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Influence of hydration of food additive polysaccharides on FT-IR spectra distinction

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Abstract

Using the Fourier-transform infrared (FT-IR) spectra in the 1200–800 cm^{-1} region and chemometrics, food additive polysaccharides such as pectin, starch, galactan and carrageenan can be distinguished. However, this vibrational spectroscopy technique is sensitive to the changes in conformation and to the constraints imposed by the hydrogen bonding with water, which could result in the destruction of all spectra qualities. In order to see if the water absorbed by carbohydrates can prevent the information necessary for their distinction in the 1200–800 cm^{-1} region, six monosaccharides, three disaccharides, four carrageenans, four pectic polysaccharides, three galactans, two glucans and one commercial mixture of carrageenan–pectin were submitted to a 96% relative humidity environment and were also dissolved in saturated water solutions, and their spectra were compared with those obtained from the dry samples. The application of a principal component analysis (PCA) to the FT-IR spectra showed that the distinction of sugars and polysaccharides by FT-IR in the 1200–800 cm^{-1} region can be achieved with no apparent loss of information in samples that can contain water. When the samples are in aqueous solution, the water influence is included in PC1 and the FT-IR spectral information of carbohydrates, previously given in PC1 and PC2 in dried samples, can be achieved in PC2 and PC3.
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1. Introduction

Polysaccharides and their derivatives have always been of major interest in food industry because of their important role in the appearance, texture and taste of food products and due to their established properties in the promotion of longer shelf-life and as nutraceuticals.

Besides, the many methods concerning the identification or the determination of these compounds in foods, versatile and rapid analyses are desirable. Mid infrared spectroscopy appears as a very useful tool in the authentication of foods from raw materials to final products. Using the Fourier-transform infrared (FT-IR) spectra in the 1200–800 cm^{-1} region in tandem with chemometrics, food additive polysaccharides such as pectin, starch, glucomannan and carrageenan can be distinguished (Černá, Barros, Nunes, Rocha, Delgadillo and

Čopíková, 2003). However, this vibrational spectroscopy is sensitive to the changes in conformation and to the constraints imposed by the hydrogen bonding with water (Kačuráková & Mathlouthi, 1996). Polysaccharides are highly hygroscopic molecules (Whistler & BeMiller, 1997). Water sorption by polysaccharides has been described as a very important aspect of the physical behaviour of these polymers (Mazeau & Rinaudo, 2004). Infrared spectroscopy is so sensitive to hydrogen bonds that this property was the basis for its use for the observation of the conformational changes that occur when hyaluronan polysaccharide passed from the solid semi-crystalline state to the aqueous state (Haxaire, Maréchal, Milas & Rinaudo, 2003). An excess of water, nevertheless, would result in saturated bands that destroy all the qualities of the spectra.

If the analytical evaluation of the authenticity of the product by means of infrared spectroscopy should be rapid and reliable, the problem of scanning spectra of samples containing different water content can rise (Kačuráková and Mathlouthi, 1996; Kačuráková & Wilson, 2001). In order to see if the water absorbed by these compounds during their storage affects the spectra, six monosaccharides, three disaccharides, four

carrageenans, four pectic polysaccharides, three galactans, two glucans and one commercial mixture of carrageenan–pectin submitted to a hydrated environment and in solution were studied in the FT-IR 1200–800 cm^{-1} region and compared with those from the dry samples.

2. Materials and methods

2.1. Samples origin

Standards. Twenty three carbohydrate standards, mono-, di- and polysaccharide standard compounds, were used to build a FT-IR dataset based in their absorbances in the region 1200–800 cm^{-1}: L-arabinose (Sigma), D-fructose (Sigma), D-glucose (Sigma), D-mannose (Sigma), D-galactose (Sigma), D-galacturonic acid (Sigma), sucrose (Lachema), maltose (Sigma), lactose (Sigma), amylose (Sigma), amylopectin (Sigma), κ-carrageenan (Fluka), ι-carrageenan (Fluka), λ-carrageenan (Fluka), commercial carrageenan, mixture of commercial carrageenan and HM pectin, HM pectin 1 (Sigma), HM pectin 2 (Sigma), potassium pectinan (PectK3) [DE=21.6%] (Sigma), potassium polygalacturonan (Sigma), arabic gum (Sigma), galactan (Koch-Light Laboratories), and arabinogalactan (Sigma).

