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Fourier transform Raman and infrared spectroscopy of pectins

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Abstract

The FT-Raman and FT-IR spectra of polygalacturonic (pectic) acid, potassium pectate and its derivatives, as well as commercial citrus and sugar beet pectins were measured and interpreted. Methyl and acetyl esters of potassium pectate derivatives have several characteristic Raman and IR bands that allow both this groups to be distinguished. The very intense Raman band at 857 cm^{-1} is sensitive to the state of uronic carboxyls and to *O*-acetylation. The wavenumber of this band decreases with methylation (min. 850 cm^{-1}) and increases with acetylation (max. 862 cm^{-1}). The acetylation of potassium pectate, as well as its acetylation together with methylation, causes drastic changes in the Raman spectra in the region below 700 cm^{-1}. Sugar beet pectin, but not citrus pectin, showed Raman bands at 1633 and 1602 cm^{-1} and IR band at 1518 cm^{-1}. All these bands rise from feruloyl groups and can be used for identification of pectins containing feruloyl groups.

Keywords: FT-Raman spectroscopy; Diffuse reflectance FT-IR spectroscopy; Pectins

1. Introduction

Pectins are widespread plant polysaccharides that are commonly used in the food industry as gelling and stabilising agents (BeMiller, 1986; Thakur, Singh, & Handa, 1997; Van Buren, 1991). Basically, pectins are polymers of (1 → 4) linked partially methyl esterified α-D-galacturonic acid. Pectins with more than 50% methyl ester groups are classified as high-methoxyl (HM) and those with less than 50% methyl ester groups as low-methoxyl (LM).

Natural pectins are not simple galacturonans. Particularly, L-rhamnose residues may interrupt the galacturonic acid backbone of pectins forming rhamnogalacturonan. Several pectins are also partially *O*-2 and *O*-3 acetylated and may contain neutral sugar side chains attached to rhamnose units (Thakur et al., 1997; Thibault, 1986). In some cases, feruloyl groups are linked to neutral sugar side chains by ester bonds (Colquhoun, Ralet, Thibault, Faulds, & Williamson, 1994; Ralet, Thibault, Faulds, & Williamson, 1994; Rombouts & Thibault, 1986; Thibault, 1986).

The structure of pectins influences their physical–chemical properties significantly as well as their applications in food technology. Among various analytical methods, mid-infrared FT-IR spectroscopy is an excellent tool for structural and quantitative analysis of polysaccharides, including pectins (Kačuráková & Wilson, 2001).

FT-IR spectra of natural pectins and their derivatives have been recorded for KBr discs (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998; Kamnev, Colina, Rodriguez, Ptitchkina, & Ignatov, 1998; Manrique & Lajolo, 2002), in emulsions with nujol (Kamnev et al., 1998), for films (Filippov, 1978, 1992), and powders (Engelsen & Nørgaard, 1996a,b), D_2O-phosphate buffer solutions (Bociek & Welti, 1975), water solutions and gels (Wellner, Kačuráková, Malovíková, Wilson, & Belton, 1998).

FT-IR methods for determining the content of different groups, such as free carboxyls, carboxylate anions, methyl esters, acetyls and amides, in pectin solutions

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Abbreviations: DAc, degree of acetylation; DAm, degree of amidation; DM, degree of methylation (methoxylation, esterification); FT, fourier transformed; HM, highly methylated (methoxylated, esterified); IR, infrared; LM, low methylated (methoxylated, esterified); UA, uronic acids.

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(Bociek & Welti, 1975) and films (Filippov, 1984; Filippov & Kohn, 1986; Filippov & Vaskan, 1987a,b) have been presented. Coimbra et al. (1998) has studied uronic acid and neutral sugars in pectic samples using FT-IR and multivariate analysis. The degree of methylesterification of pectins have been estimated by FT-IR using second derivative algorithm and peak decomposition (Chatjigakis et al., 1998) as well as using an outer product PLS1 regression (Barros et al., 2002).

Diffuse reflectance FT-IR has obvious advantages in the analysis of pectin derivatives compared with other techniques. This infrared technique has been successfully applied to record and analyse spectra of dried powder pectins for the quality control of commercial samples (Engelsen & Nørgaard 1996a,b), and for the determination of the polygalacturonic acid content in pectin extracts and the degree of esterification of pectin (Chatjigakis et al., 1998; Gnanasambandam & Proctor, 2000; Monsoor, Kalapathy, & Proctor, 2001a,b). Diffuse reflectance FT-IR is especially attractive since commercial pectins and their derivatives are slightly soluble or insoluble in common solvents used in FT-IR. For example, hydrophobically modified amidated derivatives of HM citrus pectin (CP) have been prepared and analyzed by diffuse reflectance FT-IR spectroscopy (Sinitsya, Čopíková, Prutyanov, Skoblya, & Machovič, 2000).

In contrast, Raman spectroscopy is not a widespread method for the analysis of pectins, but it is widely applied in structural investigations of polysaccharides. Raman spectra of amylose (Cael, Koening, & Blackwell, 1973, 1975a), cellulose (Cael, Koening, & Blackwell, 1975b), carrageenans (Malfait, van Dael, & van Cauwelaert, 1987; Sekkal & Legrand, 1993), chitin (Gałat & Popowicz, 1978), and other polysaccharides (Cabassi, Casu, & Perlin, 1978; Gałat, 1980; Kačuráková et al., 1999; Zhbankov et al., 2000) have been interpreted and used in structural analysis of these polysaccharides. Engelsen et al. (1996a,b) compared FT-Raman spectra of amidated pectins with FT-NIR and FT-IR data for the determination of quality parameters. The assignment of Raman and infrared bands has been carried out in this paper.

It is evident that infrared and Raman spectroscopic methods are complementary for the structural analysis of carbohydrates (Zhbankov, Andrianov, Ratajchak, & Markhevka, 1995). Some vibration bands that are very weak or overlapped by stronger bands and cannot be detectable in IR can be indicated and studied by Raman spectroscopy and vice versa. Both these methods are rapid, non-destructive and do not need complex procedures for sample preparation.

