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Fourier Transform Infrared Spectroscopy and Chemometric Analysis of White Wine Polysaccharide Extracts

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Monovarietal white wines from Maria Gomes and Bical Portuguese Bairrada varieties were prepared according to different maceration and pectic enzyme clarification procedures. The polysaccharide-rich extracts, obtained by wine concentration, dialysis, and lyophilization, were fractionated by graded ethanol precipitation. A wide range of fractions rich in polysaccharides were obtained. Using the spectral region between 1200 and 800 cm^{-1} of the FTIR spectra of the wine polysaccharide dry extracts, using PCA and CCA chemometric methods, it was possible to discriminate the extracts on the basis of their polysaccharide composition. Moreover, it was possible to identify the wine-making processes involved and their influence on the wine polysaccharides. Furthermore, a calibration model using a PLS1 was proposed for the quantification of mannose in the samples obtained by precipitation with 60% ethanol aqueous solutions. This information will allow an expeditious assessment and monitoring of the polysaccharide composition and modifications that occur during the wine-making processing and evolution.

KEYWORDS: FTIR spectroscopy; wine polysaccharides; mannose; multivariate analysis

INTRODUCTION

Polysaccharides are one of the main groups of wine macromolecules. They play an important role in wine stability (*1*), and their effects depend on their composition, structure, and concentration in wines. They originate both from grape and microorganisms. Arabinans (*2*), type II arabinogalactans (*3*), rhamnogalacturonans, and galacturonans (*4*) arise from native cell-wall pectic polysaccharides of grape berry after degradation by pectinases during the maturation of grape and during the first steps of wine-making. Yeasts produce mannans and mannoproteins during and after fermentation (*5*). Glucans are produced by *Botrytis cinerea*, which may infect grape berries (*6*).

The methodologies usually used for the analysis of the polysaccharides are time-consuming and expensive. FTIR as been used as an important source of information for a quick evaluation of polysaccharide composition in vegetable samples (*7*−*11*). In wine, FTIR has been proposed and implemented for routine analysis of a large number of parameters, such as alcohol, volatile acidity, pH, tartaric acid, lactic acid, SO_2, glucose and fructose, acetic acid, citric acid and polyphenols (*12*). Also, a rapid method for discrimination of red wine cultivars based on FTIR spectra of the phenolic extracts in the region of 1640−950 cm^{-1} was recently proposed (*13*). As far as we know, no reference to the possible use of FTIR for the determination of polysaccharides has yet been reported.

The polysaccharides of Maria Gomes and Bical wines prepared according to different technological procedures gave a wide range of fractions rich in polysaccharides. Following the work carried out on the identification and quantification of cell wall pectic and hemicellulosic polysaccharides in the spectral region between 1200 and 850 cm^{-1} (*7*, *8*, *11*), this paper shows the potential of FTIR associated with the appropriate chemometric methods to discriminate polysaccharides in wine dry extracts, allowing the quantification of mannose in the samples. The final intent of these studies is (a) to be able to predict the type of polysaccharides according to their spectra, (b) to quantify the main type of constituent monosaccharide residues present in the samples, and (c) to study the possibility of extrapolating the results to more complex systems such as the whole wine sample. This information will allow an expeditious assessment and the monitoring of the polysaccharide composition and modifications that occur during the wine-making processing and evolution.

MATERIALS AND METHODS

Wine Samples. Maria Gomes (MG) and Bical (B) grape varieties, from the Portuguese Bairrada Appellation, were used. Parcels of 40 kg were racked, pressed, and sulfited (60 mg/L). For each variety, five procedures of wine preparation were used: the reference wine (RM0) was prepared without skin contact and without clarification with

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enzymes; a second wine was prepared with enzyme clarification of the musts with Ultrazym (Novo Nordisk) pectic enzymes, 30 mg/L (CM0); a third wine was prepared with the introduction of a skin-contact step (maceration) of 12 h (RM1); a fourth wine was prepared with the use of skin contact of 12 h and must clarification, as described (CM1); and a fifth wine was prepared with the use of skin contact of 24 h and must clarification (CM2). The musts were inoculated with active dry yeast VL1, and the fermentation occurred, in triplicate, at 20 °C in 5 L glass vessels with a headspace of 250 mL. After fermentation and deposition of the suspended solids, the liquids were transferred to 0.75 L bottles and sulfited. The bottles were stoppered and stored at room temperature until use.

Polysaccharide Recovery. The wine (500 mL) was rotary-evaporated under reduced pressure at 35 °C to eliminate the ethanol and concentrate the total solids present to a volume of 25 mL. The material was then dialyzed to remove the tartaric acid and other small molecules. The dialysate was concentrated, frozen, and freeze-dried to give the wine polymeric material as a powder.

Ethanol Fractionation. The polymeric material (60 mg) was dissolved in 6 mL of water. Absolute ethanol (9 mL) was added, and the solution (60% ethanol, assuming additive volumes) was stirred for 1 h at 4 °C. This solution was then centrifuged, and the residue obtained was removed (fraction Et60). To the supernatant was added 15 mL of absolute ethanol; the solution (80% ethanol) was stirred for 1 h at 4 °C and centrifuged, and the residue obtained (fraction Et80) was removed from the supernatant solution. Each precipitate was dissolved in water, rotary-evaporated, frozen, and freeze-dried (*14*).

