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Use of FT-IR spectroscopy as a tool for the analysis of polysaccharide food additives

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Abstract

This work purposes the characterisation of food additive polysaccharides such as starch, glucomannan and carrageenan by the use of the information of the principal components of the FT-IR spectra in the 1200–800 cm^{-1} wavenumber region. The application of a PCA to this spectral region showed that several features could be obtained: (a) Separation between Glc and Gal, both monomeric and polymeric, and identification of their characteristic wavenumbers. (b) Identification of the specific absorbance wavenumbers for sucrose, Fru, Ara, and Man. (c) Distinction of pectic polysaccharides from the remaining carbohydrate samples. (d) Separation within κ-, ι-, and λ-carrageenans. These results show that the FT-IR spectroscopy in the 1200–800 cm^{-1} wavenumber region can be a very reliable technique for food authentication of polysaccharide-based additives and be used for a quick screening of polysaccharides used as additives in foodstuffs.

Keywords: FT-IR spectroscopy; Additives; Glucan; Carrageenan; Pectin; Additives; Guican; Carrageenan; Pectin

1. Introduction

Polysaccharides and their derivatives are widely used in food processing technologies as gelling agents and thickeners. Starch, carrageenan and pectin are examples of the most used polysaccharides in food industry. Starch is an important thickening and binding agent used in the production of puddings, soups, sauces, etc.; carrageenan utilisation in food processing is based on its ability to gel, to increase the solution viscosity and to stabilise emulsions and various dispersions, such as chocolate milk; pectin with a high ester content can set into a gel in the presence of sucrose and is widely used in marmalade and jelly production and low ester pectin can set into a gel in the presence of Ca^{2+} (Belitz & Grosch, 1999).

The authentication of food is a major concern for the consumer and for the food industry at all levels of the food processing chain, from raw materials to final products. Among the complex food constituents, the identification of the added polysaccharides could be a key factor if a rapid and reliable method is attainable. Wet classical chemical methods of polysaccharide determination are time consuming, and not always straight forward, hence not suitable for a widespread routine application in the food industry.

The vibrational spectra have been found important applications in the analysis and identification of sugars in food industries (Mathlouthi & Koenig, 1986). Mid infrared spectroscopy, is a rapid, versatile, and sensitive tool that have been used, by simple spectra analysis, for elucidating the structure, physical properties and interactions of carbohydrates (Kačuráková & Wilson, 2001), to study pectic polysaccharides and hemicelluloses extracted from plants (Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000), and to detect structural and compositional changes occurring in the cell walls of grapes during processing (Femenia, Sánchez, Simal, & Rosselló, 1998). The carbohydrates show high absorbencies in the region 1200–950 cm^{-1}, that is within the so-called fingerprint region, where the position and intensity of the bands are specific for every polysaccharide, allowing its possible identification (Filippov, 1992). Due to absorbance

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overlapping in this region, it has been very difficult to assign the absorbencies at specific wavenumbers to specific bonds or functional groups.

Pectins, polysaccharides composed by a linear backbone of (1 → 4)-α-D-GalA*p* interspersed by α-(1 → 2)-Rha*p* residues and with side chains constituted mainly by β-D-Gal*p* and α-L-Ara*f* residues, have been the most extensively studied polysaccharide by IR spectroscopy (Kačuráková & Wilson, 2001). Mid infrared spectroscopy has also been applied successfully in the studies on the conformation and structure of starch (Gałat, 1980). The gelation and retrogradation of the two main constituents of starch, amylose (linear (1 → 4)-α-D-glucan) and amylopectin (branched (1 → 4)- and (1 → 4,6)-α-D-glucan), have been studied by infrared spectroscopy (Wilson & Belton, 1988; Wilson, Goodfellow, & Belton, 1988; Wilson, Kalichevsky, Ring, & Belton, 1987), where characteristic bands for starch gels have been found at 1046 and 1019 cm^{-1}. The carrageenans are characterised by an alternating repeating (1 → 4)-linked disaccharide structure consisting of 3,6-anhydro-α-D-galactopyranosyl-(1 → 3)-β-D-galactopyranosyl. A sulphate group at positions C2, C4 or C6 can substitute each residue. The carrageenans, depending on the sulphate substitutions, can be defined as: kappa (κ) (β-D-Gal*p*-4-sulphate and 3,6-anhydro-α-D-Gal*p*), iota (ι) (β-D-Gal*p*-4-sulphate and 3,6-anhydro-α-D-Gal*p*-2-sulphate), lambda (λ) (non-gelling agent consisting of β-D-Gal*p*-2-sulphate and α-D-Gal*p*-2,6-disulphate). FT-IR spectra, exhibiting characteristic bands in the region 930–805 cm^{-1}, have also been employed for their identification and discrimination (Chopin & Whalen, 1993; Sekkal & Legrand, 1993). Glucomannans (linear polymers composed of β-D-Man*p* and β-D-Glc*p*) and β-glucans (polymers composed of variously linked β-D-Glc*p* residues) are also polysaccharides utilized in special nutrition foods as bulk substances.