In this work we describe the complementary use of IR and Raman spectroscopy for the analysis of pectins and their derivatives. FT-Raman and diffuse reflectance FT-IR spectra of citrus and sugar beet pectins as well as their acetylated, amidated and additionally methyl esterified derivatives were measured and the spectral changes were interpreted in connection with the state of pectic C-6 carboxyls and the degrees of methylation and acetylation.

2. Experimental

2.1. Samples preparation and analysis

Polygalacturonic (pectic) acid H-Pec, its potassium salt (pectate) K-Pec, commercial citrus and sugar beer pectins (CP and BP), five potassium pectinates KMe-Pec1-5 of various DM, pectinic acid Me-Pec, four acetylated potassium pectates K-PecAc1-4 of various DAc, acetylated potassium pectinate KMe-PecAc and acetylated pectinic acid Me-PecAc are specified in Table 1.

Initial samples of commercial pectins (CP and BP) were converted into various forms of C-6 carboxyls according to the scheme in Fig. 1: pectinic acid (HMe–), potassium pectinate (KMe), pectic acid (H–), potassium pectate (K–) and partially substituted pectinamides as the ammonium salt (NH_4Am–) and acid (HAm–). All reactions were carrried out in heterogeneous conditions with continuous mixing. After the solids settled, the liquid phase was removed. The samples were then filtered and washed successively with 40% ethanol and then with 98% ethanol. All purified samples were air dried at 60 °C and kept under vacuum over P_2O_5.

The content of anhydrogalacturonic acid (GA) in the samples was measured by photometry with *m*-hydroxybiphenyl at 520 nm (Blumenkrantz & Asboe-Hansen, 1973). This value expressed the total content of uronic carboxylic groups as galacturonic units. The degree of methylation (DM) was determined by photometry with chromotropic acid at 570 nm (Filippov & Kuzminov, 1971). The degree of acetylation (DAc) of samples K-PecAc1-3 and BP was measured by the Hestrin method, i.e. photometry with hydroxylamine at 540 nm (Downs & Pigman, 1976). The DAc value of samples K-PecAc4, KMe-PecAc and Me-PecAc4 were determined by solid state NMR (Sinitsya, Čopíková, & Pavlíková, 1998). The degree of amidation (DAm) of amidated pectins HAm-CP and HAm-BP was estimated by elemental analysis (Sinitsya et al., 2000). The degrees of methylation (DM), amidation (DAm) and acetylation (DAc) were expressed as the relative content (% for DM and DAm, mol/mol for DAc) of methoxyl, amide or acetyl groups, respectively.

2.2. Vibration spectroscopy

FT-Raman spectra of powder samples were recorded by using Bruker FT-Raman (FRA 106/S, Equinox 55/S) spectrometer equipped with a quartz beam splitter,

Table 1
Pectin samples

Sample	UA (%)	DM (%)	DAc (mol/mol)	Specification
H-Pec	91	0	0	Polygalacturonic acid from orange peel[a]
K-Pec	86	0	0	Potassium pectate[b]
Kme-Pec1	86	22	0	Potassium pectinate[c]
KMe-Pec2	88	49	0	Potassium pectinate[a]
KMe-Pec3	87	58	0	Potassium pectinate[c]
KMe-Pec4	89	64	0	Potassium pectinate[c]
KMe-Pec5	90	73	0	Potassium pectinate, HM citrus GENU pectin type B rapid set[b]
Me-Pec	90	90	0	Pectinic acid[c]
K-PecAc1	84	0	0.20	Acetylated potassium pectate[b]
K-PecAc2	ND	0	0.38	Acetylated potassium pectate[c]
K-PecAc3	50	0	0.85	Acetylated potassium pectate[c]
K-PecAc4	71	0	1.10	Acetylated potassium pectate[c]
KMe-PecAc	25	39	1.40	Acetylated potassium pectinate[c]
Me-PecAc	26	90	1.67	Acetylated pectinic acid[c]
CP	88	49	0	Citrus pectin[a]
HAm-CP[d]	ND	20	0	Dam = 28%
BP	83	47	0.36	Sugar beet GENU pectin type BETA[b]
HAm-BP[d]	ND	19	0.21	Dam = 24%

[a] Koch-Light Lab, England.
[b] Copenhagen Pectin Factory, Denmark.
[c] Institute of Chemistry, Slovak Academy of Science, Slovak Republic.
[d] Forms of CP and BP: KMe, pectinate; HMe, pectinic acid; K, pectate; H, pectic acid; NH_4Am, amidated pectinate; HAm, amidated pectinic acid; ND, not determined.

a liquid nitrogen cooled germanium detector and excitation at 1064 nm from a Nd:YAG laser. The laser power was set at 100 mW, and 256 scans were accumulated with a spectral resolution of 2.0 cm^{-1}. Diffuse reflectance FT-IR spectra of the samples were obtained using Nicolet 740 spectrometer with a DCT 680 cell (Nicolet Analytical Instruments), 100–200 scans were accumulated with a spectral resolution of 4.0 cm^{-1}. All spectra were 10-point filtered and the polynomial baseline corrected using Omnic 3.1a (Nicolet Analytical Instruments) and Origin 6.0 (Microcal Origin) software. The second derivatives of the Raman and IR spectra were used for wavenumber determination of overlapped bands.

3. Results and discussion

3.1. Pectic acid and potassium pectate

FT-Raman and FT-IR spectra of H-Pec and K-Pec are shown in Fig. 2. The assignments of vibration bands are presented in Table 2. The most intense Raman band at 2940 cm^{-1} was assigned to the stretching of C–H bonds of pyranoid ring carbons. In FT-IR spectra, the broad and intense O–H stretching band of hydroxyls and bound water (3600–3200 cm^{-1}), which was not observed in FT-Raman, overlapped the infrared C–H stretching band. FT-IR spectrum of H-Pec had a smaller broad shoulder near 2600 cm^{-1} assigned to the O–H stretching vibration in free carboxyls COOH bonded by hydrogen bonds into dimers (Filippov, 1978). This band was also absent in the corresponding FT-Raman spectrum.

