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Quantification of polymeric mannose in wine extracts by FT-IR spectroscopy and OSC-PLS1 regression

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Abstract

FT-IR spectroscopy has being a widespread technique in the agro-industry for the quick assess of food components, including the wine. Using the region of wavenumbers 1200–800 cm^{-1} of the FT-IR spectra wine polysaccharides, Partial Least Squares Regression (PLS1) independent calibration models were built for mannose quantification in complex matrices from white and in red wine extracts. With PLS1 it was not possible to build a calibration model that included both white and red wine extracts. However, a predictive ability of the model for quantification of mannose from mannoproteins based on this FT-IR spectral region was achieved by the application of orthogonal signal correction (OSC)-PLS1.

Keywords: FT-IR spectroscopy; White wine; Red wine; Wine polysaccharides; Mannose; Mannoproteins; OSC-PLS1

1. Introduction

Polysaccharides, is one group of wine macromolecules which, depending on their composition, structure and concentration, is relevant for explaining and controlling wine stability (Segarra, Lao, López-Tamames, & Torre-Boronat, 1995) and retention of aroma compounds (Goubet, Le Quere, & Voilley, 1998). They are originated both from grape and microorganisms. Arabinans (Belleville, Williams, & Brillouet, 1993), type II arabinogalactans (Pellerin, Vidal, Williams, & Brillouet, 1995), rhamnogalacturonans and galacturonans (Pellerin, Doco, Vidal, Williams, Brillouet, & O'Neill, 1996) arise from native cell-wall pectic polysaccharides of grape berry after degradation by pectic enzymes during grape maturation and during the first steps of wine making. Yeasts produce mannans and mannoproteins during and after fermentation (Waters, Pellerin, & Brillouet, 1994), whereas glucans are produced by *Botrytis cinerea*, which may infect grape berries (Dubourdieu & Ribereau-Gayon, 1981).

The methodologies usually used for the analysis of the polysaccharides, which include glycosidic-linkage hydrolysis, and derivatisation for formation of volatile sugar derivatives for gas chromatography analysis, are time consuming and expensive. To overcome these drawbacks, FT-IR as been used as an important source of information for a quick evaluation of polysaccharide composition in vegetable samples (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998; Coimbra, Barros, Rutledge, & Delgadillo, 1999; Ferreira, Barros, Coimbra, & Delgadillo, 2001; Kacuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000; Kacuráková & Wilson, 2001). In wine, FT-IR have been proposed and implemented for routine analysis of a large number of parameters, such as ethanol, volatile acidity, pH, tartaric acid, lactic acid, SO_2, glucose and fructose, acetic acid, citric acid and polyphenols (Dubernet, Dubernet, Dubernet, Coulomb, Lerch, & Traineau, 2000). Also, a rapid method for discrimination of red wine cultivars based on FT-IR spectra of the phenolic extracts in the 1640–950 cm^{-1} region was proposed (Edelmann, Diewok, & Lendl, 2001) and, more recently, the possible use of FT-IR for identification of polysaccharides was reported (Coimbra, Gonçalves, Barros, & Delgadillo, 2002).

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Following the work carried out on the quantification of mannose from mannoproteins in purified white wine extracts (Et60) by FT-IR and PLS1 regression (Coimbra et al., 2002), the aim of this work is to propose a chemometric methodology to improve the predictive ability of the model based on the FT-IR spectral region between 1200 and 800 cm^{-1} for a wider range of samples, including samples from different and more complex matrices, such as the whole polymeric material, and from red wines. In many cases, and for such complex matrices, the use of PLS1 regression is not suitable as the inherent complicated variations can hinder relevant information. Therefore, pre-processing techniques should be used to enhance or sort-out the relevant information making the models simpler and easier to interpret. Orthogonal Signal Correction (OSC) is one of many pre-processing filter that aim to remove strong systematic variations in a given independent set of variables that are not correlated with the dependent variables. That is, the filter removes from the dependent variables (y) structures that are orthogonal to the independent set of variables (X) (Wold, Antti, Lindgren, & Öhman, 1998).

The final intent of these studies is to be able to predict the type of polysaccharides according to their spectra on such complex matrices, to quantify the main type of constituent monosaccharide residues present in wine extracts, and to study the possibility of using the results in more complex systems such as the whole wine. This information will allow an expeditious assessment and monitoring of polysaccharide composition and modifications that occur during the winemaking processing.