The application of chemometrics to the FT-IR spectra was established as a reliable and fast method for the determination of important gel-forming parameters in amidated pectins (Engelsen & Norgaard, 1996), and allowed the classification of corn starches (Dupuy, Wojciechowski, Huvenne, & Legrand, 1997) and commercial carrageenans (Jurasek & Phillips, 1998). It was also proved to be useful for a quick evaluation of cell wall monosaccharide composition of polysaccharides of pectic (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998) and hemicellulosic origin (Coimbra, Barros, Rutledge, & Delgadillo, 1999). Moreover, it was applied as a rapid method to screen cell wall mutants of *Arabidopsis* (Chen et al., 1998), to detect structural and compositional changes occurring in the cell walls of pears after processing (Ferreira, Barros, Coimbra, & Delgadillo, 2001), and to determine the degree of methylesterification of pectic polysaccharides in plant cell wall extracts (Barros et al., 2002).

Following the work carried out on the detection of hydrocolloids in confectionery jellies and food supplements (Čopíková, Synytsya, Černá, Kaasová, & Novotná, 2001) by FT-IR spectroscopy, this paper proposes, with the use of a PCA, the identification of the specific wavenumbers that contribute for the discrimination of the different polysaccharides constituent of carbohydrate-based additives according to their FT-IR spectra within the 1200–800 cm^{-1} region.

2. Materials and methods

2.1. Samples origin

Standards. Twenty seven carbohydrate standards, mono-, di- and polysaccharide standard compounds, were used to build a FT-IR model based in their absorbencies in the region 1200–800 cm^{-1}: D-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), starch (Sigma), β-glucan of barley (Megazyme), 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 [DE = 89.7%] (Sigma), potassium pectinan [DE = 64.3%] (Sigma), potassium pectinan [DE = 21.6%] (Sigma), potassium polygalacturonan (Sigma), galactan of arabic gum (Sigma), galactan (Koch-Light Laboratories), and arabinogalactan (Sigma).

Confectionery jellies and food supplements. Eight samples were analysed using the FT-IR model: five polysaccharide samples isolated from confectionery jellies (samples 1–5 in Tables 1–3), and three food supplement polysaccharide samples: yeast wall glucomannan *Saccharomyces cerevisiae* (sample 6), yeast extracellular glucomannan *Saccharomyces cerevisiae* (sample 7), and a β-glucan mixture, resultant from *Saccharomyces cerevisiae* (sample 8a), and *Lactobacillus* species (sample 8b). According to the manufacturers, the confectionery jellies contain pectin, in addition to other sugars (Table 1). The carbohydrate relative composition of confectionery jellies is specified in Table 2. The food supplement polysaccharides were analysed directly from the raw material. The confectionery jelly polysaccharides were previously isolated before analysis: the confectionery jellies were dissolved in distilled water to form of a 10% solution (w/v). To 100 ml of this solution, 300 ml of 96% ethanol was added, the solution (72% ethanol) was stirred for 1 day at 5 °C and the high molecular weight polysaccharides were recovered by centrifugation and washed with 80% ethanol up to negative reaction (phenol–sulphuric acid test) for carbohydrates. The polysaccharides were then dissolved in distilled water to form a 2% aqueous solution (w/v) and the produced solution was freeze-dried.