H-Pec has a very strong IR (1762 cm^{-1}) and a weak Raman (1740 cm^{-1}) bands assigned to C=O stretching of COOH. FT-Raman spectrum of K-Pec had two carboxylate stretching bands: the weaker asymmetric (1607 cm^{-1}) and the stronger symmetric (1405 cm^{-1}) ones. Very intense corresponding IR bands (asymmetric mode is stronger than symmetric) were observed at 1633 and 1419 cm^{-1}, respectively. In-plane deformation band of water $\delta(H_2O)$, which is seen as a shoulder at 1645 cm^{-1} in FT-IR spectrum of H-Pec, overlaps asymmetric carboxylate stretching band

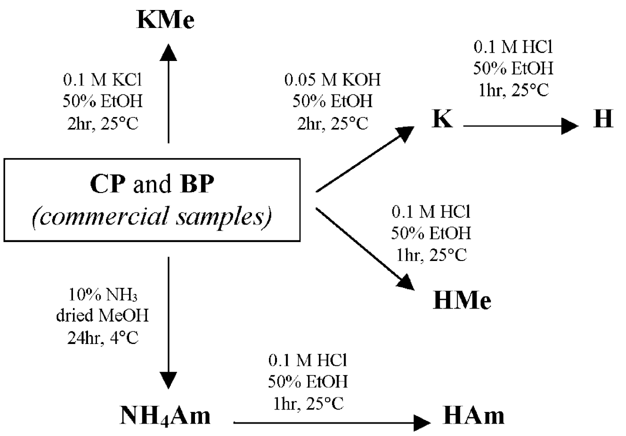

Fig. 1. Preparation of various forms of citrus and sugar beet pectins (CP and BP).

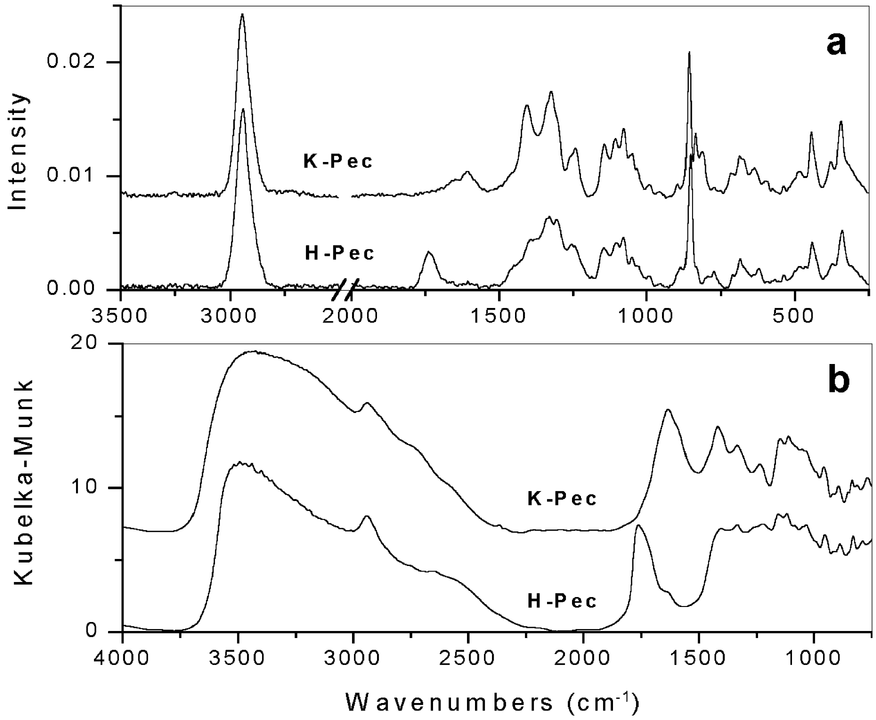

Fig. 2. FT-Raman and FT-IR spectra of polygalacturonic (pectic) acid H-Pec and potassium pectate K-Pec.

of K-Pec that may influence the position of the last band (Filippov, 1976, 1978).

The very strong and sharp Raman band was observed at 853 cm^{-1} for H-Pec and at 857 cm^{-1} for K-Pec (Table 2). The corresponding IR band was not found, as it might be very weak and overlapped by an intense absorption at 833 cm^{-1} assigned to out-off-plane vibration of hydroxyls (Filippov, 1976, 1978). It is known that the frequency of the vibration band at 830–900 cm^{-1} is sensitive to anomeric configuration of carbohydrates (Mathlouthi & Koening, 1986; Zhbankov, Adrianov, & Marchewka, 1997; Wells & Atalla, 1990; Engelsen & Nørgaard, 1996a). The band at 825–860 cm^{-1} corresponds to equatorial anomeric H (α-anomers and α-glycosides), whereas the band at 880–900 cm^{-1} corresponds to axial anomeric H (β-anomers and β-glycosides). Therefore, the Raman band at 853–857 cm^{-1} strongly indicates α-glycosidic bonds in H-Pec and K-Pec (Engelsen & Nørgaard, 1996a).

Previous literature interpretations of the carbohydrate vibration band mentioned above have been significantly influenced by its anomeric sensitivity. For example, Cael et al. (1973) assigned the vibration at 845 cm^{-1} calculated by normal co-ordinate analysis for α-D-glucose to C1–H deformation coupled to a mixed CH_2 vibration. Sivchik and Zhbankov (1978) reported later that this band results from a complex vibration of the backbone and the contribution of the C1–H group in this vibration is minimal. We assigned the Raman band at 853–857 cm^{-1} (H-Pec and K-Pec) to C6–C5–O5–C1–O1 backbone vibration according to Engelsen & Nørgaard (1996a) because the corresponding band of aldopyranoses has been expected to be sensitive both to the deuteration of hydroxyls and to the oxidation of C-6 carbon. The slight but detectable shift (+4 cm^{-1}) of this band observed for K-Pec confirms in comparison with H-Pec that the conversion of free acid groups COOH into carboxylate anions COO^- also effects the position of this skeletal band. Similar slight shifts were also detected for two intense skeletal Raman bands at 441 and at 340 cm^{-1} (H-Pec) in the far-infrared region and assigned to the C–O–C torsion deformation (Table 2).