2. Material and methods

2.1. Wine samples

Fernão-Pires (Maria Gomes) and Bical, white grape varieties, and Baga, red variety, from the Portuguese Bairrada Appellation, were used. Wines from each white variety were obtained with different technological procedures: with and without skin-contact and/or clarification of the musts with enological pectic enzymes, as described by Coimbra et al. (2002). For the red variety, one wine was prepared with a carbonic maceration step of 7 days, and a second wine was prepared with a carbonic maceration of 14 days.

2.2. Polysaccharide recovery

The wines (500 mL) were rotary-evaporated under reduced pressure at 35 °C to eliminate the ethanol and concentrate the total solids present until a volume of 25 mL. The material was then dialysed in order 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.

2.3. 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, 15 mL of absolute ethanol was added; the solution (80% ethanol) was stirred for 1 h at 4 °C, centrifuged, and the residue obtained (fraction Et80) was removed from the supernatant solution (fraction EtSN). Each precipitate was dissolved in water, rotary evaporated, frozen, and freeze-dried (Coimbra, Delgadillo, Waldron, & Selvendran, 1996).

2.4. Sugars analysis

Neutral sugars were analysed as their alditol acetates by GC-FID (Blakeney, Harris, Henry, & Stone, 1983; Harris, Blakeney, Henry, & Stone, 1988). Uronic acids (Ur. Ac.) were determined colorimetrically by a modification of the method of Blumenkrantz and Asboe-Hansen (1973).

2.5. FT-IR spectroscopy and multivariate analyses

FT-IR spectra were acquired for each sample of polymeric material and fractions obtained by ethanol precipitation (three replicates) between 4000–400 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 analysed by a program developed in the Institut National Agronomique Paris-Grignon in collaboration of the University of Aveiro (Barros, 1999). For multivariate analyses, Partial Least Squares (PLS1), and Orthogonal Signal Correction-PLS1 were applied in the 1200–800 cm^{-1} FT-IR region. Each spectrum was SNV (Standard Normal Deviates) corrected.

3. Results and discussion

3.1. Characterisation of wine polysaccharide samples

The samples used in this study were the wine polymeric material and the fractions obtained from it that were precipitated with 60% and 80% ethanol, in a total of 60 samples (data sets 1 and 2), where the majority of them were the wine polymeric material (46 samples). The samples used had a wide sugars composition range (Table 1). The main sugars present in these samples were mannose (Man, 3–89 mol%), arabinose (Ara, 1–40 mol%), galactose (Gal, 1–47 mol%), uronic acid (Ur. Ac., 0–57 mol%), rhamnose (Rha, 0–8 mol%), and glucose (Glc, 1–46 mol%). The amount of carbohydrates in each fraction varied from 15% (sample 56) to 97% (sample 6).

Fig. 1a shows typical FT-IR spectra of the wine polymeric material extracts. The spectra show high

Table 1
Sugar composition of wine polysaccharide samples from Bical, Fernão-Pires and Baga varieties