Sugars Analysis. Neutral sugars were released by Saeman hydrolysis (*15*) and analyzed as their alditol acetates by GLC (*16*, *17*) using a Carlo Erba GC 6000 Vega series 2, in splitless mode, with an FID detector. A 30 m DB-225 column (J&W Scientific) with i.d. of 0.25 mm and 0.15 μm film thickness was used. With the injector and detector operating at 220 and 230 °C, respectively, the following oven temperature program was used: 220 °C for 1 min and 230 °C for 6.5 min, with a rate of 20 °C/min. Linear velocity of the carrier gas (H_2) was set at 50 cm/s at 220 °C. Hexuronic acids were determined colorimetrically according to a modification (*14*) of the method of Blumenkrantz and Asboe-Hansen (*18*).

FTIR Spectroscopy. FTIR spectra were acquired for each sample/fraction (three replicates) between 4000 and 400 cm^{-1} at 8 cm^{-1} resolution with 128 co-added scans, using a single-reflectance ATR accessory (GoldenGate). The spectra were converted to JCAMP-DX format and analyzed with a program developed in the Institut National Agronomique Paris-Grignon in collaboration with the University of Aveiro (*19*). For multivariate analysis purposes, the fingerprint region between 1200 and 800 cm^{-1} was used, as it contains information about polysaccharide characteristics (*7*, *8*). For chemometric analyses, PCA, CCA, and PLS1, each spectrum was autoscaled (mean centered and standardized) and the data sets were centered by column (wavenumber) for PCA and CCA.

MATHEMATICAL SECTION

Principal Component Analysis. PCA is essentially a descriptive method. This method is, normally, the first step in the data exploration that allows a visualization of the main variability aspects of a data set, without the constraint of an initial hypothesis concerning the relationship within samples and between samples and responses (variables). The main goal of this procedure is to find relationships between the different parameters (objects and variables) and/or the detection of possible clusters within objects and/or variables.

To find the main sources of variability of a data set and the relationships between and within objects and variables, the initial matrix, defined by $\mathbf{X}(n, m)$, is decomposed into matrixes that represent the object space (spectra), the variable space (wavenumbers) and a error matrix (variation not accounted for by the extracted principal components).

Table 1. Origin and Sugar Composition of Wine Polysaccharide Fractions

origin	mol % Rha	Ara	Xyl	Man	Gal	Glc	HexA	total sugars (mg/g)
polymeric material, Maria Gomes variety								
MG-RM0	3	22	0	25	25	4	22	781
MG-CM0	4	5	0	56	8	11	15	587
MG-RM1	3	30	1	14	29	3	20	631
MG-CM1	3	5	0	65	7	6	14	455
MG-CM2	2	5	0	61	5	14	13	509
polymeric material, Bical variety								
B-RM0	4	38	1	12	20	3	22	490
B-CM0	6	21	1	32	12	6	22	226
B-RM1	4	22	0	22	25	3	23	633
B-CM1	5	19	5	31	10	3	27	412
B-CM2	3	11	1	41	9	12	23	502
polymeric material precipitated in 60% ethanol, Maria Gomes variety								
MG-RM0 Et60	3	9	0	49	16	2	20	861
MG-CM0 Et60	3	4	0	69	5	2	17	753
MG-RM1 Et60	2	17	1	24	31	0	26	606
MG-CM1 Et60	2	3	0	87	4	1	3	585
MG-CM2 Et60	1	3	0	82	2	2	10	489
polymeric material precipitated in 60% ethanol, Bical variety								
B-RM0 Et60	6	23	0	24	26	3	17	444
B-CM0 Et60	8	12	1	43	9	4	24	296
B-RM1 Et60	4	16	0	35	28	2	14	704
B-CM1 Et60	8	15	3	46	5	1	22	574
B-CM2 Et60	2	7	0	64	7	4	16	799
polymeric material precipitated in 80% ethanol, Maria Gomes variety								
MG-RM0 Et80	3	28	0	7	45	3	15	959
MG-CM0 Et80	6	10	1	26	22	11	24	427
MG-RM1 Et80	4	35	0	4	38	3	16	643
MG-CM1 Et80	6	11	1	29	17	11	25	290
MG-CM2 Et80	3	8	0	35	9	4	42	401
polymeric material precipitated in 80% ethanol, Bical variety								
B-RM0 Et80	4	36	0	4	34	5	18	611
B-CM0 Et80	8	22	1	21	19	14	15	298
B-RM1 Et80	4	27	1	15	29	3	19	642
B-CM1 Et80	5	25	0	19	17	3	31	408
B-CM2 Et80	3	9	0	16	10	6	56	940

The decomposition is formalized by

$$\mathbf{X}(n, m) = \mathbf{T}(n, k)\mathbf{P}(k, m)^{\mathbf{T}} + \mathbf{E}(n, m) \quad (1)$$

where **T** is the scores matrix, **P** is the loadings matrix, **E** is the error matrix, n is the number of objects (spectra), m is the number of variables (in this study, wavenumbers), and k is the number of principal components used (*20*).

