

Use of FT-IR spectroscopy to follow the effect of processing in cell wall polysaccharide extracts of a sun-dried pear

D. Ferreira^{a,b}, A. Barros^a, M.A. Coimbra^{a,*}, I. Delgadillo^a

^aDepartment of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

^bEscola Superior Agrária de Viseu, Instituto Superior Politécnico de Viseu, 3500 Viseu, Portugal

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Abstract

The sun-dried pear is a traditional Portuguese food product with unique organoleptic characteristics. In order to understand the modifications that occur during the drying process, cell wall extracts were prepared from dried and fresh tissues. Cell wall polymers were solubilised from the CWM by sequential extraction with aqueous solutions of CDTA, Na₂CO₃, and KOH, with increasing strengths, to leave the cellulosic residue. The polysaccharides present in the different extracts were characterised by sugar analysis and by FT-IR spectroscopy in the region between 1200 and 850 cm⁻¹. The spectra analysis followed by chemometrics allowed us to distinguish the fresh from the sun-dried pear extracts in CDTA, Na₂CO₃, and KOH precipitates. These extracts contain pectic polysaccharides rich in GalA and Xyl-rich hemicellulosic polysaccharides (xylans). The selection of the most important wavenumbers allowed us to identify the spectral region related to GalA in pectic polysaccharide extracts in the range between 1120 and 990 cm⁻¹. This study shows the potential of the FT-IR spectroscopy and multivariate information in the discrimination of cell wall extracts from dried and fresh pears. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Fruit drying is a very ancient practice for food preservation still in use nowadays. The sun-dried pear used in this study is a Portuguese agricultural product, which is highly appreciated by the consumer because of its singular organoleptic characteristics.

After harvesting, the pears are peeled and allowed to sun-dry for nearly five days. Then the pears are laid in baskets and covered with a cloth for two days. This treatment is necessary to give the pears the elasticity needed to flatten without breakage. After this, the pears are subjected to a second sun-drying process. The final product is a small dried pear with a reddish-brown colour and peculiar elastic

properties. These kinds of traditional agricultural product are socially and economically important in the interior regions of Portugal, contributing to the diversification of agricultural production.

Technologies of the drying processes are still under development (Ratti & Mujumdar, 1996); however, there is a paucity of information about the chemical and biochemical changes occurring naturally within the fruit tissues during this process. Modifications in fruit texture with processing have been described generally as an overall tissue change of firmness related to cell wall polymers (Stolle-Smits, Beekhuizen, Recourt, Voragen & Dijk, 1997; Femenia, Sánchez, Simal & Rosselló, 1998). The knowledge of the structure and chemical composition of the cell wall polysaccharides may highlight the modifications that occur during the drying process. This could afford a less empirical and more rational approach to the chemical and biochemical modifications involved in the different steps of the process.

The application of FT-IR and chemometrics to the study of cell wall polysaccharides has proved to be a very valuable technique for the evaluation of cell wall monosaccharide composition (Coimbra, Barros, Barros, Rutledge & Delgadillo, 1998; Coimbra, Barros, Rutledge & Delgadillo,

* Corresponding author. Fax: +351-234-370084.

E-mail address: mac@dq.ua.pt (M.A. Coimbra).

Abbreviations: Ara: arabinose; CDTA: *trans*-1,2-cyclohexane-diamine-*N,N,N',N'*-tetraacetate; CWM: cell wall material; CR: cellulosic residue; FID: flame ionisation detector; FT-IR: Fourier transform infrared; GalA: galacturonic acid; GC: gas chromatography; HexA: hexuronic acid; KOH ppt: KOH extracts insoluble in water; PC: principal component; PCA: principal component analysis; PLS: partial least squares regression; RMSEP: root mean square error of prediction; SDS: sodium dodecylsulfate; TOP: trimmed object projection; UVE: uninformative variable elimination; Xyl: xylose

1999). Recently, hexuronic acid and neutral sugars of pectic origin from olive and orange cell walls were quantified using the spectral region between 1200 and 850 cm^{-1} (Coimbra et al., 1998). In this work, the changes in cell wall polysaccharide extracts from fresh and sun-dried pears were followed using of FT-IR associated with chemometric analysis.

2. Materials and methods

2.1. Samples origin

The analysis was performed on a single lot of fresh (*Pirus communis* L. var. S. Bartolomeu) and sun-dried peeled pears. Both lots were obtained from the same producer. Harvesting and processing occurred during July/August.

Cell wall material free of intracellular polymers was prepared from fresh and dried pulps according to the methods previously described by Coimbra, Delgadillo, Waldron and Selvendran (1996). The polymers were

solubilised from the CWMs by sequential extraction with: (1) CDTA, sodium salt, (2) Na_2CO_3 , and (3) KOH with increasing strengths to leave the cellulosic residue, as shown in Fig. 1.

