadalupe & Ayestarán, 2007b), higher-molecular-weight polysaccharides, eluting between P50 and P400, corresponded to molecules with an average molecular weight higher than 47.3 kD (average of 212, 112 and 50 kD), and corresponded to a complex mixture of high-molecular-weight AGP from grape berries and high-molecular-weight MP from yeasts. Polysaccharides with an average molecular weight of 12 kD (P10) corresponded to grape RG-II dimers and lower molecular-weight AGP and MP. Signals eluting after P5 corresponded to a molecular weight of less than 6 kD and it was attributed to oligosaccharides and small fragments of AGP, MP and RG-II. Therefore, and in order to estimate the total polysaccharide content from the HRSEC profile, these last signals were not taking into account and total polysaccharides were estimated from the sum of signals with a higher molecular weight than P5. Table 5 shows the quantities of polysaccharides estimated by HRSEC-RID for the three wines analyzed. It was observed again that the estimated polysaccharide content was dependent on the wine sample ($p < 0.05$). The results of total polysaccharide content (TPC) obtained with the GC-MS method and estimated polysaccharide content (EPC) obtained by the HRSEC-RID method were compared by using a correlation matrix. Although the polysaccharide content estimated by HRSEC was considerably lower than that obtained with GC-MS, a good correlation was found between the two methods ($r = 0.746$, $p < 0.05$), indicating that the former could serve as a rapid and simple method for total wine polysaccharide estimation. It is important to notice that HRSEC-RID provided almost half of the value obtained by the chromatographic method and thus it led to an underestimation of real wine polysaccharides; however, it could be valid for comparative purposes.

4. Conclusions

Factors affecting the yield of polysaccharide precipitation were carefully evaluated, concluding that concentration of wine samples was essential to ensure the quantitative precipitation of all wine polysaccharides The suitability of gas chromatography with mass spectrometry detector (GC-MS) for determining the content of wine monosaccharides and thus polysaccharide families was proved. It provided good values of quantification and detection limits and suitable values of repeatability, reproducibility and overall recoveries. The comparison of the results obtained by GC-MS and GC-FID revealed that both methods were suitable for determining the content of monosaccharides in wine samples but MS detector showed to be more sensitive and selective than FID, allowing lower detection limits which may be useful when quantifying rare RG-II monosaccharides which are present in musts and wines in very low concentrations. A further advantage of the MS detection is that it provides unambiguous identification and quantification of the complex mixtures of monosaccharides present in grape derived beverages. Finally, three wine samples were analyzed by GC-MS, GC-FID and HRSEC-RID. No significant differences were found for polysaccharide families quantified by MS or FID, indicating that both methods provided similar results. The good correlation observed between the polysaccharide values obtained with the GC method and the HRSEC-RID method (r = 0.746, p < 0.05) indicated that the latter could serve as a rapid and simple method for estimating the content of total wine polysaccharides although it does not provide information about the concentration of specific polysaccharide families.

Acknowledgements

The authors would like to thank the *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria* (INIA) for the funding provided for this study through the project RTA2009-00029-C02-01 (with FEDER funds).

References

Arnous, A., & Meyer, S. (2009). Quantitative prediction of cell wall polysaccharide composition in grape (*Vitis vinífera L.*) and apple (*Malus domestica*) skins from acid hydrolysis monosaccharide profile. *Journal of Agricultural and Food Chemistry*, *57*, 3611-3619.

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.

Boulet, J. C., Williams, P., & Doco, T. (2007). A Fourier transform infrared spectroscopy study of wine polysaccharides. *Carbohydrate Polymers*, *69*, 79-85.

Carvalho, E., Mateus, N., Plet, B., Pianet, I., Dufourc, E., & de Freitas, V. (2006). Influence of wine pectic polysaccharides on the interactions between condensed tannins and salivary proteins. *Journal of Agricultural and Food Chemistry*, *54*, 8936-8944.

Chalier, P., Angot, B., Delteil, D., Doco, T., & Gunata, Z. (2005). Interactions between aroma compounds and whole mannoproteins isolated from *Saccharomyces cerevisiae* strains. *Food Chemistry*, *100*, 22-30.