Canonical Correlation Analysis. CCA seeks to identify and quantify the relationships between two sets of variables or domains (**X** and **Y**) (*21*). The method maximizes the correlation between the linear combination of the variables in one domain with the linear combination of the variables in the other domain. The procedure starts by finding the pair of linear combinations (between both domains) having the largest correlation. Next, it seeks a pair of linear combinations having the largest correlation but uncorrelated to the first pair of linear combinations found. This procedure is repeated until extraction of all canonical factors has been accomplished. The pairs of linear combinations are called canonical variables, and their correlations are called canonical correlations. The canonical correlation is a measure of the strength between the obtained pairs of linear combination between the two domains (**X** and **Y**). The aim of CCA is to concentrate the information of both domains into a small number of canonical variables that should represent almost the same information present in the initial variables. This method has the properties of being a data reduction technique and also of being a predictive technique.

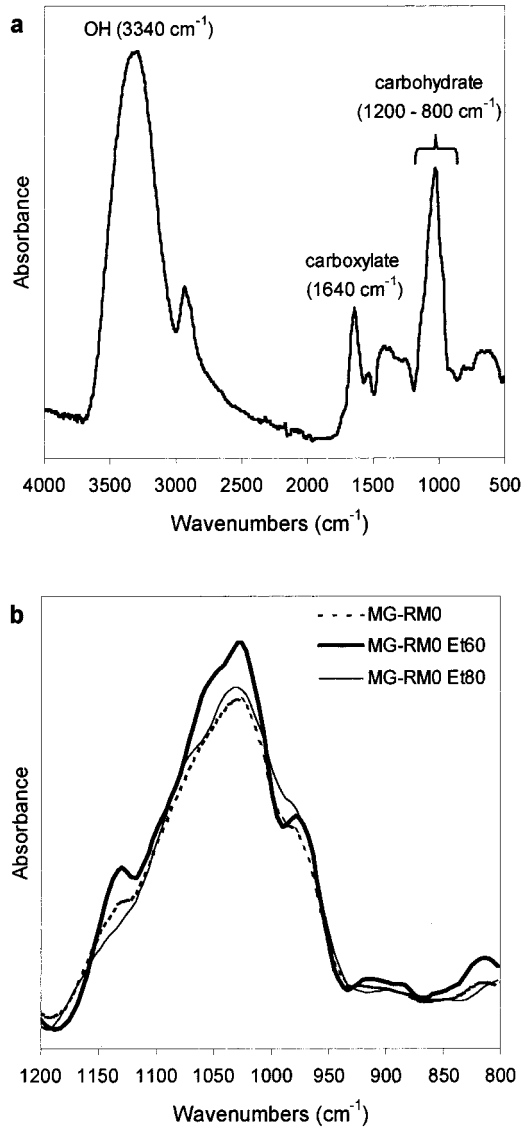


Figure 1. FTIR spectra of Maria Gomes RM0 wine polymeric material (GoldenGate-ATR): (a) spectrum of the 4000–500 cm^{-1} region of the polymeric material; (b) spectra of the 1200–800 cm^{-1} region of the polymeric material and Et60 and Et80 extracts.

Partial Least Squares Regression 1. The PLS1 regression model is used to establish a relationship between a set of independent variables **X** and a dependent variable **y**, often in the case where the number of variables (independent and dependent) is large (*22*). This procedure performs a PCA [by means of noniterative partial least squares (NIPALS)] on the independent variables matrix, maximizing at the same time the correlation of the extracted PCs with the dependent variable vector. The relationship is formalized by means of the equation

$$\mathbf{y} = \mathbf{Xb} + \mathbf{E} \qquad (2)$$

where **y** is the vector of the dependent variable (in this study, sugar content in mol %), **X** is the matrix of the independent variables (in this study, the spectra are arranged by rows and the wavenumbers by columns), **b** is the regression coefficients vector, and **E** represents the error not accounted for by the model.

RESULTS AND DISCUSSION

Selection of Samples. The samples used in this study were obtained from monovarietal wines of two grape varieties, Maria Gomes (MG) and Bical (B). For each variety, five procedures of wine preparation were used: the reference wine (RM0) was prepared without skin contact and without clarification with enzymes; a second wine (CM0) was prepared with enzyme clarification of the musts; a third wine (RM1) was prepared with the introduction of a skin-contact step (maceration); a fourth (CM1) wine and a fifth (CM2) wine were prepared with the use of skin contact and must clarification for 12 and 24 h, respectively. The polymeric material obtained was fractionated by ethanol precipitation in two fractions: those containing the material insoluble in 60% ethanol (Et60) and those containing the material insoluble in 80% ethanol (Et80). **Table 1** shows the sugars composition of the 10 wine polymeric extracts and the 20 ethanol-precipitated materials, for a total of 30 samples.