2.2. Carbohydrate analysis

All the extracts were submitted to a treatment with 72% H_2SO_4 for 3 h at room temperature followed by dilution to 1 M and hydrolysis for 2.5 h at 100°C (Coimbra et al., 1996). The neutral sugars released were derivatised as their alditol acetates and separated by gas chromatography, as described by Coimbra et al. (1996), using a Carlo Erba ICU 600 with a split injector (split ratio 1:60), an FID detector and a 30 m column DB-225, with an ID of 0.25 mm and 0.15 μm film thickness. The analyses were carried out under the following conditions: injector 220°C, detector 230°C, oven temperature program 220°C for 4 min, 220–230°C at 25°C/min and 230°C for 6 min. The flow rate of the carrier gas (H_2) was set at 1 ml/min at 220°C. Hexuronic acids were quantified colorimetrically by a modification (Coimbra et al., 1996) of the method described by Blumenkrantz and Asboe-Hansen (1973).

2.3. FT-IR spectra

FT-IR spectra were obtained at a resolution of 8 cm^{-1} . The samples, in triplicate, were incorporated into KBr (spectroscopic grade) and pressed into a 1 mm pellet. The 256 scans were co-added before Fourier transformation. Spectra were recorded in the absorbance mode from 4000 to 400 cm^{-1} , using a Nicolet Magna-IR 550.

2.4. Data pre-treatment

Each spectrum was baseline corrected and the absorbance was normalised between 0 and 1. The spectra were transferred via a JCAMP.DX format (Rutledge & McIntyre, 1992) into the data analysis software package developed by A. Barros and D. N. Rutledge in Institut National Agronomique, Paris-Grignon, as described in Coimbra et al. (1998, 1999).

3. Results and discussion

3.1. Sugar analysis of cell wall extracts

Table 1 shows the sugar composition of the extracts obtained by sequential extraction of fresh and sun-dried pear pulps with aqueous solvents. The CDTA and carbonate extracts are rich in HexA and Ara, characteristic of pectic polysaccharides. The fresh pear CDTA extracts contain a relatively higher amount of HexA than the processed ones; on the contrary, for the carbonate extracts, the amount of HexA is higher in the sun-dried pears than in the fresh ones. The hemicellulosic polysaccharides were extracted with KOH solutions. These polymers are rich in Xyl and Glc.

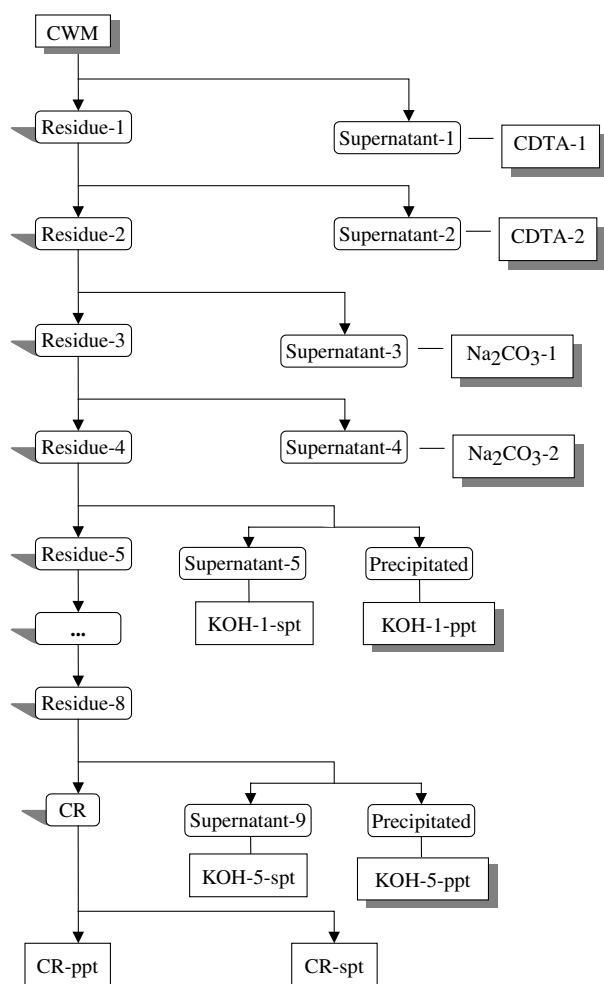


Fig. 1. Experimental design of the sequential extraction of cell wall materials from pear pulps. ppt, precipitate; spt, supernatant; CR, cellulosic residue.