The results of chemical analysis of the samples were as described by Černá et al. (2003).

2.2. Hydratation of samples

All standards and lyophilised polysaccharides were kept over phosphorus pentoxide and their infrared spectra were collected. This collection had been equilibrated at 25 °C and relative humidity of air 96% in a desiccator for 72 h. The samples were labelled as 'hydrated' and their infrared spectra were acquired. Then, to each sample, a few drops of water were added and the saturated solution or gel was created. These samples were labelled as 'solution' and their infrared spectra were collected. The water content of saccharides in dry and hydrated states is presented in Table 1.

2.3. FT-IR spectroscopy and chemometric analysis

The FT-IR spectra of the three collections of samples were obtained using a Golden Gate single reflection diamond ATR system in a Bruker IFS-55 spectrometer. The spectra were recorded at the absorbance mode from 4000 to 400 cm^{-1} (mid infrared region) at the resolution of 8 cm^{-1}. Five replicate spectra (128 co-added scans) were collected for each sample. The measured spectra were transferred via a JCAMP.DX format into the data analysis software package for PCA and each spectrum, within the 1200–800 cm^{-1} region, was SNV (standard normal deviates) corrected. The PCA analysis could allow the characterisation of the sample relationships (scores plans or axis) and, at the same time, the recover of their subspectral profiles (loadings).

Table 1
Water content of the samples used in dry and hydrated states

	Water content (%)	
	Dry state	Hydrated state
Arabinose	0.12	10.6
Fructose	0.25	39.5
Glucose	0.15	23.5
Mannose	0.18	47.6
Galactose	0.16	64.5
Galacturonic acid	0.15	11.8
Sucrose	0.012	13.4
Maltose	0.13	8.5
Lactose	0.015	6.0
Amylose	0.12	20.1
Amylopectin	0.11	13.8
κ-Carrageenan	0.39	77.1
λ-Carrageenan	0.31	32.7
Pectin HM 1	0.51	23.2
Pectin HM 2	0.53	48.3
Galactan	0.32	30.0
Arabic gum	0.55	50.6
Galactan	0.39	80.6
Arabinogalactan	0.13	20.7

2.4. Water content analysis

The water content in dry and hydrated samples was determined by the Karl–Fischer method (ThermoOrion AF8) using the reagent Hydranal-Composite 5 from Sigma-Aldrich. The Karl–Fischer apparatus was first calibrated with deionized water. The sample (1 g) was weighed accurately and transferred into automated titrating chamber. The titration was carried out according to the procedure of Isengard and Präger (2003). Experiments were made with three sample replicates and coefficients of variation of less than 2% were obtained for all samples.

The moisture content in solutions was determined by refractometry.

3. Results and discussion

3.1. Hydrated samples

Fig. 1 shows the PCA scores scatter plot and PCA loadings plot of the FT-IR spectra of dry and hydrated mono-, di-, and polysaccharides in the 1200–800 cm^{-1} region. The distribution of the samples in the PC1×PC2 axes (Fig. 1a) was done according to their composition in glucose (PC1 negative), galactose (PC1 positive), galacturonic acid (PC2 negative) and sucrose (PC2 positive), independently of the monomeric or polymeric nature of the samples, as previously observed by Černá et al. (2003). Also, it can be observed that the presence of water in amounts that ranged from 6 to 40% (Table 1) did not change significantly this separation trend. The exception was mannose (containing 48% of water) that was shifted from PC1 negative to PC1 positive.