Table 1
Characteristics of studied samples, according to the manufacturer labels

Sample No.	Sample specification	Sample components
1	Pectin jelly with fruit flavour	Sugar, glucose syrup, water, pectin, citric acid, acidity regulator (sodium citrate), flavours, colours
2	Peach flavoured jellies	Sugar, glucose syrup, water, pectin, citric acid, sodium citrate, natural peach flavour, colour
3	Fruit flavoured gums with real fruit juices	Sugar, glucose syrup, water, pectin, acids (citric acid, lactic acid), acidity regulators (potassium sodium tartrate, trisodium citrate, calcium lactate, sodium polyphosphate), fruit juices
4	Fruit jelly	Sugar, glucose syrup, water, pectin, citric acid, sodium citrate, natural flavours, natural colours
5	Pectin jelly with fruit flavour	Sugar, glucose syrup, water, pectin, citric acid, acidity regulator (sodium citrate), moisturiser (sorbitol), flavour, colours

2.2. Carbohydrate analysis

The monosaccharides and disaccharides present in original jelly samples were analysed by HPLC, equipped with a pump (DeltaChrom SDS 030) and connected to a RI detector (RefractoMonitor IV). The confectionery jelly was dissolved in distilled water to form a 10% solution (w/v) and injected in a 20 μl loop using a valve. The separation was performed with a Separon SGX NH_2 column (4 × 250 mm) protected with a precolumn (3.3 × 30 mm). The conditions of analysis were as follows: column temperature at 25 °C, using deionised water–acetonitrile (25:75, v/v) as eluent, with a flow of 1.0 ml/min. Data acquisition and peak processing were performed with DATA Apex software.

Neutral sugars were released from the isolated confectionery jelly polysaccharides and from the food supplements by Saeman hydrolysis (Selvendran, March, & Ring, 1979) and analysed as their alditol acetates by gas chromatography (Blakeney, Harris, Henry, & Stone, 1983; Harris, Blakeney, Henry & Stone, 1988) using a Hewlett-Packard 5890 gas chromatograph with a split injector (split ratio 1:60) and a FID detector. A DB-225 capillary column (30 m length, 0.25 mm internal diameter and 0.15 μm film thickness) was used. With the injector and detector operating at 220 and 230 °C, respectively, the following temperature program was used: 220 °C for 4 min and 230 °C for 7.5 min with a rate of 25 °C/min. Hydrogen was used as the carrier gas. Uronic acid was determined colorimetrically by a modification (Coimbra, Delgadillo, Waldron, & Selvendran, 1996) of the Blumenkrantz and Asboe-Hansen (1973) method. The absorbencies were measured at 520 nm on a Shimadzu UV/Vis spectrophotometer. The absence of monosaccharides in the isolated polysaccharide extracts was confirmed using the same procedure but without the acid hydrolysis step.

Table 2
The content of dry matter ($s = 0.02$) and sugars in confectionery jelly samples ($s = 0.1$)

Sample No.	Content (%)				
	Dry matter	Fructose	Glucose	Sucrose	Maltose
1	80.50	4.65	5.68	55.13	7.23
2	81.20	1.87	8.64	40.16	6.91
3	80.34	4.96	13.71	34.97	6.62
4	81.05	6.46	12.19	43.17	5.53
5	80.38	3.70	6.11	55.50	11.23

s: standard deviation.

2.3. FT-IR spectroscopy and chemometric analysis

The FT-IR spectra of the isolated polysaccharides and standards were obtained using a Golden Gate single reflection diamond ATR system in a Brucker 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

Table 3
Sugar composition of polysaccharides isolated from confectionery jellies and food supplement polysaccharide samples (expressed as mol%)

Isolated polysaccharide	Ara	Man	Gal	Glc	Ur. Ac.
Confectionery jellies					
1	1	1	2	75	19
2	0	0	2	97	0
3	0	0	1	97	1
4	1	0	1	98	0
5	1	0	2	94	2
Food supplements					
6	0	67	0	33	0
7	0	81	0	19	0
8a	0	22	17	60	0
8b	0	3	49	48	0

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 auto-scaled (centred and divided by the standard deviation). The PCA analysis could allow the characterisation of the sample relationships (scores plans or axis) and, at the same time, the recover of their sub-spectral profiles (loadings).