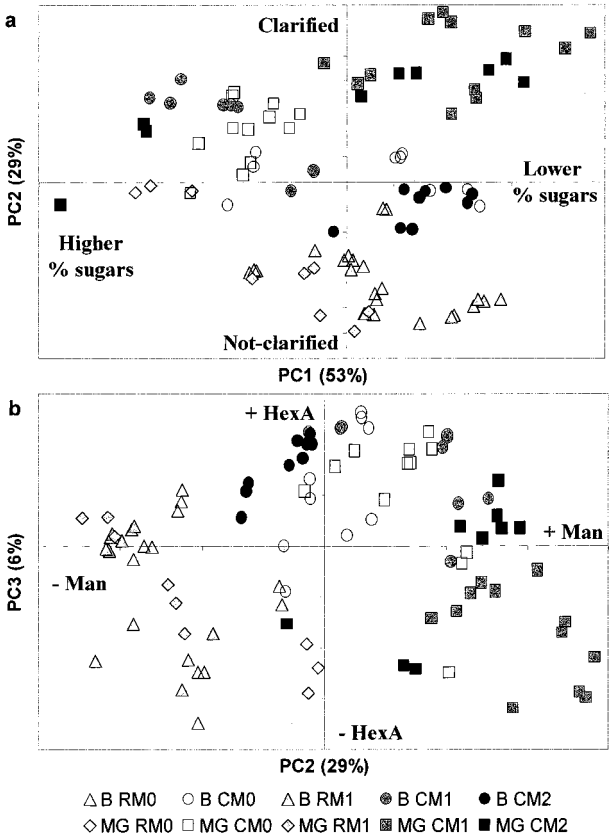


Figure 2. PCA scores scatter plot of the FTIR spectra of the ethanol 60% precipitated fractions: (a) PC1 vs PC2; (b) PC2 vs PC3 (axes cross each other at the origin).

The main sugars present are mannose (Man), arabinose (Ara), galactose (Gal), hexuronic acid (HexA)—the experimental name given to the galacturonic acid residues of pectic polysaccharides, rhamnose (Rha), and glucose (Glc). The Et60 fractions were richer in Man, and the Et80 fractions were richer in Ara and Gal. However, their sugar composition showed significant variations according to the wine-making technology used and variety.

Figure 1a shows a typical FTIR spectrum of the wine polymeric material extract. The spectrum shows high absorbance at wavenumbers characteristic of polysaccharides: 3440 cm^{-1} OH and 1200−800 cm^{-1} carbohydrate (*23*). To correlate the carbohydrate region (**Figure 1b**) with the different polysaccharides present in the wine samples different multivariate analyses were applied.

PCA of the FTIR Spectra of the Fractions Precipitated in 60% Ethanol. The scores scatter plots of PC1 versus PC2

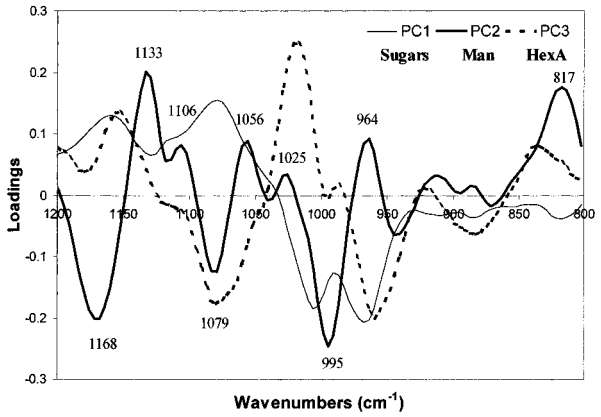
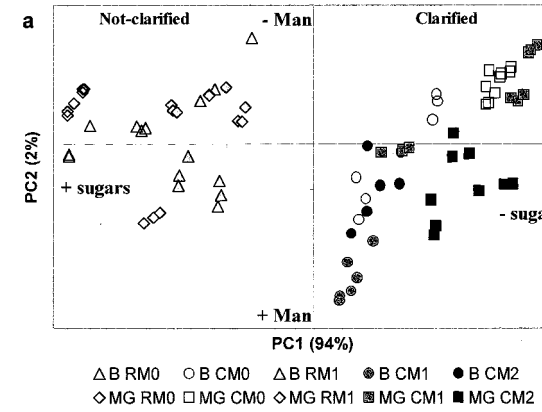


Figure 3. PCA loadings plot of the FTIR ethanol 60% fractions (PC1, PC2, and PC3).