Sample number and origin	Data set	Mol%						Total sugars (%, w/w)
		Rha	Ara	Man	Gal	Glc	Ur. Ac.	
Bical white wine								
1 Et80	1	4	35	3	36	3	19	63
2 Polymeric material	1	4	40	4	33	4	16	64
3 Polymeric material	2	4	32	4	33	8	19	57
4 Polymeric material	1	5	29	10	28	3	23	70
5 Polymeric material	1	4	39	11	19	3	22	47
6 Polymeric material	1	2	8	15	11	7	57	97
7 Polymeric material	2	3	35	16	21	4	21	49
8 Polymeric material	2	6	22	20	16	2	32	42
9 Polymeric material	1	4	28	21	16	5	26	35
10 Polymeric material	1	3	11	21	10	6	50	94
11 Polymeric material	1	4	24	21	27	3	21	59
12 Polymeric material	2	4	22	22	24	4	24	68
13 Et60	1	7	23	22	26	2	19	47
14 Polymeric material	1	3	22	23	25	4	22	63
15 Polymeric material	1	7	21	28	26	3	15	49
16 Polymeric material	1	5	21	36	13	5	18	29
17 Polymeric material	2	3	12	39	10	10	24	51
18 Polymeric material	1	2	12	41	9	17	18	50
19 Polymeric material	1	8	12	41	10	5	24	33
20 Et60	2	1	15	42	28	4	10	40
21 Polymeric material	1	5	9	42	7	9	28	50
22 Polymeric material	1	6	12	46	9	3	23	26
23 Et60	1	2	4	62	11	7	13	27
Fernão-Pires white wine								
24 Polymeric material	1	3	37	3	40	2	14	60
25 Polymeric material	1	5	33	4	35	4	20	59
26 Polymeric material	2	4	36	4	38	3	14	79
27 Polymeric material	2	3	29	5	47	2	14	90
28 Polymeric material	1	3	27	7	44	2	18	95
29 Polymeric material	1	3	31	14	30	3	17	58
30 Polymeric material	1	5	10	19	23	12	30	41
31 Polymeric material	1	2	22	21	24	3	26	85
32 Polymeric material	1	3	23	24	24	4	23	77
33 Polymeric material	1	6	12	24	25	11	21	40
34 Polymeric material	1	2	20	29	26	5	17	73
35 Polymeric material	1	3	7	31	10	5	43	32
36 Polymeric material	1	6	9	35	19	11	19	39
37 Polymeric material	1	3	7	37	11	2	41	48
38 Polymeric material	1	2	10	37	6	4	41	44
39 Polymeric material	1	6	14	39	17	8	15	26
40 Polymeric material	1	5	6	55	9	10	15	55
41 Polymeric material	1	3	4	56	9	11	17	63
42 Polymeric material	2	4	5	56	8	13	14	58
43 Et60	1	1	9	64	17	2	7	65
44 Polymeric material	1	1	6	67	8	4	14	43
45 Polymeric material	1	3	4	68	4	2	20	77
46 Polymeric material	1	3	4	70	5	2	15	72
47 Polymeric material	1	3	3	70	4	2	18	69
48 Polymeric material	1	2	4	79	2	1	12	50
49 Polymeric material	2	1	3	82	2	2	10	47
50 Et60	1	0	2	85	1	2	9	33
51 Et60	2	0	1	85	3	1	8	55
52 Et60	1	2	3	89	3	1	3	70
Baga red wine								
53 Et80	1	4	29	11	45	10	0	25
54 Et80	1	3	31	11	45	8	2	58
55 EtSN	1	3	33	12	8	34	0	36
56 EtSN	1	2	26	13	10	46	0	15
57 Polymeric material	2	3	23	34	22	15	0	27
58 Polymeric material	1	3	18	39	21	13	0	38
59 Et60	1	2	8	70	10	9	2	35
60 Et60	1	2	6	77	8	5	0	30

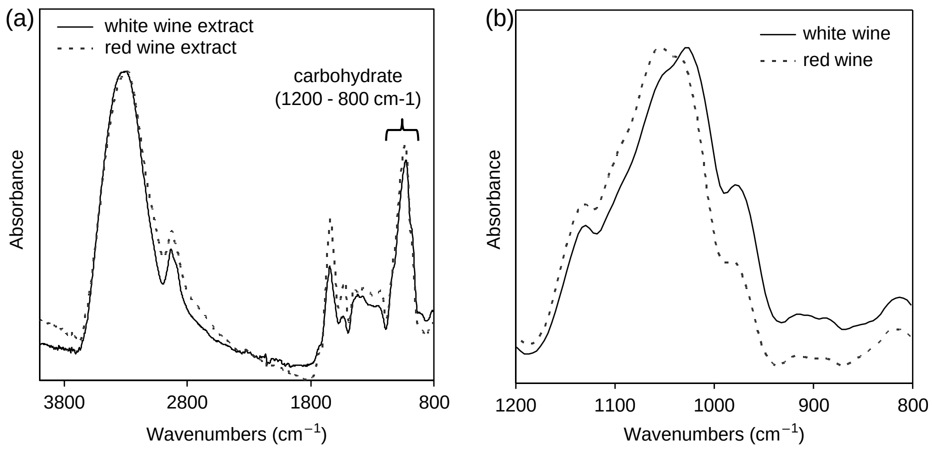

Fig. 1. FT-IR spectra of monovarietal white (Fernão-Pires) and red (Baga) wine polymeric material. (a) 4000–800 cm^{-1} region; (b) 1200–800 cm^{-1} region.

absorbance at wavenumbers characteristic of wine polysaccharides. Slight differences are observed in the carbohydrate region between the white and red wine (Fig. 1b).