3.2. Potassium pectate and its derivatives

FT-Raman and FT-IR spectra of Me-Pec and pectinates of various DM are shown in Fig. 3, whereas the corresponding spectra of Me-PecAc, KMe-PecAc and acetylated pectates of various DAc are demonstrated in Fig. 4. The spectra of potassium pectate K-Pec, which represents a basic non-derived structure of galacturonan, are also shown in both cases.

According to the results obtained, C-6 methylation and (or) *O*-2,3 acetylation of pectate led to peak height decreasing at 3500–3200 cm^{-1} (O–H stretching) in FT-IR, and to peak height increasing at 2940–2960 cm^{-1} (C–H stretching) in both FT-Raman and FT-IR (Figs. 3 and 4). The last effect is caused by the contribution of methyl groups in these pectate derivatives. The ν(OH) decrease was moderate on methylation, more extensive on acetylation, and extremely significant when both methyl and acetyl esters are present. The O–H stretching band of Me-Pec, K-PecAc4 and Me-PecAc showed a peak height of about 63,

Table 2
FT-Raman and FT-IR band wavenumbers (in cm^{-1}) for polygalacturonic (pectic) acid H-Pec and potassium pectate K-Pec (Engelsen & Nørgaard, 1996a; Filippov, 1976, 1978, 1980; Kačuráková et al., 1999; Wellner et al., 1998)

H-Pec		K-Pec		Assignment
FT-Raman	FT-IR	FT-Raman	FT-IR	
	3493		3425	$\nu(OH)$
2941	2942	2945	2941	$\nu(CH)$
	2653			$\nu(OH)_{COOH}$
1740	1762			$\nu(C{=}O)_{COOH}$
	1645			$\delta(H_2O)$
		1607	1633	$\nu_{as}(COO^-)$
		1405	1419	$\nu_s(COO^-)$
1393	1403			$\nu,\delta(C-OH)_{COOH}$
1330	1335	1324	1334	$\delta(CH)$
1254	1253sh	1242	1236	$\delta(CH)$
	1226			$\delta(OH)_{COOH}$
1145	1156	1144	1146	$\nu(COC)_{\text{glycosidic bond, ring}}$
1105	1119	1106	1112	$\nu(CC)(CO)$
1079	1085	1078	1083	$\nu(CO) + \delta(OH)$
1050		1049		$\nu(CC)(CO)$
1030	1034	1033	1036sh	$\nu(CC)(CO)$
990	990sh			$\gamma(COOH)_{dimers}$
		992	992	$\delta(COO^-)$
953	954	957	958	$\delta(CCH)$, $\delta(COH)$
	915		917	
887	888	896	894	$\delta(CCH)$, $\delta(COH)$
853		857		(C6–C5–O5–C1–O1)
834	830	836	835	$\gamma(C-OH)_{ring}$
795	790	814	815	$\gamma(C-OH)_{ring}$
775	760sh	774	769	Ring 'breezing'
750sh	738			$\gamma(C-OH)_{COOH}$
710	700sh	717	710sh	$\gamma(C-OH)_{ring}$
686	682	687	673	Low frequency vibrations of pyranoid ring
621	637	636	649	
537	534	538	544	
486		483		
441		444		T(C–O–C) def.
372		378		
340		344		T(C–O–C) def.

30 and 6% of the peak height of the corresponding band of K-Pec, respectively (Figs. 3(b) and 4(b)).

This phenomenon can be explained by the fact that carboxylic groups together with hydroxyls participate in a hydrogen bond system, which stabilises the pectin conformation in solid state. Such system becomes weaker in the the order COO^-–COOH–$COOCH_3$ that is followed by $\nu(OH)$ decreaseing (Filippov, 1978, 1980). Acetylation leads to both a decrease in the free hydroxyl content and weakening of the hydrogen bonds. Therefore, the resulting effect of acetylation on a $\nu(OH)$ decrease was much more significant than was observed in the case of methylation. Both acetylation and methylation together enhanced the effects of each other and led to a maximal $\nu(OH)$ decrease.

The spectra obtained confirmed that the vibration bands of methyl and acetyl esters had different positions and properties in both FT-Raman and FT-IR spectra (Table 3) that permitted these groups to be distinguished. In FT-Raman spectra of pectinates and Me-Pec, the C–H stretching vibration band increased gradually and shifted from 2940 to 2960 cm^{-1} with DM increasing due to the contribution of the intense asymmetric stretching vibration of methyls in methylester groups $\nu_{as}(CH_3)_{Me}$ (Fig. 3(a)).

The acetylation of pectates led to a narrowing of the Raman band at 2940 cm^{-1} assigned to asymmetric stretching vibration of methyl groups in acetyls $\nu_{as}(CH_3)_{Ac}$, while acetylated pectinic acid Me-PecAc had an intense Raman band at 2940 cm^{-1} (acetyl esters) with a smaller shoulder near 2960 cm^{-1} (methyl esters) (Fig. 4(a)). Therefore, $\nu_{as}(CH_3)$ Raman bands of methyl and acetyl esters had different positions by 20 cm^{-1} (Table 3).

The weak band at 2860 cm^{-1} was observed in IR and Raman spectra of pectinates, Me-Pec, KMe-PecAc and Me-PecAc but was absent in the spectra of K-Pec and acetylated pectates. (Figs. 3 and 4) Therefore, this band increased with methylation and did not depend on acetylation, so it was assigned to the symmetric stretching vibration of methyl groups in methyl esters $\nu_s(CH_3)_{Me}$.