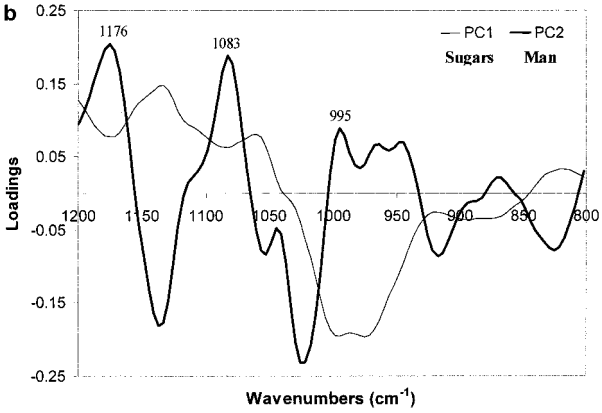
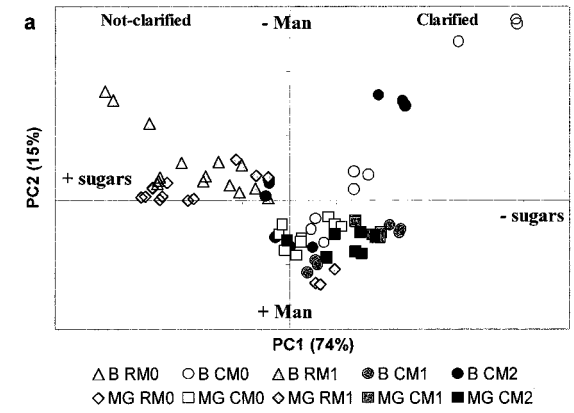


Figure 4. PCA of the FTIR spectra of the ethanol 80% precipitated fractions: (a) scores scatter plot (PC1 vs PC2) (axes cross each other at the origin); (b) loadings plot.

(a) and PC2 versus PC3 (b) of the 90 FTIR spectra of the fractions precipitated in 60% ethanol are represented in **Figure 2**. These three PCs represent 88% of the total variability present in the infrared spectra. From **Figure 2a**, it can be seen that the points along PC1 showed higher dispersion within each sample group. This may be due to the fermentation variability among fermenters. The samples located according to the PC2 axis are related to the use of the clarification step. The samples clarified are mainly located in PC2 positive, and the not-clarified samples are located in PC2 negative. According to **Table 1**, they seem to be distinguished according to their relative content of Man, as the clarified samples were richer in this sugar residue. In fact, the distribution of samples along this axis, when compared to the sugar analysis, shows that at the PC2 negative side are found samples with Man content between 24 and 35 mol %,



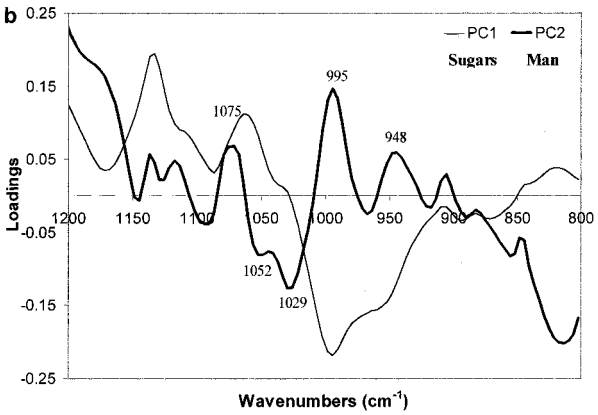


Figure 5. PCA of the FTIR spectra of the wine extracts: (a) scores scatter plot (PC1 vs PC2) (axes cross each other at the origin); (b) loadings plot.

and the positive side has samples with a Man amount between 82 and 87 mol %. Around the origin of the axis were located samples that ranged between 43 and 64 mol %. **Figure 2b** shows that along PC3 the distribution of the points is slightly related with the relative amount of HexA: samples B-CM0, B-CM1, and MG-CM0 are in PC3 positive and sample MG-CM1 is in PC3 negative. From these plots, one could see that, in Et60 fractions, the variations of sugars and Man introduced by the clarification process are more relevant for the distinction of the samples than the difference between the two white wine varieties (MG and B). The relative composition of sugars in Et60 fractions resultant from the maceration process was shown to be less influenced when compared to the clarification process.

Analysis of the PC1 loadings (**Figure 3**) shows that this profile, which accounts for 53% of the total variability, seems to be related to the total amount of sugar present in the samples. In fact, a trend is observed for samples with higher sugars content being located at the PC1 negative axis (region between 1040 and 950 cm^{-1}) and, on the opposite side (PC1 positive axis), were located samples with a lower percentage of sugars. The PC2 axis (29% of the total variability) shows the loadings profile of Man content variability. It is characterized mainly by the wavenumbers 1168, 1133, 1106, 1079, 1056, 1025, 995, 964, and 817 cm^{-1}. The loadings plot of PC3 (6% of the total variability) shows a profile with the characteristic wavenumbers of galacturonic acid of pectic polysaccharides: 1150, 1100, and 1020 cm^{-1} (*7*, *8*, *11*).