Although the hydration of the samples gave broader bands, attributed to molecular disorientation and disappearance of the crystalline structures, as was observed for model
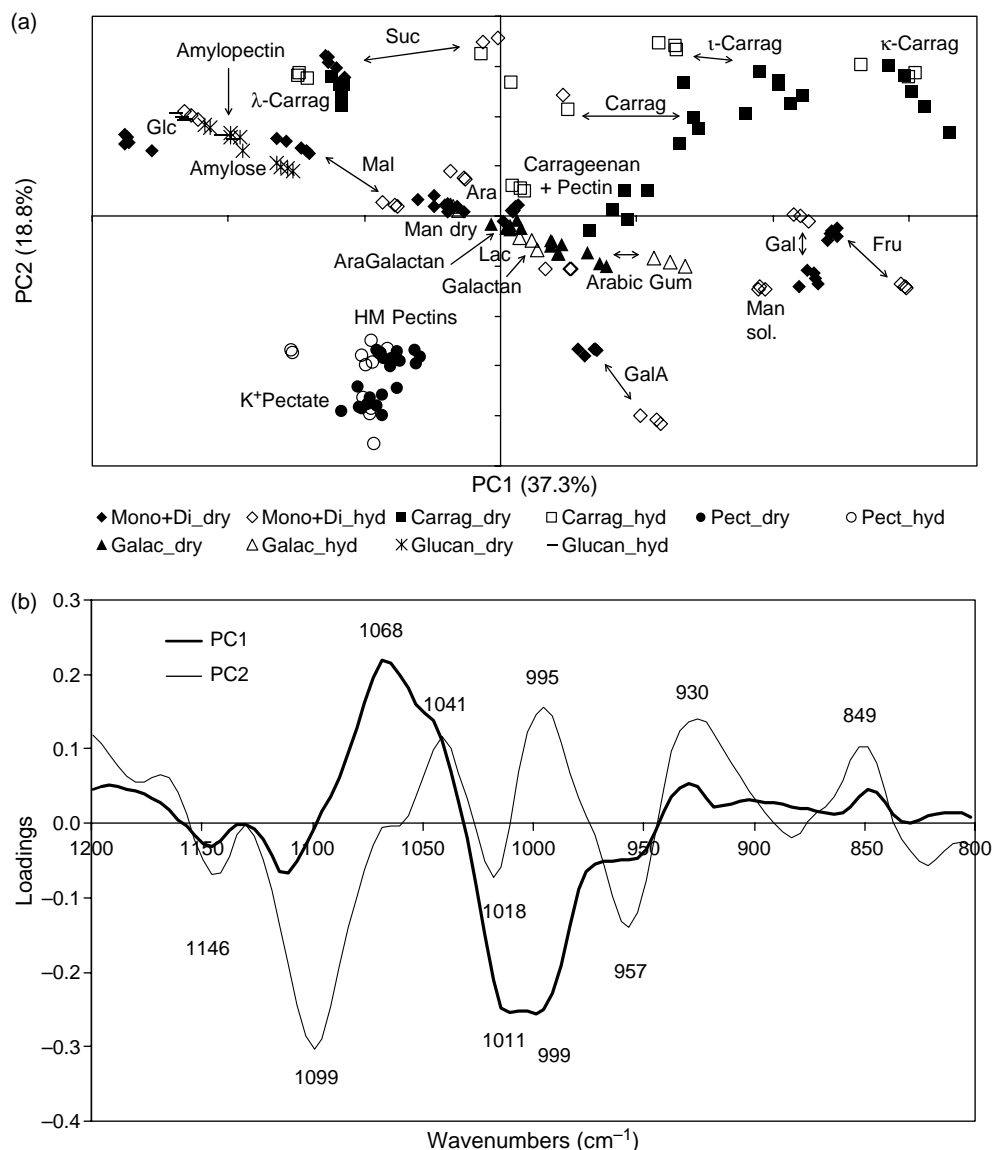
Fig. 1. PCA of FT-IR spectra in the 1200–800 cm^{-1} wavenumber region of mono-, di-, and polysaccharide standards in dry and hydrated forms. (a) Scores scatter plot (PC1 × PC2 - axes cross each other at the origin) and (b) loadings plot.