Table 1

Origin and sugar composition of cell wall extracts from fresh and sun-dried pear pulps. Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, manose; Gal, galactose; Glc, glucose; HexA, hexuronic acid

Extracts	Mol%								Total sugar (mg/g)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
<i>Fresh</i>									
f: SDS-1.5-spt	0	0	19	3	1	16	4	57	260
f: SDS-0.5-spt	0	0	25	7	1	8	2	56	577
f: CWM	1	0	21	17	2	8	36	16	722
f: CDTA-1	0	0	18	1	0	2	0	79	382
f: CDTA-2	0	0	24	2	0	3	1	71	191
f: Na ₂ CO ₃ -1	0	0	50	4	0	13	0	33	566
f: Na ₂ CO ₃ -2	0	0	52	5	0	14	0	29	569
f: KOH-1-spt	0	2	31	26	0	14	15	13	896
f: KOH-1-ppt	0	0	14	32	0	6	39	9	620
f: KOH-2-spt	0	4	6	35	2	14	31	7	1085
f: KOH-2-ppt	0	0	4	82	0	1	6	7	848
f: KOH-3-spt	0	4	5	33	3	15	35	5	897
f: KOH-3-ppt	1	0	8	69	0	3	12	7	691
f: KOH-4-spt	0	5	2	30	4	16	40	3	1014
f: KOH-4-ppt	0	0	2	85	1	2	5	5	1002
f: KOH-5-spt	0	2	17	21	9	16	27	7	859
f: KOH-5-ppt	0	0	5	70	2	2	15	6	500
f: CR-spt	0	0	44	12	0	19	3	21	908
f: CR-ppt	0	0	7	36	1	3	44	9	883
<i>Sun-dried</i>									
d: SDS-1.5spt	6	2	31	6	2	17	3	32	467
d: SDS-0.5spt	2	3	22	11	3	6	7	46	755
d: CWM	3	3	11	41	1	3	27	12	596
d: CDTA-1	1	2	26	5	1	4	1	59	820
d: CDTA-2	2	4	31	8	1	5	4	45	533
d: Na ₂ CO ₃ -1	2	3	35	7	1	4	1	47	782
d: Na ₂ CO ₃ -2	3	6	34	13	2	15	3	23	290
d: KOH-1-spt	3	1	18	29	5	8	28	8	514
d: KOH-1-ppt	0	0	7	26	0	3	49	16	599
d: KOH-2-spt	0	4	7	37	2	11	29	10	871
d: KOH-2-ppt	0	0	12	42	0	6	30	10	816
d: KOH-3-spt	0	5	3	32	3	15	34	9	904
d: KOH-3-ppt	0	0	3	62	2	2	23	8	961
d: KOH-4-spt	0	1	14	26	6	13	29	12	713
d: KOH-4-ppt	0	0	14	48	0	4	28	6	780
d: KOH-5-spt	0	0	30	35	0	5	9	21	148
d: KOH-5-ppt	0	0	15	18	0	8	50	9	428
d: CR-spt	1	2	41	9	1	11	7	28	965
d: CR-ppt	3	1	8	40	1	0	42	8	580

KOH precipitates of fresh pulp were shown to be richer in Xyl than processed ones; on the contrary, the sun-dried pears were shown to have precipitates with a high relative amount of Glc.

3.2. Multivariate qualitative analysis of FT-IR spectra

Fig. 2 shows the TOP scores scatter plot (PC2 × PC3) of the information provided by the FT-IR spectra in the 1200–850 cm⁻¹ region of all fresh and dried pear extracts. This analysis allows the differentiation between pectic polysaccharides rich in HexA (CDTA) and those with a lower content of HexA (CDTA + carbonate + SDS) and from the hemicellulosic polysaccharides (KOH) and cellulosic

residues (CR). However, it was not possible to distinguish the fresh from the processed.

3.3. Pectic and hemicellulosic extracts

The information provided by the FT-IR spectra in the region 1200–850 cm⁻¹ of fresh and dried pear extracts was studied and characterised by applying a PCA. The scores scatter plot shown in Fig. 3a allows the distinction between pectic and hemicellulosic extracts along the PC1 axis, which corresponds to nearly 52% of the variability contained in the FT-IR spectra. In the pectic extracts, it is also possible to distinguish between the fresh and the dried pear within the CDTA extracts; this distinction is