3.2. Calibration model for Man in white wines

Using the 1200–800 cm^{-1} region of the FT-IR spectra of the purified ethanol 60% precipitated polysaccharide fractions from white wines, a PLS1 regression procedure has been applied for the estimation of the Man content in the samples with a good predictive ability (Coimbra et al., 2002). To extend the model to less purified samples, a PLS1 was applied to the 156 FT-IR spectra of white wine extracts (samples 1 to 52, c.f. Table 1). It was found by internal cross validation (leave-3-out) that it was necessary to have a calibration model with 4 Latent Variables to have a predictive power. The Root Mean Square Error of Cross-Validation (RMSECV) obtained was 17% with a coefficient of determination (R^2) of 0.96. The **b** vector profile of the calibration model (Fig. 2a) shows that the most important wavenumbers related to the variability of Man were the bands located at 1129, 1056, 964, and 817 cm^{-1}, which increase as the Man content increases, and the bands located at 1160, 1079 and 995 cm^{-1}, which increase as the Man content decreases. The observed vs. estimated Man relationships is represented in Fig. 2b, showing a linear relationship between 3 and 88 mol% of Man.

3.3. Calibration model for Man in red wines

The PLS1 regression procedure was also applied to the 24 FT-IR spectra of the red wine polymeric material and ethanol fractions (samples 53 to 60, c.f. Table 1). For these samples, it was necessary to have a calibration model with 5 Latent Variables to have a predictive power. The RMSECV obtained was 14%, with a R^2 of 0.99. The **b** vector profile of the calibration model (Fig. 3a) shows that the most important wavenumbers related to the variability of Man were the bands located at 1145, 1129, 1056, 1025, 964, 921, and 821 cm^{-1}, which increase as the Man content increases, and the bands located at 1176, 1079 and 995 cm^{-1}, which increase as the Man content decreases. The observed vs. estimated Man relationships plot is represented in Fig. 3d, showing a good linear relationship between a wide range (11 to 77 mol%) of Man.

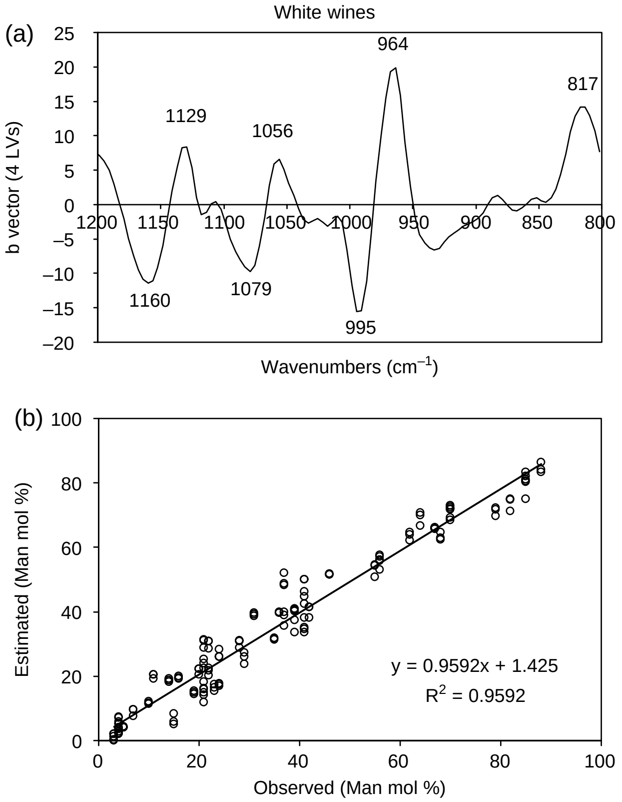

Fig. 2. PLS1 regression model for the estimation of polymeric mannose in white wine extracts (4 Latent Variables). (a) **b** vector profile; (b) relationship plot between observed vs. estimated amount of mannose.