Coimbra, C., Barros, A. S., Coelho, E., Gonçalves, F., Rocha, M. S., & Delgadillo, I. (2005). Quantification of polymeric mannose in wine extracts by FT-IR spectroscopy and OSC-PLS1 regression. *Carbohydrate Polymers*, *61*, 434-440.

De Freitas, V., Carvalho, E., & Mateus, N. (2003). Study of carbohydrate influence on protein-tannin aggregation by nephelometry. *Food Chemistry*, *81*, 503-509.

Doco, T., Quellec, N., Moutounet, M., & Pellerin, P. (1999). Polysaccharide patterns during the aging of Carignan noir red wines. *American Journal of Enology and Viticulture*, *50*, 25-32.

445 Doco, T., O´Neill, M.A., & Pellerin, P. (2001). Determination of the neutral and acidic
446 glycosyl-residue compositions of plant polysaccharides by GC-EI-MS analysis of the
447 trimethylsilyl methyl glycoside derivatives. *Carbohydrate Polymers*, *46*, 249-259.
448 Doco, T., Vuchot, P., Cheynier, V., & Moutounet, M. (2003). Structural modification of wine
449 arabinogalactans during aging on lees. *American Journal of Enology and Viticulture*, *54*,
450 150-157.
451 Doco, T., Williams, P., & Cheynier, V. (2007). Effect of flash release and pectinolytic
452 enzyme treatments on wine polysaccharide composition. *Journal of Agricultural and Food*
453 *Chemistry*, *55*, 6643-6649.
454 Dols-Lafargue, M., Gindreau, E., Le Marrec, C., Chambat, G., Heyraud, A., & Lonvaud-
455 Funel, A. (2007). Changes in red wine soluble polysaccharide composition induced by
456 malolactic fermentation. *Journal of Agricultural and Food Chemistry*, *55*, 9592-9599.
457 Dubourdieu, D., Llauberes, R. M., & Ollivier, C. (1986). Estimation rapide des constituants
458 macromoléculaires des moûts et des vins par chromatographie liquide haute pression
459 (CLHP) de tamisage moléculaire. *Connaisance Vigne Vin*, *20*, 119-123.
460 Dufour, C., & Bayonoue, C. L. (1999). Influence of wine structurally different
461 polysaccharides on the volatily aroma substances in a model solution. *Journal of*
462 *Agricultural and Food Chemistry*, *47*, 671-677.
463 Dupin, I., Stockdale, V. J., Williams, P. J., Jones, G. P., Markides, A. J., & Waters, E. J.
464 (2000a). *Saccharomyces cerevisiae* mannoproteins that protect wine from protein haze:
465 evaluation of extraction methods and inmunolocalization. *Journal of Agricultural and*
466 *Food Chemistry*, *48*, 1086-1095.
467 Dupin, I., Mc Kinnon, B. M., Ryan, C., Boulay, M., Markides, A. J., Jones, G. P., Williams,
468 P. J., & Waters, E. J. (2000b). *Saccharomyces cerevisiae* mannoproteins that protect wine

from protein haze: their release during fermentation and lees contact and a proposal mechanism of action. *Journal of Agricultural and Food Chemistry, 48,* 3098-3105.

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.

Feuillat, M., Escot, S., Charpentier, C., & Dulau, L. (2001). Élevage des vins rouges sur lies fines. Intérêt des interactions entre polysaccharides de levure et polyphénols du vin. *Revue des Œnologues, 98,* 17-18.

Fuster, A., & Escot, S. (2002). Élevage des vins rouges sur lies fines: choix de la levure fermentarie et ses conséquences sur les interactions polysaccharides pariétaux/polyphénols. *Revue des Œnologues, 104,* 20-22.

Gerbaud, V., Gabas, N., Laguerie, C., Blouin, J., Vidal, S., Moutounet, M., & Pellerin, P. (1996). Effect of wine polysaccharides on the nucleation of potassium hydrogen tartrate in model solutions. *Chemical Engineering Research and Design,* 74, 782-789.

Gerbaud, V., Gabas, N., Blouin, J., Pellerin, P., & Moutounet, M. (1997). Influence of wine polysaccharides and polyphenols on the crystallisation of potassium hydrogen tartrate. *Journal International des Sciences de la Vigne et du Vin, 31,* 65-83.