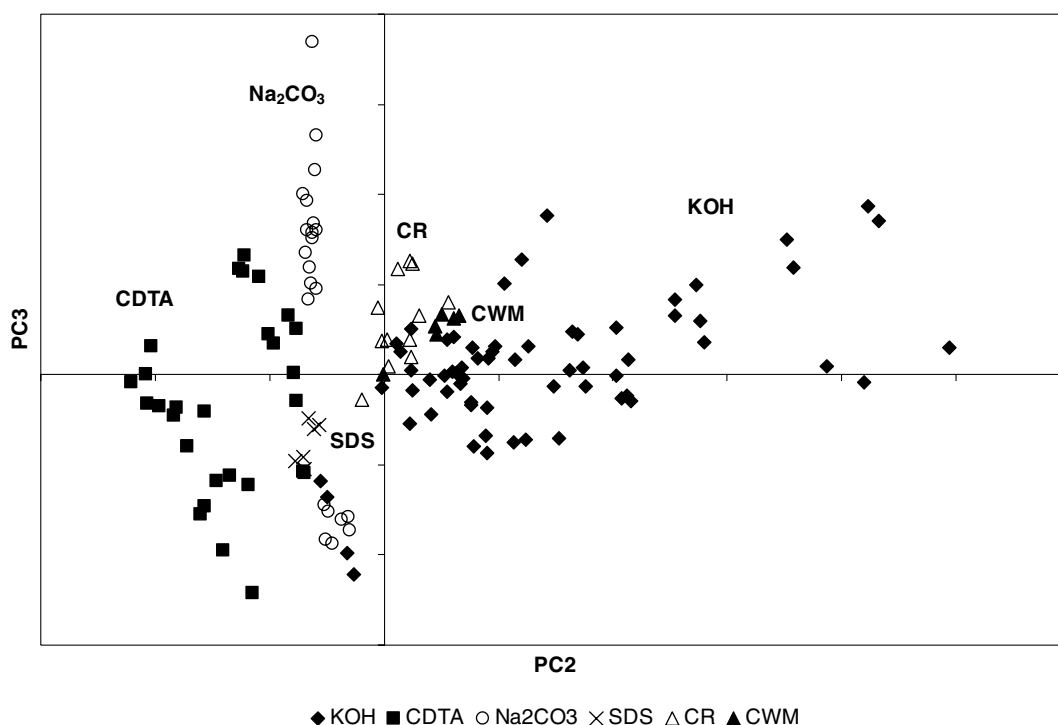


Fig. 2. Trimmed object projection (PC2 vs. PC3) of cell wall extracts from fresh and dried pear pulps.

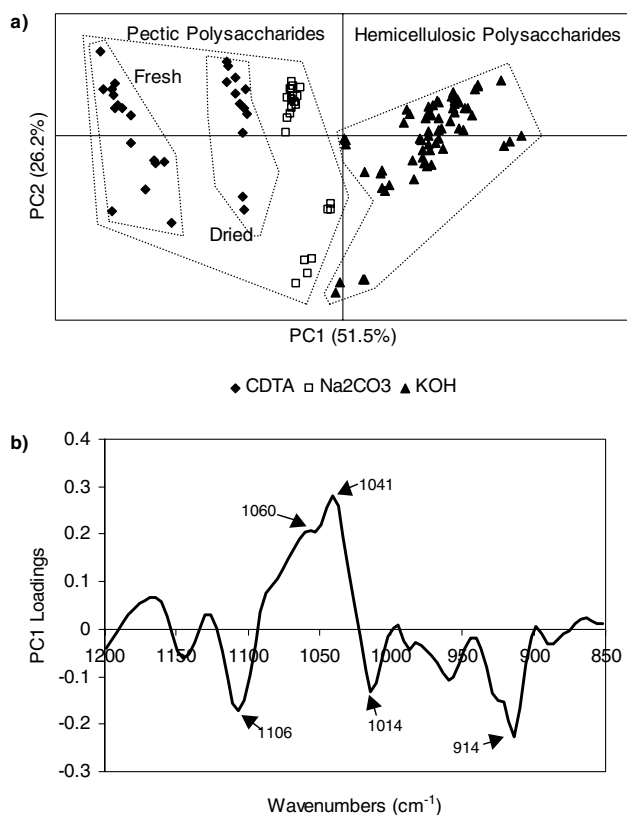


Fig. 3. PCA plots of the spectra of pectic and hemicellulosic extracts: (a) scores scatter plot (PC1 vs. PC2); and (b) loadings plot (PC1).

not possible with the carbonate extracts. From the PC1 loadings plot shown in Fig. 3b, one can note that the hemicellulosic extracts are characterised mainly by the peak located at 1041 cm^{-1} , which is attributed to Xyl (Coimbra et al., 1999), and the pectic polysaccharide extracts are characterised mainly by the absorbances at 1106 , 1014 and 914 cm^{-1} . Because all pectic polysaccharides are on the PC1 negative side, no attributions can be made to differentiate the CDTA extracts from fresh and dried pears. The absorbances at 1106 and 1014 cm^{-1} are due to the galacturonic acid (Coimbra et al., 1998, 1999). The peak located at 914 cm^{-1} is related to the non-dialysable CDTA salt (Coimbra et al., unpublished results). In order to analyse the differences between fresh and dried pears within each extract, a PCA analysis was applied to all extracts, and it was found that there were significant differences in CDTA, carbonate and KOH water insoluble extracts (results not shown). These extracts were subjected to a more detailed analysis described in the following sections.