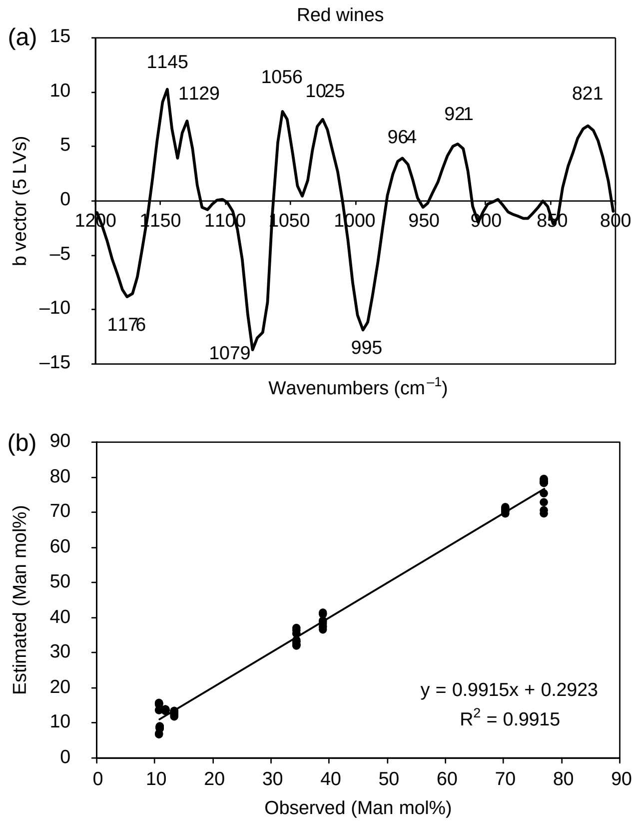

Fig. 3. PLS1 regression model for the estimation of polymeric mannose in red wine extracts (5 Latent Variables). (a) **b** vector profile; (b) relationship plot between observed vs. estimated amount of mannose.

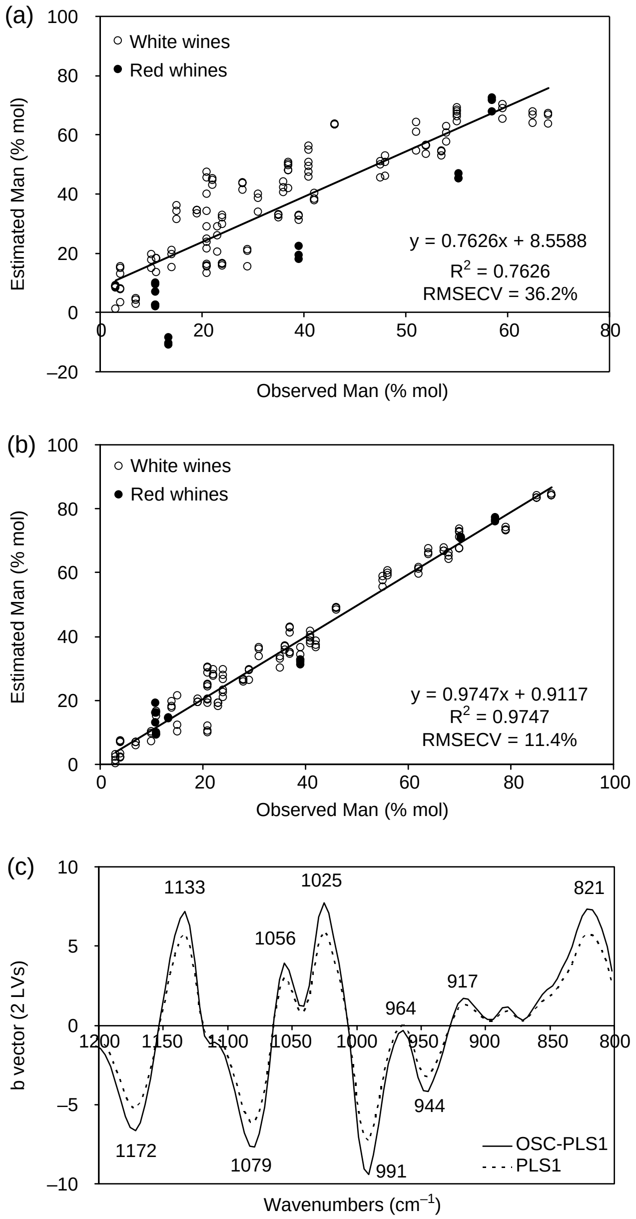

Fig. 4. Estimation of polymeric mannose in white and red wine extracts (2 Latent Variables). (a) PLS1 regression model; (b) OSC-PLS1 regression model; (c) **b** vector profiles.