For Raman and IR spectra of potassium pectinates, the carboxylate vibration bands decreased and several methyl ester bands increased with the increasing of DM (Fig. 3). In the case of acetylated pectates, the corresponding acetyl bands were observed, whereas KMe-PecAc and Me-PecAc showed the characteristic Raman and IR bands of both methyl and acetyl esters (Fig. 4). Carboxylate stretching bands of acetylated pectates did not change significantly their intensity in connection with DAc increasing.

The carbonyl vibration band of acetyls $\nu(C{=}O)_{Ac}$ at 1720 cm^{-1} (K-PecAc1) was increased with DAc and moved to 1748 cm^{-1} (K-PecAc4) (Fig. 4). The methyl scissoring bands of acetyls and methoxyls had relatively similar position in Raman and IR, but their intensities were in the relation: $\delta_{as} \ll \delta_s$ (acetyls) and $\delta_{as} \gg \delta_s$ (methoxyls) (Table 3, Figs. 3 and 4). Acetylated pectates also had a strong IR band at 1250 cm^{-1} assigned to $\nu(COC)$ of acetyl, whereas Me-PecAc had three IR bands at 1280, 1250 and 1220 cm^{-1} (Fig. 4(b)). The first and the third bands were assigned to $\nu(COC)$ of methyl esters, whereas the second band, as in case of acetylated pectates, was assigned to $\nu(COC)$ of acetyls.

The region of 1200–1000 cm^{-1} contains skeletal C–O and C–C vibration bands of glycosidic bonds and pyranoid ring. Comparing with K-Pec, the pectinates showed slight changes in both Raman and IR spectra in this region (Fig. 3). However, acetylated pectates showed marked decreasing of the intense Raman bands at 1143, 1105 and 1178 cm^{-1}. New weak bands appeared at 1205, 1169, 1153 (shoulder), 1130, 1092, 1070, 1014 and 1008 cm^{-1} in Raman spectra of acetylated pectates (Fig. 4(a)). Acetylated pectates also showed some changes in FT-IR spectra: the band at 1088 cm^{-1} increased and the band at 1112 cm^{-1} decreased with the acetylation

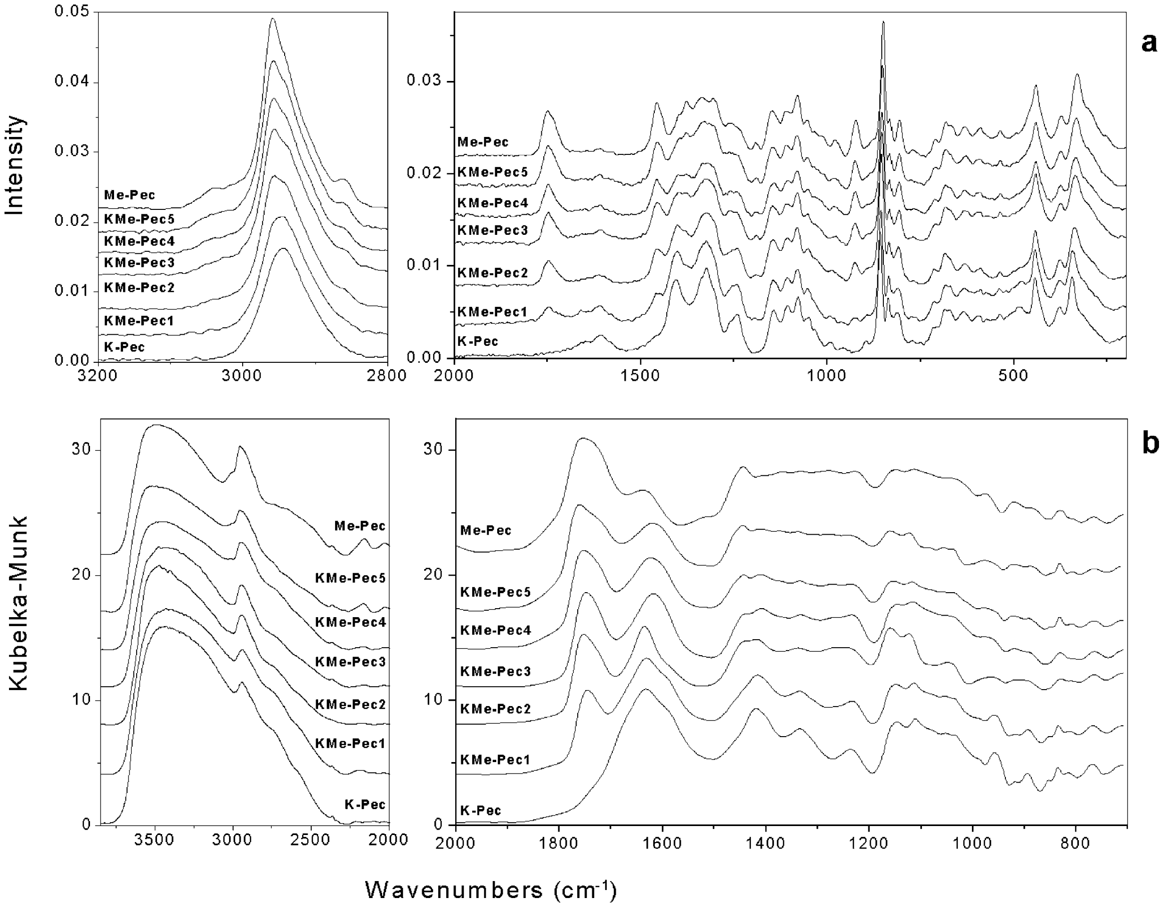

Fig. 3. FT-Raman (a) and FT-IR (b) spectra of potassium pectate K-Pec, potassium pectinates KMe-Pec1-4 and pectinic acid Me-Pec.

degree (Fig. 4(b)). The samples KMe-PecAc and Me-PecAc, which contain both acetyl and methylester groups, showed two new strong Raman bands at 1080 and 1004 cm^{-1} and a new IR band at 1064 cm^{-1} (Fig. 4). The results confirm that acetylation of pectate leads to drastic changes in pyranoid ring conformation.