González-Ramos, D., & González, R. (2006). Genetic determinants of the release of mannoproteins of enological interest by *Saccharomyces cerevisiae*. *Journal of Agricultural and Food Chemistry, 54,* 9411-9416.

Guadalupe, Z., Palacios, A., & Ayestarán, B. (2007a). Maceration enzymes and mannoproteins: a possible strategy to increase colloidal stability and colour extraction in red wines. *Journal of Agricultural and Food Chemistry, 55,* 4854-4862.

492 Guadalupe, Z., & Ayestarán, B. (2007b). Polysaccharide profile and content during the
493 vinification and aging of Tempranillo red wines. *Journal of Agricultural and Food*
494 *Chemistry*, *55*, 10720-10728.
495 Guadalupe, Z., & Ayestarán, B. (2008). Effect of commercial mannoprotein addition on
496 polysaccharide, polyphenolic and colour composition in red wines. *Journal of Agricultural*
497 *and Food Chemistry*, *56*, 9022-9029.
498 López-Barajas, M., López-Tamames, E., & Buxaderas, S. (1998). Improved size-exclusion
499 high-performance liquid chromatographic method for the simple analysis of grape juice
500 and wine polysaccharides. *Journal of Chromatography A*, *823*, 339-347.
501 Lubbers, S., Leger, B., Charpentier, C., & Feuillat, M. (1993). Effect Colloide-Protecteur
502 d'extracts de parois de levures sur la stabilite tartrique d'une solution hydro-alcoolique
503 modele. *Journal International des Sciences de la Vigne et du Vin*, *27*, 13-22.
504 Lubbers, S., Voilley, A., Feuillat, M., & Charpentier, C. (1994). Influence of mannoproteins
505 from yeast on the aroma intensity of a model wine. *Lebensmittel-Wissenschaft und-*
506 *Technologie*, *27*, 108-114.
507 Mateus, N., Carvalho, E., Luis, C., & de Freitas, V. (2004). Influence of the tannin structure
508 on the disruption effect of carbohydrates on protein–tannin aggregates. *Analytica Chimica*
509 *Acta*, *513*, 135-140.
510 Moine-Ledoux, V., & Dubourdieu, D. (1999) An invertase fragment responsible for
511 improving the protein stability of dry white wines. *Journal of the Science of Food and*
512 *Agriculture*, *79*, 537-543.
513 Moine-Ledoux, V., & Dubourdieu, D. (2002). Rôle des mannoprotéines des levures vis à vis
514 de la stabilisation tartrique des vins. In *Bulletin de l´OIV 75*, pp. 471-482.
515 Nuñez, Y. P., Carrascosa, A. V., González, R., Polo, M. C., & Martínez-Rodríguez, A. J.
516 (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.

Nuñez, Y. P., Carrascosa, A. V., González, R., Polo, M. C., & Martínez-Rodríguez, A. J. (2006). Isolation and characterization of a thermally extracted yeast cell wall fraction potentially useful for improving the foaming properties of sparkling wines. *Journal of Agricultural and Food Chemistry*, *54*, 7898-7903.

Nuñez, Y. P., Pueyo, E., Carrascosa, A. V., & Martínez-Rodríguez, A. J. (2008). Effects of aging and heat treatment on whole yeast cells and yeast cell walls and on adsorption of ochratoxin A in a wine model system. *Journal of Food Protection*, *71*, 1496-1499.

Palomero, F., Morata, A., Benito, S., González, M. C., & Suárez-Lepe, J. A. (2007). Conventional and enzyme-assisted autolysis during ageing over lees in red wines: Influence on the release of polysaccharides from yeast cell walls and on wine monomeric anthocyanin content. *Food Chemistry*, *105*, 838-846.

Palomero, F., Morata, A., Benito, S., Calderón, F., & Suárez-Lepe, J. A. (2009). New genera of yeasts for over-lees aging of red wines. *Food Chemistry*, *112*, 432-441.

Pellerin, P., & Cabanins, J. C. (1998). Les glucides et l´œnologie. In *Fondements Scientifiques et* technologiques, Vol. 1 (pp. 41-92). Lavoisier, Paris: Tech & Doc.