xylooligosaccharides by Kačuráková, Belton, Wilson, Hirsch, and Ebringerová (1998), the presence of water in these samples did not change the spectral characteristics that allowed their distinction, as can be observed in the loadings plot shown in Fig. 1b. The PC1 positive absorption band in the range 1100–1030 cm^{-1}, with a maximum at 1068 cm^{-1}, is attributed to galactose residues, and the negative absorption band in the range 1030–944 cm^{-1}, with a minimum at 999 cm^{-1}, is assigned to glucose residues. In PC2, the bands with maximum at 1041, 995, 930, and 849 cm^{-1}, are attributed to sucrose, and those with a minimum at 1146, 1099, 1018 and 957 cm^{-1}, are assigned to galacturonic acid residues. The FT-IR spectra allow also the distinction along PC1 axis of λ-carrageenan (PC1 negative) from κ- and ι-carrageenan (PC1 positive).

These results were in accordance with the studies of Kačuráková and Mathoulthi (1996), where it was shown that the general profiles of the infrared spectra (1800–900 cm^{-1}) of

mono- and oligosaccharides in aqueous solution are comparable in a concentration range between 10 and 70%. In order to see if this spectral characteristic can be retrieved even when the samples were measured dissolved in water, a second set of experiments was done with the same samples in water solution.