The vibration bands of pectins at the region of 1000–700 cm^{-1} are complex nature, so their assignment is quite difficult especially in the case of FT-IR spectra. The Raman and IR bands of K-Pec at 991 (weak in IR) and 960 cm^{-1} slightly changed in acetylated pectates, declined in pectinates and were entirely absent in Me-Pec, KMe-PecAc and Me-PecAc. These bands were assigned to in-plane deformations of carboxylate and pyranoid ring (Filippov, 1978; Wellner et al., 1998). The band at 975–978 cm^{-1} was absent in IR and Raman spectra of K-Pec and acetylated pectates, but appeared and increased with DM increasing in pectinates. We assigned this band to the $\nu(O-CH_3)$ vibration of methyl esters (Engelsen & Nørgaard, 1996; Wellner et al., 1998).

The rocking bands of methyls $\rho(CH_3)$ were observed in the Raman and IR spectra at 922 and 928 cm^{-1} for methoxyls and acetyls, respectively (Figs. 3 and 4). Samples KMe-PecAc and Me-PecAc had specific vibration bands at 1006–1004 cm^{-1} (Raman), 988 cm^{-1} (IR) and 916–911 cm^{-1} (IR) that may confirm significant structural changing in highly acetylated pectinates in comparison with pectate. In addition, two Raman (IR) bands at 836 (835) and 814 (815) cm^{-1} assigned to out-off-plan vibrations of hydroxyls $\gamma(C-OH)$ shifted to lower wavenumbers (max. by 8–10 cm^{-1}) in methylated and acetylated samples.

The positions of the very strong Raman band observed at 857 cm^{-1} for K-Pec (skeletal vibration of the pyranoid ring) seems to be sensitive both to the methylation of C-6 carboxyls and to the acetylation of C-2 and C-3 hydroxyls (Fig. 5). For potassium pectate and pectinates with increasing DM, the position of this band gradually shifted to 850 cm^{-1} (Me-Pec). In contrast, subsequent acetylation of pectate led to the gradual shift of this band to higher wavenumbers, max. 862 cm^{-1} (K-PecAc4). In the case of KMe-PecAc and Me-PecAc, which contain both methyl and acetyl esters, the wavenumber of this band was insignificantly less than that of K-PecAc4. This band was broader and weaker in the case of all acetylated derivatives in comparison with that of K-Pec or pectinates.

In the region below 700 cm^{-1} the FT-Raman spectra of pectinates changed slightly in comparison with pectate

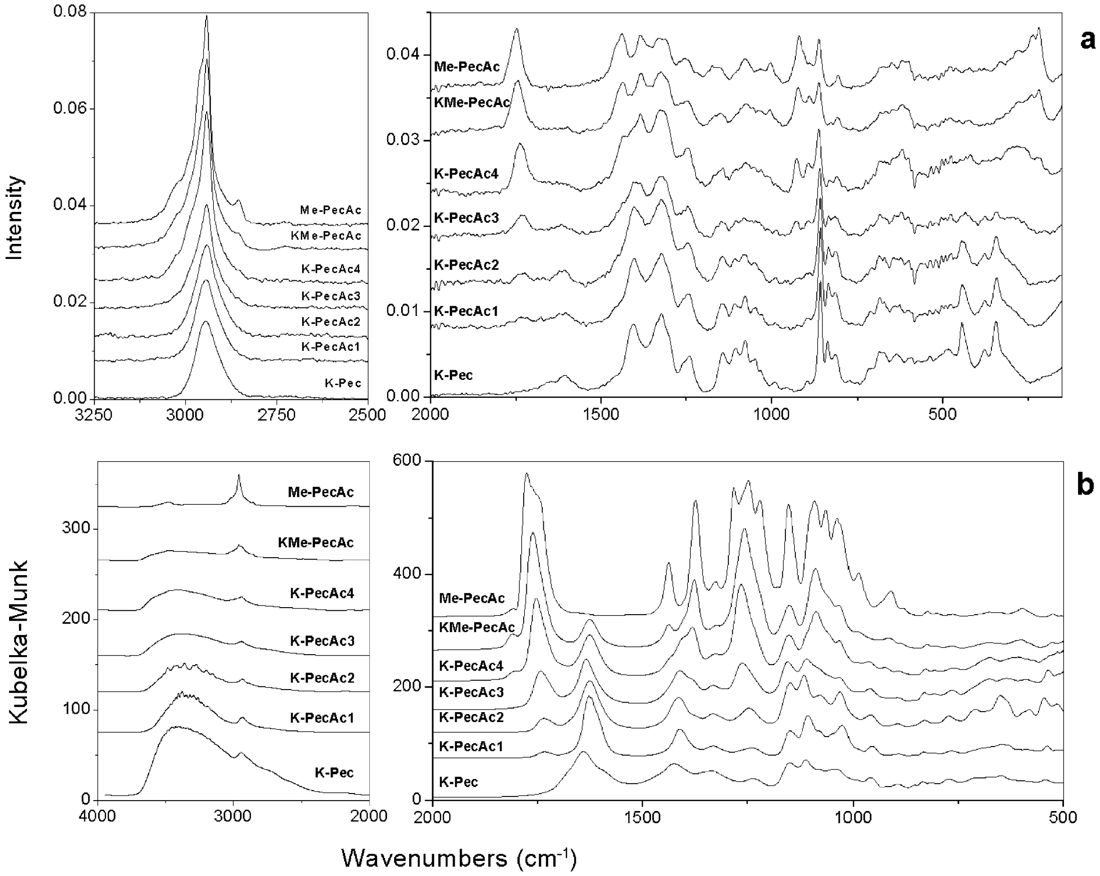

Fig. 4. FT-Raman (a) and FT-IR (b) spectra of potassium pectate K-Pec and its acetylated derivatives.