Pérez, S., Rodríguez-Carvajal, M. A., & Doco, T. (2003). A complex plant cell wall polysaccharide: rhamnogalacturonan II. A structure in quest of a function. *Biochimie*, *85*, 109-121.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2002). In *Tratado de* enología, Vol. 2: Química *del vino, Estabilización y Tratamientos* (pp. 177-255). Buenos Aires, Argentina: Hemisferio Sur.

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

542 Saucier, C., Glories, Y., & Roux, D. (2000). Interactions Tanins-colloides: Nouvelles
543 avancées concernant la notion de "bons" et de "mauvais" tanins. *Revue des Œnologues*, *94*,
544 7-8.
545 Segarra, I., Lao, C., López-Tamames, E., & de la Torre-Boronat, M. C. (1995).
546 Spectrophotometric methods for the analysis of polysaccharide levels in winemaking
547 products. *American Journal of Enology and Viticulture*, *46*, 564-570.
548 Vicens, A., Fournand, D., Williams, P., Louise, S., Moutounet, M., & Doco, T. (2009).
549 Changes in polysaccharide and protein composition of cell walls in grape berry skin (Cv.
550 Shiraz) during ripening and over-ripening. *Journal of Agricultural and Food Chemistry*,
551 *57*, 2955-2960.
552 Vidal, S., Doco, T., Williams, P., Pellerin, P., York, W. S., O'Neill, M. A., Glushka, J.,
553 Darvill, A. G., & Albersheim, P. (2000a). Structural characterization of the pectic
554 polysaccharide rhamnogalacturonan II: evidence for the backbone location of the aceric
555 acid-containing oligoglycosyl side chain. *Carbohydrate Research*, *326*, 277-294.
556 Vidal, S., Doco, T., Pellerin, P., & Moutounet, M. (2000b). Soluble polysaccharide content at
557 initial time of experimental must preparation. *American Journal of Enology and*
558 *Viticulture*, *51*, 115-121.
559 Vidal, S., Williams, P., O´Neill, M.A., & Pellerin, P. (2003). The polysaccharides of red
560 wine: total fractionation and characterization. *Carbohydrate Polymers*, *54*, 439-447.
561 Vidal, S., Francis, L., Williams, P. K., Kwiatkowski, M., Gawel R., Cheynier, V., & Waters,
562 E. (2004). The mouth-feel properties of polysaccharides and anthocyanins in a wine like
563 medium. *Food Chemistry*, *85*, 519-525.
564 Waters, E., Pellerin, P., & Brillouet, J. M. (1994). A Saccharomyces mannoprotein that
565 protects wine from protein haze. *Carbohydrate Polymers*, *23*, 185-191.

566 Wolz, S. Extraction of mannoproteins and polysaccharides and their effect on aroma.
567 Improvement of mouthfeel. (2005). *Deutsche Weinmagazin*, *22*, 21-25.

Figure captions

Figure 1: GC-MS chromatogram obtained for a wine polysaccharide extract. For extraction, chromatographic and detection conditions see Sections 2.4 and 2.5. Peak identification: Aceric acid (peak 1), 2-*O*-methyl-fucose (peak 2), 2-*O*-methyl-xylose (peak 3), apiose (peak 4), arabinose (peak 5, 6, 7, 10, 11), rhamnose (peak 8), fucose (peak 9), xylose (peak 12, 13, 14), mannose (peak 15, 19), galacturonic acid (peak 16, 23), Dha (peak 18), galactose (peak 17, 20, 21, 22), glucose (peak 24, 26), glucuronic acid (peak 25, 27), Kdo (peak 28), myo-inositol (peak 29).

Figure 2: HRSEC-RID chromatograms of total soluble polysaccharides in three wine samples. Chromatogram obtained using two serial Shodex OHpack KB-803 and KB-805 columns. Elution times for the molecular weight markers (P5→P400) are shown.