PCA of the FTIR Spectra of the Fractions Precipitated in 80% Ethanol. The scores scatter plots of PC1 versus PC2 (a) and respective loadings plot (b) of the 90 FTIR spectra of the fractions precipitated in 80% ethanol are represented in **Figure 4**. The PC1 axis, which accounts for 94% of the total variability, has a loadings plot similar to that found for PC1 of

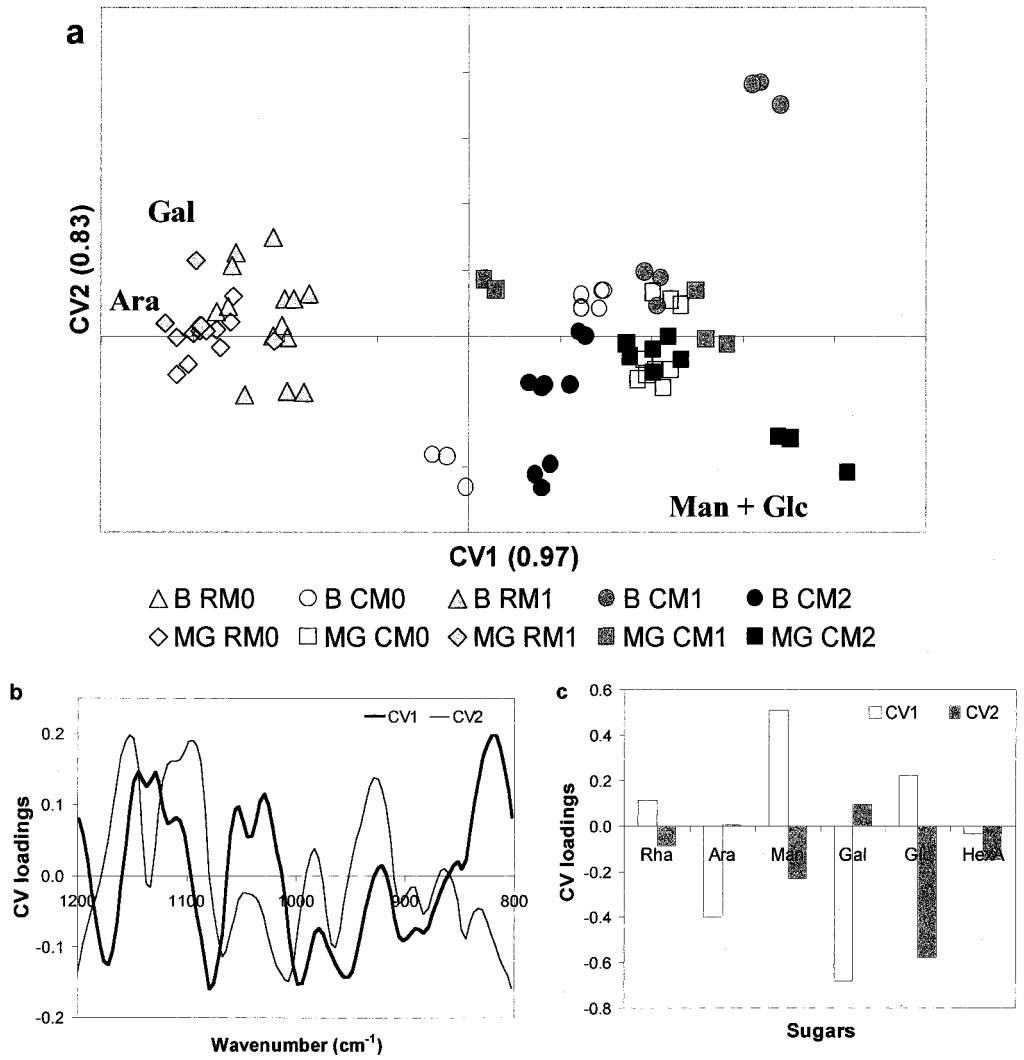


Figure 6. CCA of wine extracts: (a) projection of the wine polymeric samples in the canonical variate space (CV1 vs CV2) (axes cross each other at the origin); (b) FTIR loadings profile; (c) sugar analysis loadings plot.

Et60 fractions. From **Table 1**, it is possible to see that the samples located in PC1 negative contain higher amounts of sugars (not clarified) than the samples located in PC1 positive (clarified). PC2 represents only 2% of the total variability. However, from the loadings plot (**Figure 4b**), one can identify the Man profile, which gives an indication that samples located in the PC2 negative axis are characterized by their higher amount of Man when compared to samples located in the opposite axis direction. These observations were confirmed by the sugar analysis, although the Man content of Et80 fractions varied only from 4 to 35 mol %.

PCA of the FTIR Spectra of the Wine Polysaccharide Fractions. The identification of the Man FTIR profiles in the ethanol fractionated extracts gives the tools to extend the analysis of the wine polysaccharides to the raw wine polymeric extracts, obtained simply by removal of the ethanol and water solvents by rotary evaporation and removal of small molecules by dialysis. The scores scatter plot of PC1 versus PC2 (a) and the loadings plot (b) of the 90 FTIR spectra of the wine polymeric material are represented in **Figure 5**. As previously observed for Et60 and Et80 fractions, the PC1 axis (74% of the total variability) is related to the total amount of sugars (PC1 loadings profile and **Table 1**), distinguishing the samples clarified (PC1 positive) from those not clarified (PC1 negative). The second major source of variability (PC2, 15% of total variability) is related to Man. The samples located in the PC2 positive axis have a lower Man content when compared to the ones located on the PC2 negative side.