3.4. CDTA extracts

From the scores scatter plot shown in Fig. 4a, a distinction between the fresh and the dried pears is observed. The dried pears are represented mainly in quadrant IV, which is characterised by the bands located at 1064 and 1040 cm^{-1} in the loadings plot (Fig. 4b). According to Coimbra et al. (1998, 1999), these wavenumbers are related to the high content of Ara. This observation can be confirmed by the sugar analysis in Table 1. The extracts from the fresh pear are

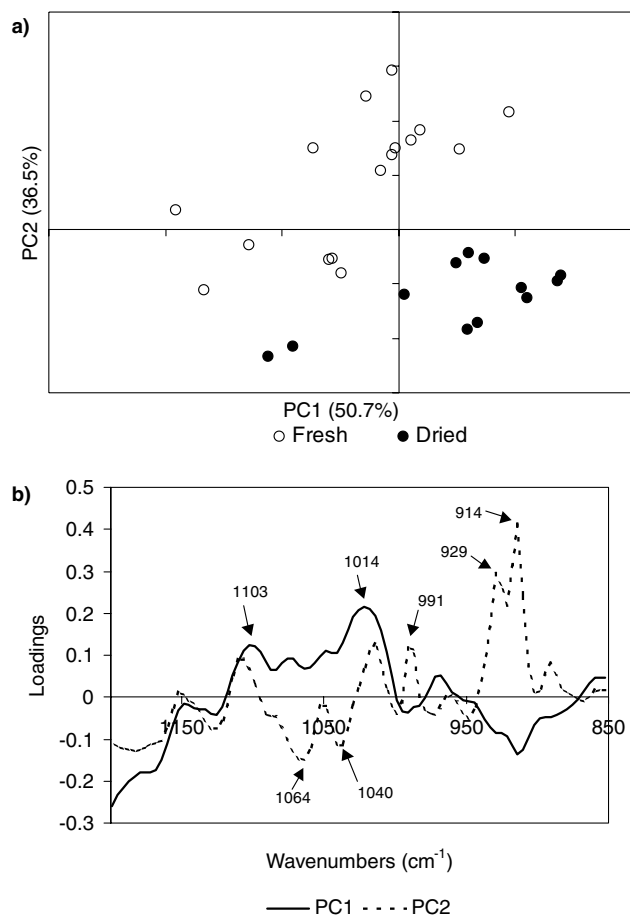


Fig. 4. PCA plots of the spectra from CDTA extracts: (a) scores scatter plot (PC1 vs. PC2); and (b) loadings (PC1 and PC2).

represented mainly in quadrants I and II, which are characterised by the peaks at 1103 and 1014 cm^{-1} , attributed to GalA, and the two intense peaks at 929 and 914 cm^{-1} , attributed to the CDTA salt. This higher content of CDTA salt in fresh pear extracts is not observed in dried pears. This behaviour of the chelating agent may be due to the presence of degraded pectic polymers with a higher amount of Ara in the side chains of dried pears.

3.5. Carbonate extracts

The scores scatter plot of carbonate extracts is shown in Fig. 5a. The PC1 axis seems to be related to the distinction between the fresh and processed pears. Despite the high variability of dried pears, it is possible to attribute some characteristic wavenumbers to this differentiation by the loadings plot shown in Fig. 5b. The dried pears seem to have more HexA content than the fresh ones (peaks located at 1100 and 1014 cm^{-1}). The region related to Ara content (peaks around 1040–1060 cm^{-1}), seems to be linked to the fresh pears. These results are in accordance with the sugar analysis shown in Table 1, where it is observed that the dried extracts are richer in HexA than the fresh ones.