Figure 1

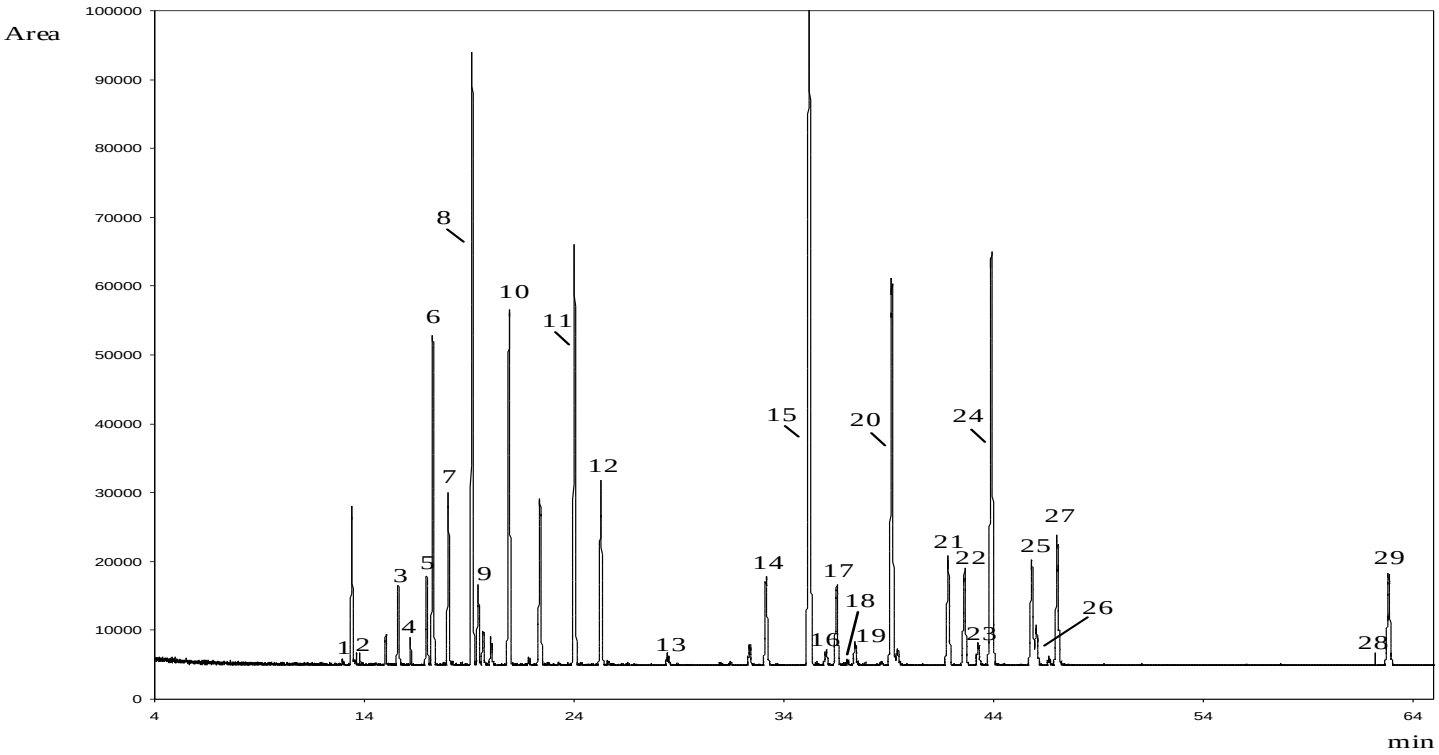
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Figure 2