3. Results and discussion

3.1. PC1

Twenty seven carbohydrate standards were used to build a FT-IR model based in their absorbencies in the region 1200–800 cm^{-1}: six monosaccharides (Ara, Fru, Gal, GalA, Glc, and Man), three disaccharides (lactose, maltose, and sucrose), four glucans (amylose, amylopectin, barley β-glucan, and starch), five carrageenans (ι-, λ-, κ-carrageenan, commercial carrageenan, and commercial carrageenan–pectin mixture), three galactans (arabic gum, arabinogalactan, and galactan), and six pectins having different degrees of methylesterification.

The scores scatter plot of PC1 vs. PC2 (Fig. 1a) indicates that the distribution of the samples in the PC1 axis was done according to their composition in Glc (PC1 negative) and Gal (PC1 positive). Glc-rich compounds like, starch, amylose, amylopectin, β-glucan, maltose, sucrose, and Glc, were all placed in the negative side of PC1, independently of their monomeric or polymeric nature. On the other side, polysaccharides such as the carrageenans (except λ-carrageenan) and galactans, and the monosaccharides Fru and GalA, were placed in the positive side of PC1. Lactose (β-D-Gal*p*-(1 → 4)-D-Glc*p*) was placed near the origin, i.e. had no influence concerning this axis. Based on the scores scatter plot sample distribution, the positive absorption band in the range 1100–1030 cm^{-1}, with maximum at 1068 cm^{-1} in PC1 loadings plot (Fig. 2) can be attributed to Gal, and the band in the range 1030 − 944 cm^{-1}, with minimum at 998 cm^{-1} can be attributed to Glc, as previously defined by Kačuráková et al. (2000) and Kačuráková and Mathlouthi (1996).

3.2. PC2

According to the scores scatter plots of PC1 vs. PC2 and PC2 vs. PC3 (Fig. 1a and b) PC2 distinguish the spectra of the pectin samples and GalA (PC2 negative), from all the others, especially the carrageenans (PC2 positive). Pectins with different degree of methylesterification were observed together in the same region, not significantly separated. Carrageenans (PC1 and PC2 positive) were placed differently from Gal (PC1 positive and PC2 negative) in the scores plan, except λ-carrageenan, that was located on PC1 negative and PC2 positive. This may be due to the higher content in sulphate and absence of 3,6-anhydro-Gal in this carrageenan when compared to the others. Commercial carrageenan is probably κ-carrageenan. This distinction of the pectic samples can be also seen in the loadings plot of PC2 by the absorbencies at the negative side at 1145, 1100, 1018, and 960 cm^{-1} and by the absorbencies at the positive side at 1064 and 1045 cm^{-1}, as previously described for pectic polysaccharides (Coimbra et al., 1998, 1999).

3.3. PC3 and PC5

The scores scatter plots of PC2 vs. PC3 and PC3 vs. PC5 (Fig. 1b and c) allowed to see in PC3 a distinction of the spectra ι- and κ-carrageenans (PC3 positive) from the monosaccharides Ara, Fru, and Gal, and the disaccharide sucrose (PC3 negative). GalA and Glc were in the centre and Man was slightly PC3 positive. PC5 separate Man and λ-carrageenan (PC5 positive) from Ara and sucrose (PC5 negative). The separation of the ι- and κ-carrageenans (PC1, PC2 and PC3 positive and PC5 negative) were due to the absorbencies at 929 and 848 cm^{-1}, which are in accordance with Chopin and Whalen (1993). Sucrose (PC1, PC3 and PC5 negative and PC2 positive) was related to the wavenumber 995 cm^{-1} and Fru (PC1 and PC5 positive and PC3 negative) was related to the wavenumbers 1064 and 1045 cm^{-1} (Kačuráková & Mathlouthi, 1996). Ara (PC3 and PC5 negative) was related to the wavenumbers 1052 and 979 cm^{-1} (Coimbra et al., 1998) and Man (PC3 and PC5 positive) was related to the wavenumbers 1072 and 1033 cm^{-1} (Kačuráková et al., 2000).