CCA of the FTIR Spectra of the Wine Polysaccharide Fractions. Application of the PCA to the FTIR spectra showed that they contain information related to the sugar composition of the fractions. To establish a direct relationship between the spectral information and the sugar composition of the wine fractions, a CCA was applied to these two domains. From **Figure 6c**, one can observe that the first canonical variate axis (CV1), which presents a high correlation between the two domains (0.97), is characterized by samples with a higher relative amount of both Man and Glc (on the CV1 positive axis) and with a higher relative amount of Ara and Gal (on the CV1 negative axis). From **Figure 6a**, it is possible to observe that the CV1 axis separates the samples submitted to the clarification step (CV1 positive) from those not clarified (CV1 negative). The CV1 loadings of the FTIR spectra show a similar profile of PC2 in the Et60, related to the Man relative content. This shows that Man is the most important source of variability in the wine polymeric material.

CCA of the FTIR Spectra of the Fractions Precipitated in 60% Ethanol. To define more precisely the contribution of the relative amount of Man to the FTIR spectral profiles, a CCA was applied to the Man-richest samples, the Et60 fractions. By comparing the CV scatter plot (**Figure 7a**) to the CV loadings sugar profiles (**Figure 7c**), it was possible to correlate the sample

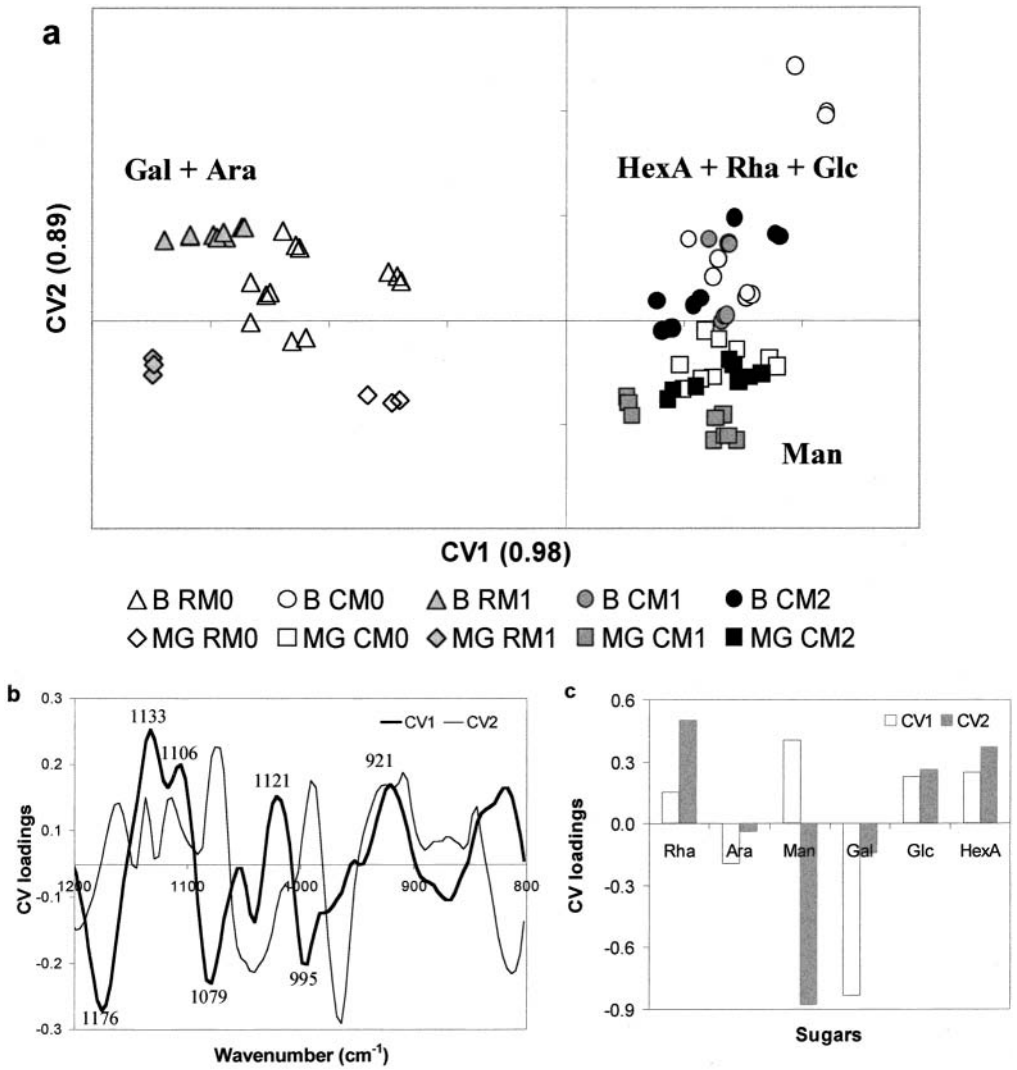


Figure 7. CCA of the wine ethanol 60% samples: (a) projection of the samples in the canonical variate space (CV1 vs CV2) (axes cross each other at the origin); (b) FTIR loadings profiles; (c) sugar analysis loadings plot.