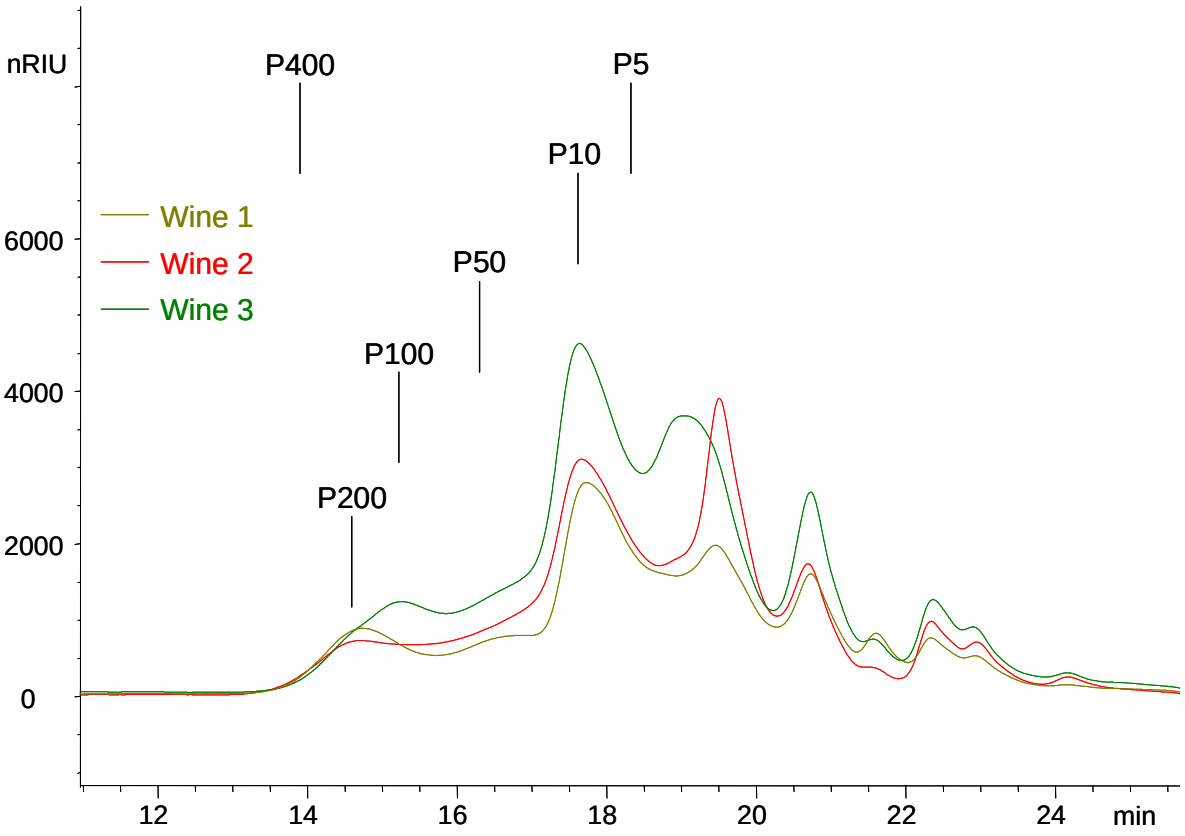
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Table 1. Calibration curve data[1], calculated detection (LOD) and quantification (LOQ) limits[1] and precision (repeatability and reproducibility) of the GC-MS detection

Compound	Equation[2]	Sd $_{slope}$[3]	Correlation coefficient (r^2)	Linear range (µg)	LOD (µg)	LOQ (µg)	Repeatability (%)[4]	Reproducibility (%)[5]
Aceric acid							9	1
2-*O*-methyl fucose							10	1
2-*O*-methyl xylose	A = 0.1278 C	0.0015	0.998	1.3 -275	0.4	1.3	12	1
Apiose							3	9
Arabinose	A = 0.5083 C	0.0067	0.998	0.7-1550	0.2	0.7	11	5
Rhamnose	A = 0.6812 C	0.0959	0.960	0.4-1500	0.1	0.4	11	13
Fucose	A = 0.6456 C	0.0109	0.997	0.6-525	0.2	0.6	10	7
Xylose	A = 0.5602 C	0.0083	0.997	0.4-500	0.1	0.4	9	9
Mannose	A = 0.6312 C	0.0861	0.970	2.5-1500	0.1	0.5	8	4
Dha[6]							5	8
Galactose	A = 0.2630 C	0.0031	0.998	1.6-1500	0.5	0.6	6	4
Galacturonic acid	A = 0.1741 C	0.0016	0.999	1.9-1300	0.6	0.9	6	14
Glucose	A = 0.8499 C	0.0165	0.996	1.5-1500	0.5	1.5	8	3
Glucuronic acid	A = 0.2382 C	0.0029	0.998	4.4-475	0.9	4.4	5	2
Kdo[6]	A = 0.1216 C	0.0002	0.999	1.7-125	0.5	1.7	10	13

[1] Values calculated for the monosaccharide standards.
[2] A denote the peak area and C denote concentration in mg.
[3] n = 3 replicates.
[4] calculated as RSD values, n = 6 replicates.
[5] calculated as RSD values, n = 3 replicates.
[6] Dha: 3-deoxy-D-*lyxo*-heptulosaric acid, Kdo: 3-deoxy octulosonic acid

Table 2. Recoveries for the three wine samples

Compound	Wine 1	Wine 2	Wine 3
	% recovery	% recovery	% recovery
Mannose	99.37	80.64	88.73
Galactose	84.13	87.33	89.22
Glucuronic acid	116.45	106.63	95.3
TM[1]	90.25	94.54	103.5

[1] TM: total monosaccharide content.