3.4. Calibration model for Man in white and red wines using PLS1

In order to assess if it is possible to build a regression model for Man using both types of wines (red and white) a PLS1 calibration model was built using 141 FT-IR spectra in the 1200–800 cm^{-1} region from 47 samples (data set 1 in Table 1) and using 39 FT-IR spectra from 13 samples (data set 2 in Table 1) as the external set for the validation of the calibration model. To estimate the optimal number of Latent Variables (LVs) an internal cross-validation procedure was applied using the leave-3-out approach. The optimal dimensionality was found to be a model with 2 LVs with a RMSECV of 36.2%. In Fig. 4a it is shown the plot of the actual vs. estimated values for the polymeric Man using this PLS1 model. As it can be seen from this plot and from the RMSECV, the regression model obtained does not give satisfactory prediction ability. In fact, using data set 2 as the external set for the validation of the calibration model, a root mean square error of prediction (RMSEP) of 41.4% was obtained. This lower predictive calibration model was possibly related to the heterogeneous nature of the non-carbohydrate material present in the red and white wine extracts. These compounds can originate FT-IR signal complexities that can introduce major sources of variation that had very small or no predictive ability to the criterion of interest (Man quantification). In these cases, one common approach is to use different data pre-treatments to reduce the effect of variabilities not related to the factor of interesting. Among several methodologies that can be used, Orthogonal Signal Correction (OSC) is one of the most promising as it can be used to remove from the spectra a certain number of factors that are orthogonal (not related) to the criterion of interest (Man) (Fearn, 2000; Wold et al., 1998). This data pre-treatment procedure aims to improve the predictive ability of subsequent PLS1 regression models.

Table 2
Percentage of explained variability for PLS1 and OSC-PLS1 optimal models

	PLS1		OSC-PLS1	
	X	y	X	y
LV1	32.9%	58.7%	40.5%	69.4%
LV2	55.4%	17.6%	59.4%	28.0%
Total	88.3%	76.3%	99.9%	97.4%

3.5. Calibration model for Man in white and red wines using OSC-PLS1 regression

The methodology for selection of the optimal model dimensionality using the OSC was as follow: (a) The OSC procedure was applied to the calibration data set by removing 1, 2, to n factors. (b) After each removed factor a PLS1 regression was applied to the corrected data set and their predictive ability was assessed by cross-validation (leave-3-out).

It was found that by removing from the calibration set 11 factors an optimal PLS1 model with 2 LVs with a RMSECV of 11.4% (Fig. 4b) was obtained, showing a significant increase in the model predictive power when compared to the PLS1 calibration model. Fig. 4b shows the plot of the observed vs. estimated values for the polymeric Man using the OSC-PLS1 model, where a better linear trend was obtained when compared to that of Fig. 4a. Using data set 2 as the external set for the validation of this calibration model, a RMSEP of 14.6% was obtained, which is much smaller than the 41.4% obtained from the PLS1 calibration model. The amount of explained variability for each Latent Variable for both PLS1 and OSC-PLS1 proposed calibration models is shown in Table 2. The OSC-PLS1 explains 97.4% of the variability contained in the y vector (polymeric Man values) whereas PLS1 only explains around 76.3%. Fig. 4c represents the **b** vectors plot for the PLS1 and OSC-PLS1 with 2 LVs where it can be seen that the only difference of the **b** vector plot between PLS1 and OSC-PLS1 is the intensity of the bands, not their positions. The characteristic bands for the quantification of polymeric Man can be identified as the bands located at 1133, 1056, 1025, 917, and 821 cm^{-1}, which are positively related to Man content, and the bands located at 1172, 1079 and 991 cm^{-1}, which are negatively related to Man content.

4. Concluding remarks

The results presented in this work showed that OSC-PLS1 is a multivariate tool that can be used to improve the predictive ability of the model for quantification of mannose from mannoproteins based on the FT-IR spectral region between 1200–800 cm^{-1}, allowing it to be used for a wider range of samples, including white and red wine extracts from complex matrices.

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