3.4. Identification of isolated polysaccharides from confectionery jellies and food supplements (glucomannans and β-glucan mixture)

Isolated polysaccharides from confectionery jellies (1–5) were placed in the PC1 negative side (Fig. 1a), near starch. In PC2, PC3 and PC5 they were placed near the origin, always near starch. According to these results, the polysaccharides isolated from confectionery jellies should be considered starch-based materials, in accordance with the positive I_2/KI test for starch and with the sugar analysis (Table 3). According to the sugar analysis, confectionery jelly polysaccharides were rich in polymeric Glc. These results contrasted with the information of pectin as jellifying agent, labelled by the manufacturer. Exception was sample 1, which showed uronic acids, however in very low quantity. The polysaccharides isolated from sample 1 contained 19% Ur.Ac, leading to the assumption that it could be a mixture of starch and pectin. According to the scores scatter plot (Fig. 1a and b) sample 1 was placed between starch and pectin, which allowed confirming its polysaccharide composition.

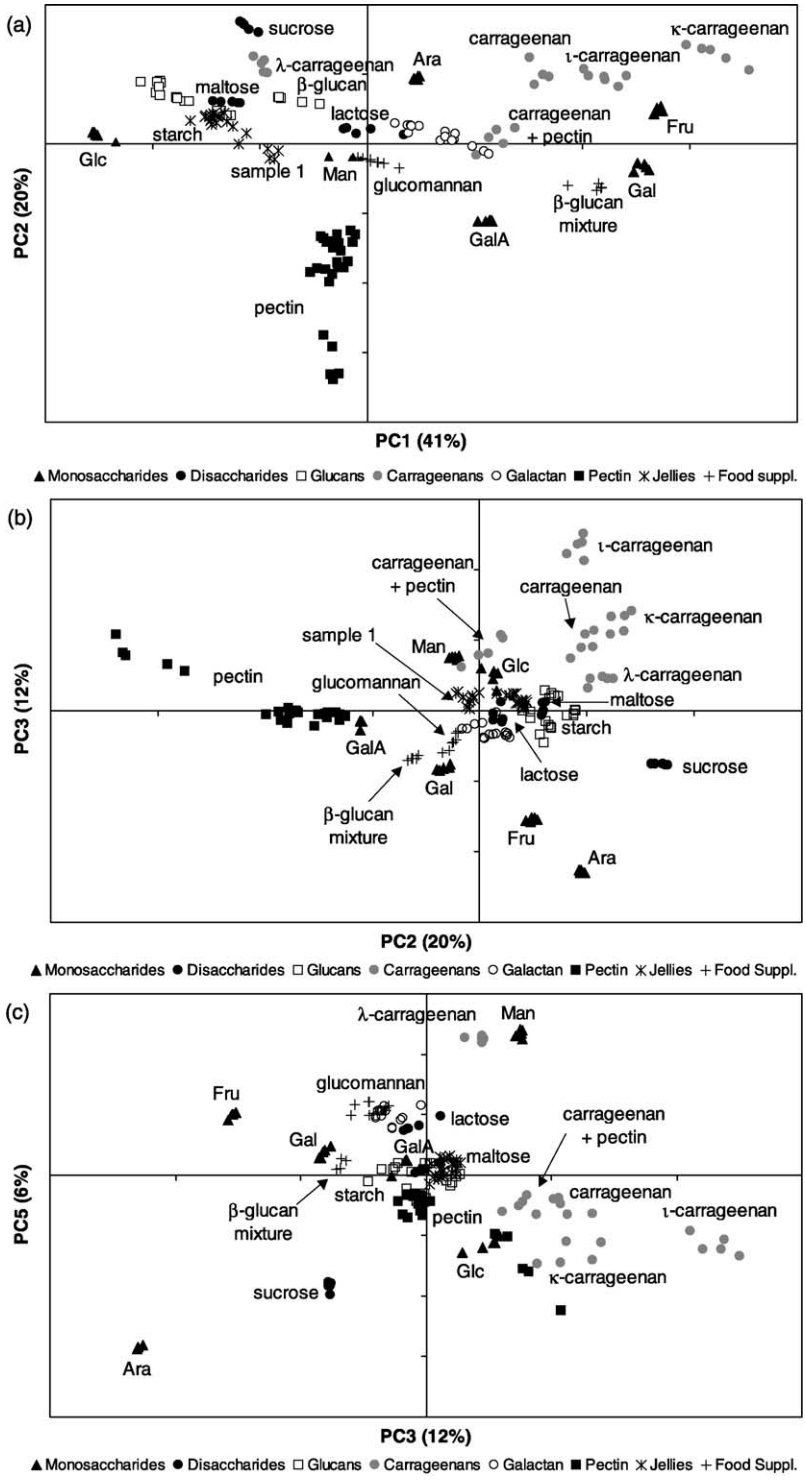

Fig. 1. PCA scores scatter plot of the FT-IR spectra of mono-, di-, and polysaccharide standards, confectionery jelly polysaccharides and food supplements (glucomannan and β-glucan mixture) in the 1200–800 cm^{-1} wavenumber region. (a) PC1 vs. PC2; (b) PC2 vs. PC3; (c) PC3 vs. PC5 (axes cross each other at the origin).

The glucomannans had a ratio Glc/Man of 1:2 and 1:4 for the wall and extra-cellular glucomannans, respectively (Table 3). No distinction was possible to achieve between them by the FT-IR spectra. They were placed always near the origin in the PC1 and PC2 scores scatter plots, near Man. In PC3, the glucomannans were in opposite direction of Man and in PC5, where Man is more separated from the other samples in the PC5 positive, the glucomannans were also placed in PC5 positive.

The bacterial β-glucan mixture was the result of a blend of samples 8a and 8b. According to the sugars composition shown in Table 3, one polysaccharide was composed of