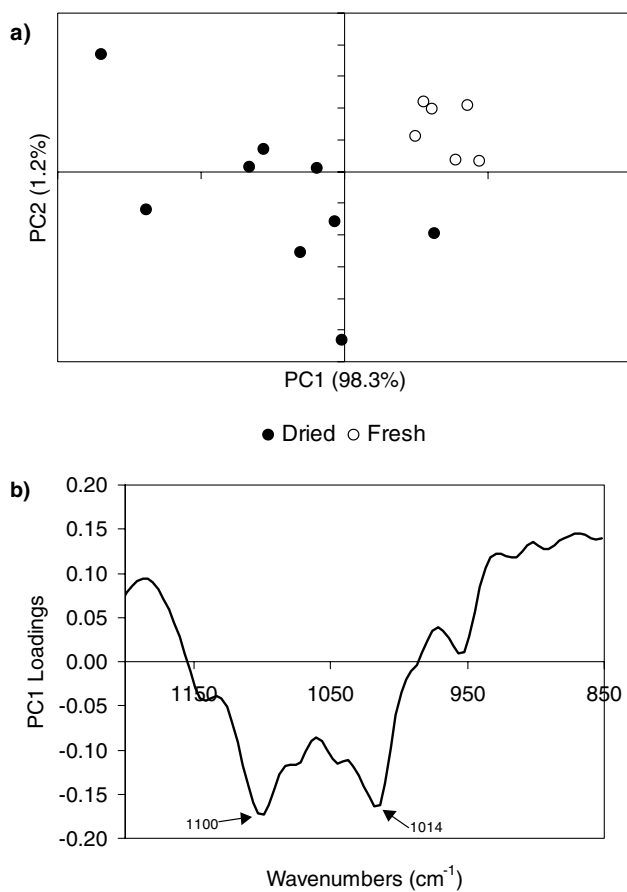


Fig. 5. PCA plots of the spectra from carbonate extracts: (a) scores scatter plot (PC1 vs. PC2); and (b) loadings (PC1).

3.6. KOH extracts insoluble in water

The scores scatter plot shown in Fig. 6a allows us to differentiate the fresh from the dried pears in the hemi-cellulosic-rich polysaccharide extracts. The loadings plot of the PC1 axis (Fig. 6b) shows a peak located at 1041 cm^{-1} , which is related to the Xyl-rich polymers, according to Coimbra et al. (1999). In fact, this result suggests that the fresh pear is richer in Xyl polymers than the dried pear, and this is in accordance with the sugar analysis shown in Table 1.

3.7. Multivariate quantitative analysis

Calibration models for the quantification of GalA have been proposed already for olive pectic polysaccharides (Coimbra et al., 1998). In this section a calibration model for the quantification of GalA in pear pectic polysaccharides is built and the most important wavenumbers are identified.

The data set containing 44 pectic polysaccharide spectra in the region 1200–850 cm^{-1} was used to build the calibration model. The PLS1 model used was built using two latent variables, determined by internal cross-validation — leave-one-out. A calibration curve with an RMSEP of

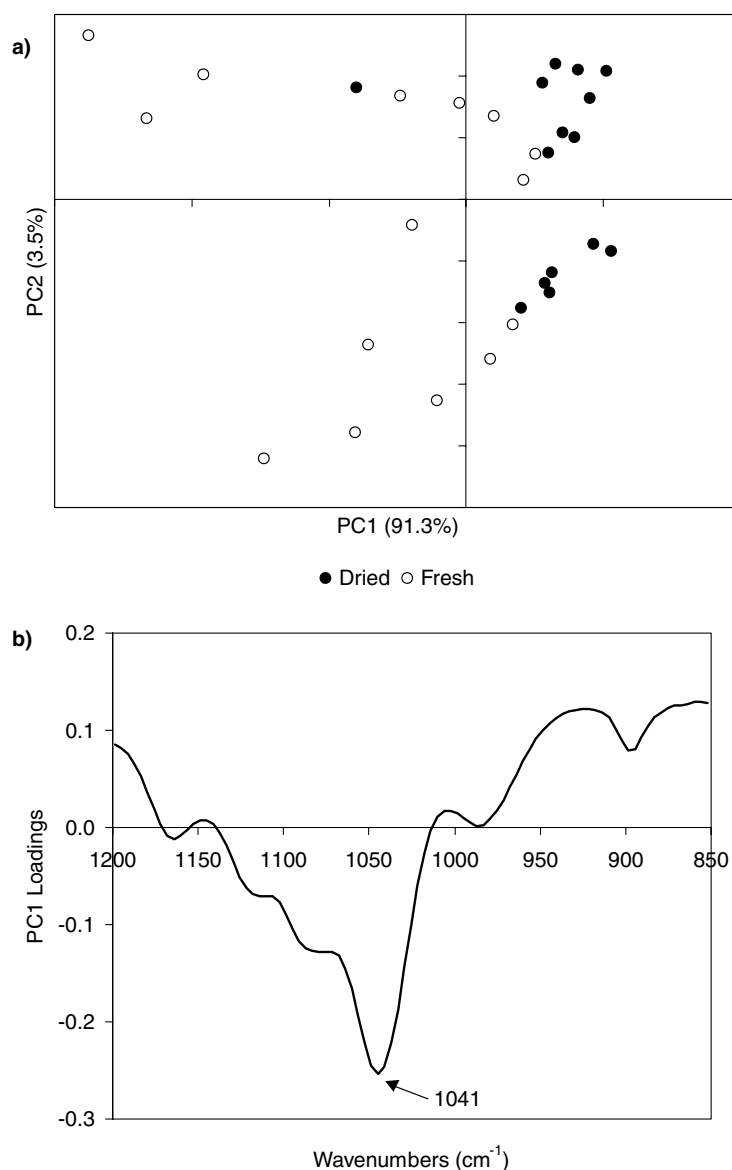


Fig. 6. PCA plots of the spectra from KOH water insoluble extracts: (a) scores scatter plot (PC1 vs. PC2); and (b) loadings plot (PC1).