Table 3. Monosaccharide composition (mg L^{-1}) of three wines determined by GC-MS of their TMS derivatives

Compound	Wine 1	Wine 2	Wine 3
Aceric acid	1.35 ± 0.14	6.1 ± 0.1	5.3 ± 1.1
2-*O*-methyl fucose	1.8 ± 0.4	7.0 ± 0.2	1.7 ± 0.1
2-*O*-methyl xylose	1.99 ± 0.35	4.8 ± 0.8	4.1 ± 0.2
Apiose	1.54 ± 0.20	10.3 ± 2.4	5.1 ± 0.9
Arabinose	70 ± 8	174 ± 5	186 ± 10
Rhamnose	90.7 ± 7.4	226 ± 47	53 ± 13
Fucose	3.2 ± 0.2	7.0 ± 0.2	4.7 ± 0.9
Xylose	15.0 ± 3.6	11.0 ± 0.1	25.4 ± 1.7
Mannose	179 ± 13	275 ± 35	150 ± 23
Dha[1]	2.53 ± 0.37	14 ± 2	7.7 ± 2.5
Galactose	174 ± 26	228 ± 13	237 ± 19
Galacturonic acid	198 ± 6	72 ± 11	377 ± 29
Glucose	136 ± 9	280 ± 31	315 ± 24
Glucuronic acid	4.3 ± 0.4	14.2 ± 0.1	13.6 ± 1.4
Kdo[1]	4.9 ± 1.6	4.2 ± 0.26	2.18 ± 1.1

Values are means ± standard deviations (n = 3).
[1] Dha: 3-deoxy-D-*lyxo*-heptulosaric acid, Kdo: 3-deoxy octulosonic acid.

Table 4. Polysaccharide concentration (mg L^{-1}) of three wines determined by GC of their TMS derivatives

		Polysaccharide families				
Sample	GC detection	AGP[1]	MP[1]	RG-II[1]	GL[1]	TPC[2]
Wine 1	GC-MS	324 ± 26^{a}	179 ± 13^{a}	147 ± 16^{a}	182 ± 13^{a}	832 ± 36^{a}
Wine 2		578 ± 13^{b}	275 ± 35^{b}	171 ± 13^{a}	9 ± 1^{b}	1033 ± 44^{b}
Wine 3		477 ± 14^{c}	150 ± 23^{a}	181 ± 13^{a}	361 ± 30^{c}	1169 ± 41^{c}
Wine 1	GC-FID	321 ± 31^{a}	160 ± 16^{a}	158 ± 18^{a}	201 ± 17^{a}	840 ± 42^{a}
Wine 2		553 ± 36^{b}	244 ± 39^{b}	197 ± 17^{b}	3.82 ± 1^{b}	999 ± 56^{b}
Wine 3		476 ± 21^{c}	132 ± 23^{a}	184 ± 17^{b}	390 ± 28^{c}	1184 ± 46^{c}

Values are means ± standard deviations (n = 3). For each detection, means in the same column and the same letter are not significantly different ($p < 0.05$).

[1] AGP: arabinans and arabinogalactan-proteins, MP: mannoproteins, RG-II: rhamnogalacturonans-II, GL: oligomers of homo- and rhamnogalacturonans.

[2] TPC: total polysaccharide content estimated as the sum of AGP, MP, RG-II and GL.

Table 5. Estimated polysaccharide concentration (mg L^{-1} of pullulans) of three wines determined by HRSEC-RID on two serial Shodex molecular exclusion columns

	Mean molecular mass (kD)				
	212	112	50	12	EPC[1]
Wine 1	89.6 ± 1.8		68.2 ± 1.3	267.6 ± 5.2	425.4 ± 5.7
Wine 2	75.6 ± 1.2		99 ± 0.9	285 ± 4.8	459.9 ± 5.0
Wine 3		126.9 ± 2.7	107.1 ± 3.1	371.7 ± 13.7	605.7 ± 14.5

[1] EPC: estimated polysaccharide concentration as the sum of polysaccharides of different molecular mass. Values are means ± standard deviations (n = 3).