3.2. Solution samples

Fig. 2 shows the PCA scores scatter plots and PCA loadings plot of the FT-IR spectra of dry, hydrated and solution forms of mono-, di-, and polysaccharides in the 1200–800 cm^{-1} region. The distribution of the samples in the PC1 axis (Fig. 2a) was done as a function of the water content in the samples, as all samples measured in dry or hydrated forms were placed in PC1 negative and the majority of the samples measured in solution were placed in PC1 positive; the exceptions were fructose, mannose and sucrose. As these samples were already in solution after the first
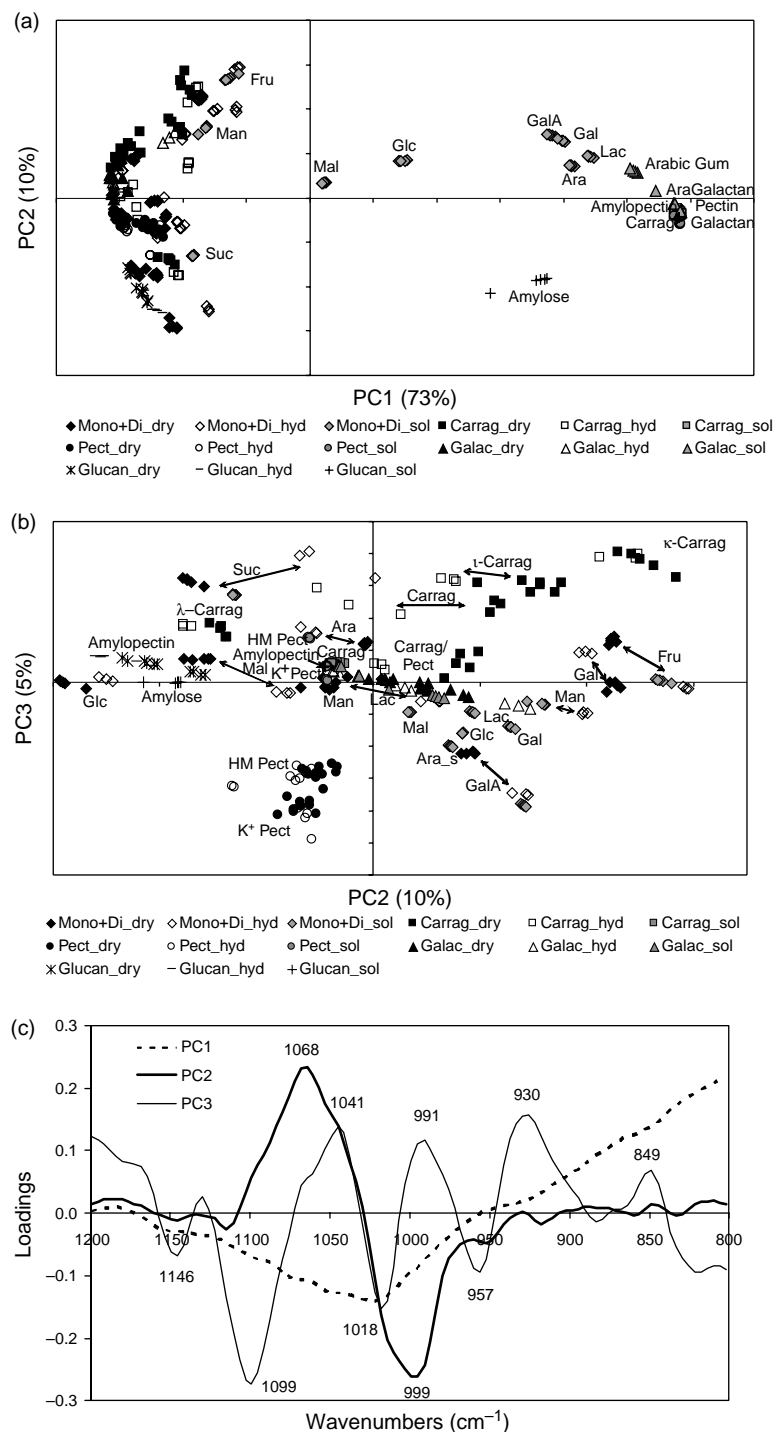
Fig. 2. PCA of FT-IR spectra in the 1200–800 cm⁻¹ wavenumber region of mono-, di-, and polysaccharide standards in dry, hydrated and solution forms. (a) PC1 ×
PC2 scores scatter plot. (b) PC2 × PC3 scores scatter plot. (c) Loadings plot.

experiment, no water was added, which explains why they were located near the respective 'hydrated' samples. The loadings profile of PC1 (Fig. 2c) shows a smooth curve similar to that observed in the distinction of samples containing different amount of sugars (Coimbra, Gonçalves, Barros, & Delgadillo, 2002).

Fig. 2b shows that the distinction between the samples can be obtained by the PC2×PC3 scatter plot. For the majority of

polysaccharides, significant shifts can be observed when these samples were analysed in solution. With the exception of amylose, whose spectra did not change significantly with the water content, the spectra of all other polysaccharides (amylopectin, carrageenans, pectins and pectates, and galac-tans) were placed near the PC2 and PC3 origin, not allowing their distinction. However, all dry and hydrated samples were placed according to the scores scatter plot of PC1 × PC2 shown

in Fig. 1a. Also, the loadings plot of PC2 and PC3 was similar to the loadings plot of PC1 and PC2, respectively, of Fig. 1b, which shows that the variability of the samples could be recovered in PC2 and PC3 when samples in solution are included.

3.3. Concluding remarks

The application of a PCA to the FT-IR spectra in the 1200–800 cm^{-1} region of monosaccharides, disaccharides and polysaccharides in dry, hydrated and in solution forms showed that although hydration affects the spectra, the distinction of sugars and polysaccharides by FT-IR in the 1200–800 cm^{-1} region can be achieved with no apparent loss of information. When the samples are in aqueous solution, the water influence is included in PC1 and the FT-IR spectral information of carbohydrates, previously given in PC1 and PC2 in dried samples, can be achieved in PC2 and PC3.

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