11.4% and a coefficient of determination (R^2) of 0.89 was obtained (Fig. 7a). Fig. 7b shows the B coefficients from the calibration PLS1 model. This profile is very similar to the B coefficients of GalA obtained from calibration models of other sources of pectic polysaccharides, such as olive and orange (Coimbra et al., 1998).

In order to identify the most important wavenumbers for the quantification of GalA, two variable selection procedures were applied in PLS1: (1) uninformative variable elimination (UVE); and (2) variable selection. The performance of both methods was then compared to the classical PLS1 calibration model. As can be observed from Table 2, both the RMSEPs and R^2 are very similar. In addition, the UVE-PLS1 gives the best result although the observed difference is not significant. The variables selected by the two methods are almost the same (bars

in Fig. 8). This is an important factor since two different approaches were used for the selection of variables. Results strongly suggest that these set of variables are, in fact, the ones that characterise the GalA content in pectic polysaccharides.

3.8. Concluding remarks

The analysis of the FT-IR spectra by the use of chemometrics (TOP and PCA), allowed us to distinguish fresh from sun-dried pear extracts. The polysaccharides that contribute to this distinction are the pectic polysaccharides rich in GalA and Xyl-rich hemicellulosic polysaccharides (xylans). This methodology suggests that it is possible to follow the effect of sun-drying in cell wall polysaccharide extracts of pears using FT-IR spectroscopy. The selection of

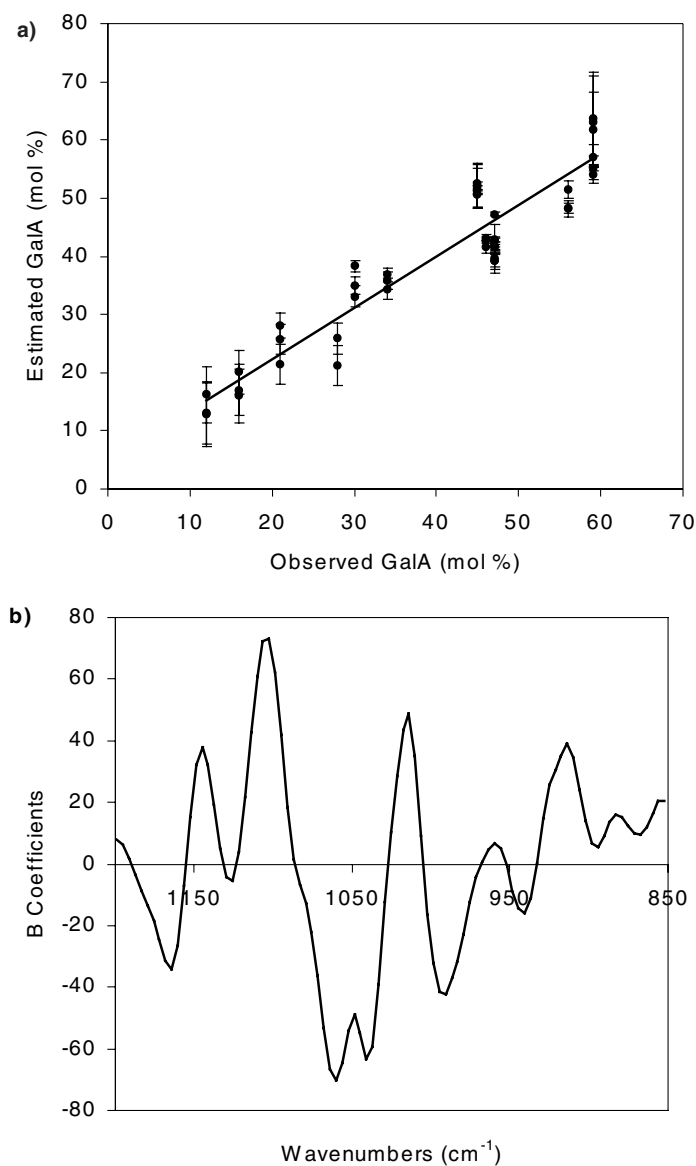


Fig. 7. Calibration model for the quantification of GalA: (a) calibration curve and estimated errors; and (b) coefficients profile.