distribution to the presence of several sugars. The samples located in the first quadrant (CV1 and CV2 positives) are richer in HexA, Rha, and Glc. Man is present in higher amounts in the samples located in the fourth quadrant (CV1 positive and CV2 negative). The samples richer in Gal and Ara are located in the CV1 negative axis. The high correlations presented in both CV axes, and a closer observation of the CV scatter plots, allow one to see that clarified samples of the MG wine variety are richer in Man. On the other hand, the samples of B wine variety are richer in HexA, Rha, and Glc in clarified samples and richer in Gal and Ara in nonclarified samples. **Figure 7a** shows, in the CV1 negative axis, for the nonclarified samples, the distinction between the macerated and nonmacerated wines. This figure shows also, along the CV2 axis, the distinction between the two wine varieties, as all MG samples were located in CV2 negative and all B samples were located in CV2 positive. According to **Figures 7b**,**c**, the distinction between clarified and nonclarified wines (CV1) and between the varieties (CV2) is related to the relative amount of Man.

Calibration Model for Man. On the basis of the previous studies of PCA and CCA of the wine polymeric material samples and ethanol-precipitated fraction, Man was found as the most important sugar to identify the polymeric material present. The high correlations observed between the FTIR spectra and the sugar analysis suggest that it should be possible to build a calibration model for this sugar for future prediction in new wine samples. For calibration purposes only the ethanol 60% fractions were used because they had the higher total sugars content when compared to the ethanol 80% and wine polymeric fractions. A PLS1 regression procedure was applied between the FTIR spectra of ethanol 60% fractions (in the 1200−800 cm^{-1} region) and the obtained Man content. It was found by internal cross-validation (leave-three-out) that it was necessary to have a calibration model with four latent variables to have a predictive power. The root mean square error of prediction (RMSEP) obtained was 7.5%, with a coefficient of determination (R^2) of 0.97. **Figure 8a** shows the **b** vector profile of the calibration model. This profile shows the most important wavenumbers related to the variability of Man. One can see that the bands located between 1150 and 1100 cm^{-1} and the band located at 975 cm^{-1} increase as the Man content increases. On the other hand, the bands located at 1070 and 1000 cm^{-1} increase as the Man content decreases. This subset of wavenumbers could be used as indicators of the Man variability in new samples, which will allow a much easier characterization of new samples regarding Man. The calibration curve is represented in **Figure 8b**, showing a good linear relationship within a wide range (11−90 mol %) of Man, which is an indication of the wide applicability that this model could have for future new samples.

Conclusions. The results presented in this work showed that FTIR spectroscopy (in the region between 1200 and 800 cm^{-1})

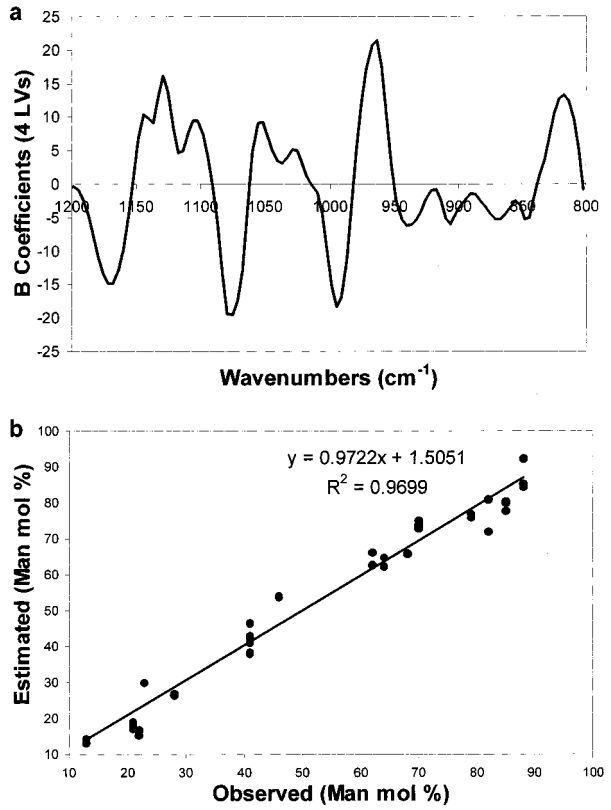


Figure 8. PLS1 model with four latent variables for the determination of Man in white wine samples (a) *b* vector profile; (b) calibration curve.

could be used to characterize white wine polysaccharide composition. It was possible to identify the wine-making process involved (must clarification and/or maceration) and its influence on the amount and type of wine polysaccharides. The models also allowed the analysis of the different types of variability present in the spectra and their correlation to several types of sugars, mainly Man. A calibration model was also proposed for the quantification of the major sugar, Man, and the results have shown that a model with good predictive power (R^2 = 0.97) could be achieved. Finally, the results show that it is possible to use the FTIR combined with multivariate techniques for an in-depth characterization of white wine polymeric fractions.

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