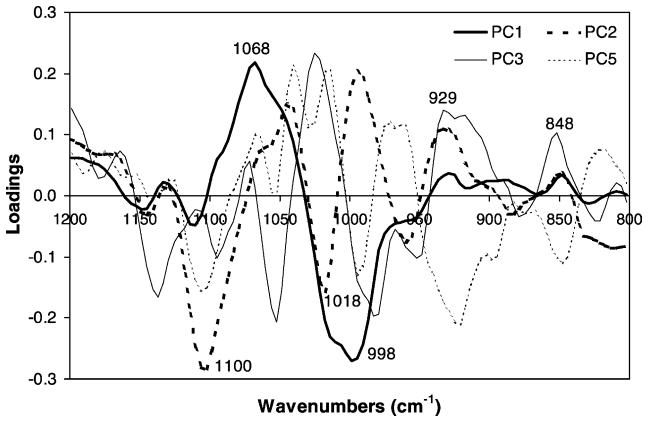

Fig. 2. PCA loadings plot (PC1, PC2, PC3, and PC5) of the FT-IR spectra of mono-, di-, and polysaccharide standards, confectionery jelly polysaccharides and food supplements (glucomannan and β-glucan mixture) in the 1200–800 cm^{-1} wavenumber region.

equimolar amounts of Gal and Glc, and the other one was composed of Glc, Man and Gal in a ratio of 3:1:1. This mixture of polysaccharides was placed in the PC1 positive side in the opposite direction of the β-glucan of barley, near Gal. Also in PC2, PC3 and PC5 it was placed near Gal. According to the relative position of the β-glucan mixture in relation to the lactose in the score scatter plots, the equimolar amounts of Glc and Gal cannot be attributed to the presence of lactose.

3.5. Concluding remarks

The application of a PCA to the FT-IR spectra in the 1200–800 cm^{-1} region of carbohydrate food additives showed that a distinction was possible. The major variability (41%) was explained by PC1, that separate Glc (negative band at 998 cm^{-1}) from Gal (positive band at 1068 cm^{-1}) based carbohydrates. No distinction was obtained from monosaccharides, disaccharides and polysaccharides. In this axis, pectin and glucomannan do not interfere. Pectin was separated in PC2, by the absorbencies located at it negative side: 1145, 1100, 1018, and 960 cm^{-1}; and by the absorbencies located at the positive side: 1064 and 1045 cm^{-1}. The ι- and κ-carrageenans were distinguished from all other carbohydrates, including λ-carrageenan, due to the absorbencies at 929 and 848 cm^{-1}. Absorption at specific wavenumbers were also observed for sucrose (995 cm^{-1}), Fru (1064 and 1045 cm^{-1}), Ara (1052 and 979 cm^{-1}), and Man (1072 and 1033 cm^{-1}). These results show that the FT-IR spectroscopy in the 1200–800 cm^{-1} wavenumber region can be used as a very reliable and quick tool for food authentication of carbohydrate-based additives.

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