Table 2

Statistical results for the quantification of GalA using different procedures of calibration (full region and selected variables)

	PLS1 (full 1200–852 cm ⁻¹) region (91 variables)	UVE - PLS1 (21 variables)	VS - PLS1 (14 variables)
RMSEP (%)	11.4	10.7	11.4
R^2	0.89	0.90	0.89
Latent variables	2	2	2

the most important wavenumbers, by two independent chemometric techniques, allowed us to define the region between 1120 and 990 cm⁻¹ as the range for the spectral identification of GalA in pectic polysaccharides.

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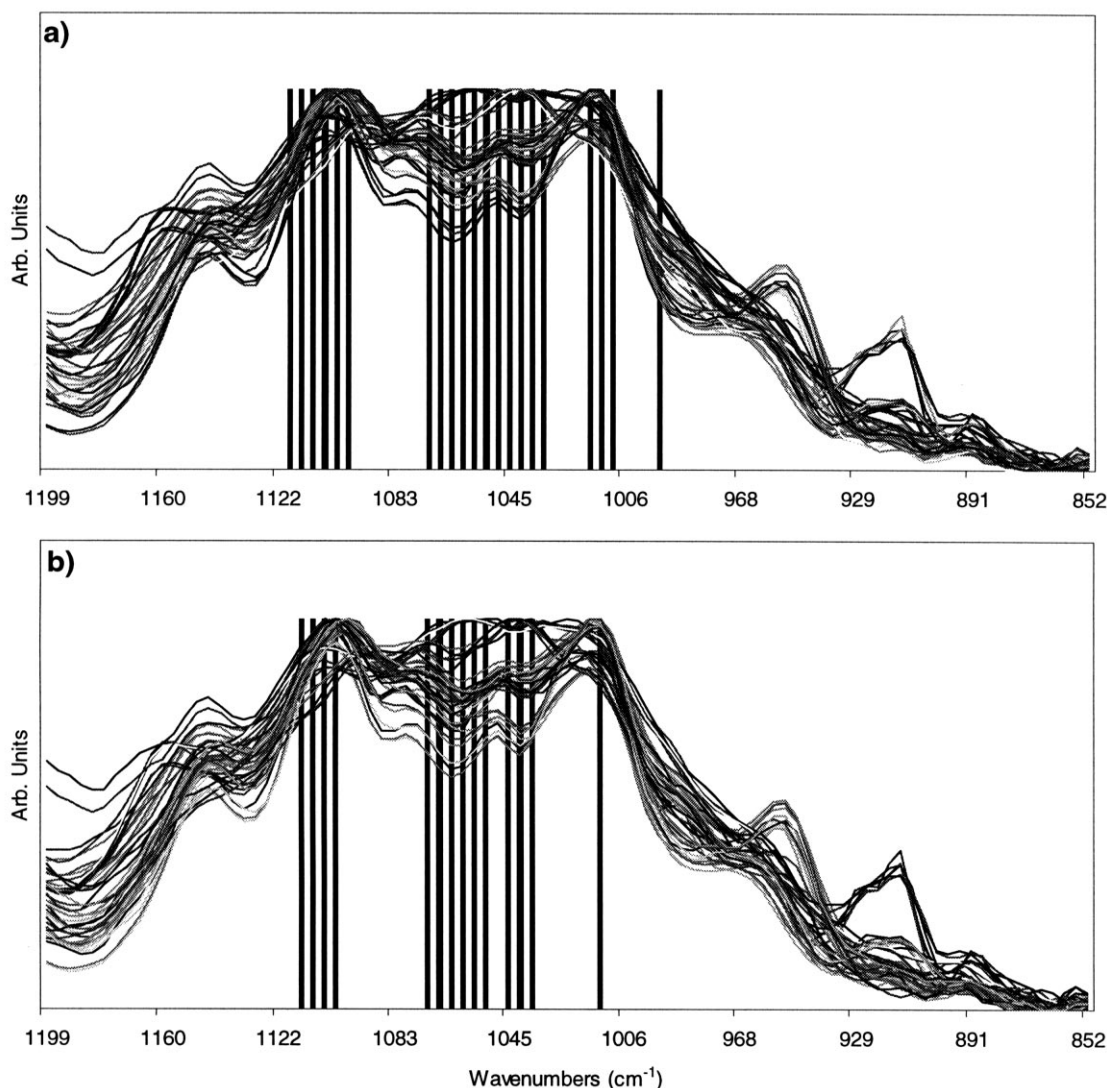


Fig. 8. Most important wavenumbers for the quantification of GalA: (a) UVE-PLS1; and (b) VS-PLS1.

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