(Fig. 3(a)). The skeletal band at 344 cm^{-1} in pectate shifted gradually to lower frequencies with methylation reaching to 331 cm^{-1} in Me-Pec. Acetylation of pectate led to drastic spectral changes in the far-IR region of Raman spectrum (Fig. 4(a)). The strong to medium bands at 444, 378 and 344 cm^{-1} gradually disappeared and several new well-distinguished narrow bands appeared at 534, 520, 503, 492, 477, 465, 450, 433 and 420 cm^{-1} in acetylated pectates. Moreover, several broad and structured bands appeared at 330–180 cm^{-1} in acetylated pectates. The Raman intensity at this region markedly increased and several new strong bands appeared at 277, 235 and 219 cm^{-1} in acetylated pectinates.

3.3. Citrus and sugar beet pectins

The FT-Raman and FT-IR spectra of various carboxylic forms of citrus and sugar beet pectins (CP and BP) are shown in Fig. 6.

The regions of carbonyl stretching vibrations (1800–1500 cm^{-1}) in Raman and corresponding IR spectra of the different forms of CP were very similar to each other (Fig. 6(a) and (b)). However, as was noted above for H-Pec and K-Pec, Raman C=O and COO^- stretching vibrations were much less intensive than the corresponding infrared bands. HMe-CP and KMe-CP had stretching vibration bands of methyl ester group near 1750 cm^{-1} (νC=O) that decreased after alkali hydrolysis. HMe-CP and H-CP had a single ν(C=O) band at 1750–1730 (FT-Raman) and at 1770–1760 cm^{-1} (FT-IR).

FT-Raman spectra of KMe-CP and K-CP had a weak band at 1612 cm^{-1} and a strong band at 1405 cm^{-1} (Fig. 6(a)). In the case of K-Pec, these two bands were

Table 3
FT-Raman and FT-IR band wavenumbers (in cm^{-1}) of methyl and acetyl ester groups in pectins (Engelsen & Nørgaard, 1996a; Filippov, 1976, 1978, 1980; Kačuráková et al., 1999; Wellner et al., 1998)

Methyl esters		Acetyl esters		Assignments
FT-Raman	FT-IR	FT-Raman	FT-IR	
2960	2970	2940	2960	$\nu_{as}(CH_3)$
2860	2865			$\nu_s(CH_3)$
1750	1754	1720–1738	1730–1752	ν(C=O)
1458	1444	1440	1435	$\delta_{as}(CH_3)$
1378	1371	1385	1388	$\delta_s(CH_3)$
1260	1282		1255	ν(COC)
1192	1220			ν(COC)
979	976			$\nu(O{-}CH_3)$
922	923	928	926	$\rho(CH_3)$

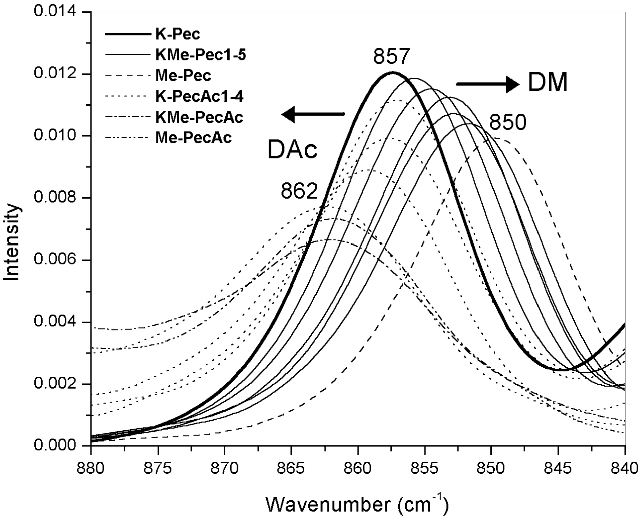

Fig. 5. FT-Raman spectra of potassium pectate K-Pec and its derivatives in the region of 880–840 cm^{-1}. The arrows show the shifts of frequency maximum depending on methylation (DM) or acetylation (DAc) degrees.

assigned to $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$, respectively. In FT-IR spectra, the first band is much more intensive than the second one (Fig. 6(b)). Two weak bands at 1683 cm^{-1} (amide I) and 1598 cm^{-1} (amide II) appeared in the Raman and IR spectra of amidated forms, i.e. NH_4Am-CP and HAm-CP. An intense asymmetric stretching vibration of carboxylate near 1612 cm^{-1} overlapped the second band in NH_4Am-CP.

The FT-IR spectra of BP and its carboxylic forms were quite similar to those of CP (Fig. 6(d)). In contrast, the FT-Raman spectrum of BP as well as most of its forms had two sharp bands at 1630 and 1603 cm^{-1} that overlapped the carboxylic bands of pectin backbone (Fig. 6(c)). These two specific bands of BP were assigned to the aromatic system and the double bond vibrations of ferulate esters attached to neutral sugar side chains of this pectin. In the case of K-BP, the ferulate bands were shifted to lower frequencies and observed at 1618 and 1583 cm^{-1}. The shift could be explained by dissociation of phenolic hydroxyls by the alkali treatment on feruloyl residues.

Various forms of BP had a specific weak and sharp IR band centred at 1517 cm^{-1}. The intense band of carboxylate symmetric stretching overlapped this band in K-BP and NH_3Am-BP. All the bands indicating ferulic acids have been detected in vibration spectra of cell-wall pectins and xylan-type polysaccharides by Kačuráková et al. (1999). The fact that only specific types of pectin, such as those obtained from spinach lives or sugar beet pulp (Thibault, 1986), contain phenolic acids could be

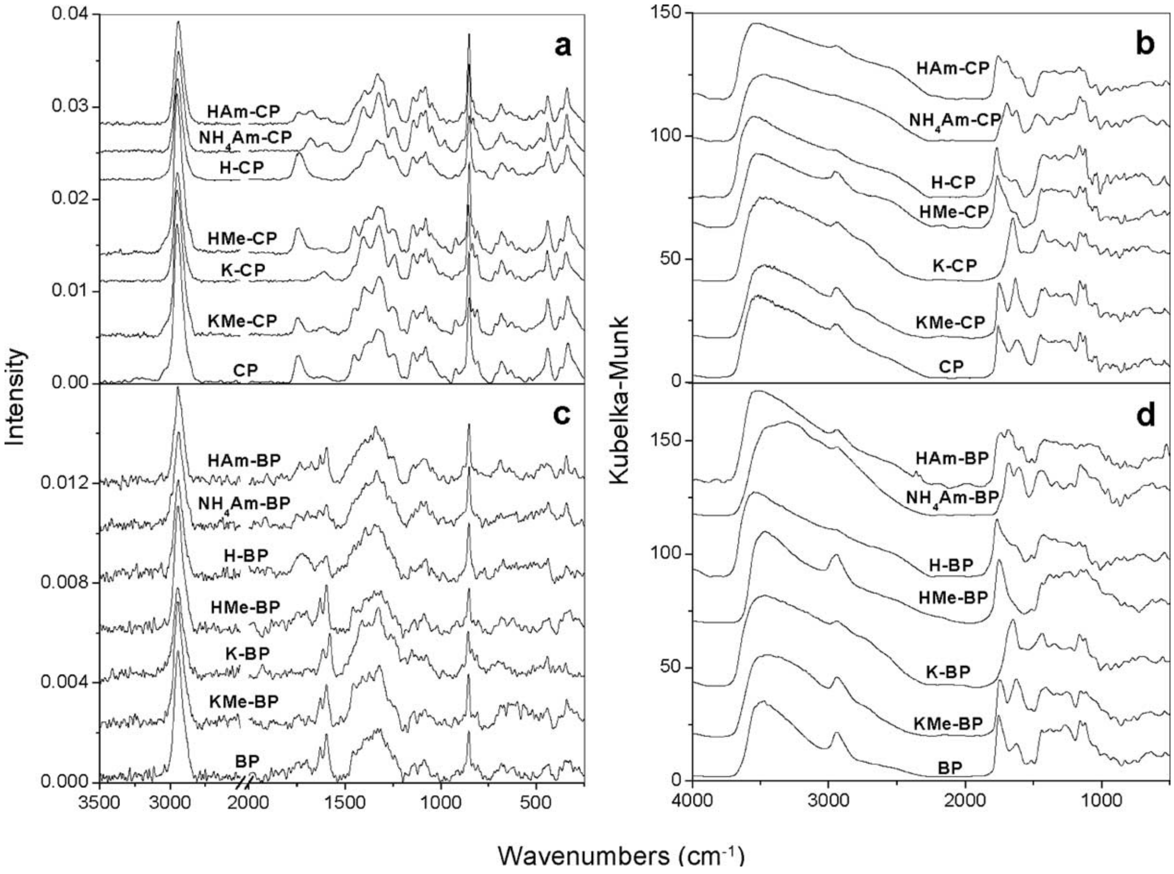

Fig. 6. FT-Raman (a) and (c) and FT-IR (b) and (d) spectra of citrus pectin CP (a) and (b) and sugar beet pectin BP (c) and (d) at various forms.

Table 4
The wavenumber (in cm^{-1}) of the very strong Raman band assigned to complex skeletal vibration for studied samples (Engelsen & Nørgaard, 1996a)

Sample	Wavenumber (cm^{-1})	Sample	Wavenumber (cm^{-1})
H-Pec	853	CP	852
K-Pec	857	K-CP	857
KMe-Pec1	856	KMe-CP	855
KMe-Pec2	854	NH_4Am-CP	854
KMe-Pec3	853	H-CP	852
KMe-Pec4	853	HMe-CP	852
KMe-Pec5	852	HAm-CP	853
Me-Pec	850	BP	854
K-PecAc1	857	K-BP	859
K-PecAc2	858	KMe-BP	856
K-PecAc3	859	NH_4Am-BP	855
K-PecAc4	862	H-BP	855
KMe-PecAc	862	HMe-BP	854
Me-PecAc	862	HAm-BP	854

interesting for pectin identification by vibration spectroscopy.

According to the FT-Raman spectra of both CP and BP samples, the position of C6–C5–O5–C1–O1 skeletal band mentioned above is sensitive to the modification of C-6 carboxyls (Table 4, Fig. 6(a) and (c)). In comparison with the commercial sample of CP, the wavenumbers of this band slightly increased for salt forms and slightly decreased for acid forms. The maximal difference (5 cm^{-1}) was observed between K-CP (857 cm^{-1}) and HMe-CP (852 cm^{-1}). De-esterification of CP also influenced the position of this band. KMe-CP and HMe-CP had rather-lower wavenumbers in comparison with K-CP and H-CP, respectively. The various forms of BP sample showed the changes in band position similar to those of the corresponding CP forms (Table 4).

4. Conclusion

This study illustrates the complementary application of FT-Raman and FT-IR spectroscopy in structural analysis of pectins. We suggest that these two vibration spectroscopic methods possess together more complete characterisation of pectin samples than each method can alone. While FT-IR spectroscopy is sensitive to functional groups of these polysaccharides, i.e. hydroxyls, carboxyls, esters and amides, FT-Raman spectroscopy, is better at analysis complex skeletal vibrations of polysaccharide chains, i.e. glycosidic bonds and pyranoid rings. Both FT-Raman and FT-IR spectroscopic methods are valuable tools for non-destructive qualitative and quantitative analysis of pectins. These vibration spectroscopic methods may be also applied in detecting of minor components integrated into the structure of pectin samples such as proteins, neutral sugars or phenolic compounds.

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