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Pectin functionalised by fatty acids: Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopic characterisation

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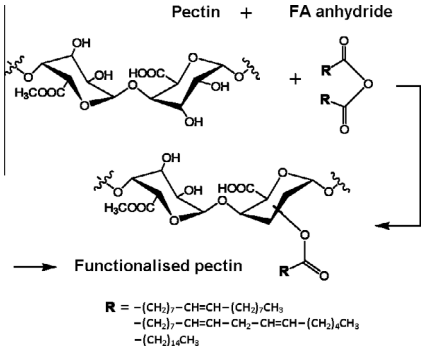
HIGHLIGHTS

- Pectin was esterified at its —OH moieties with oleic, linoleic and palmitic acids.
- DRIFT spectra showed characteristic changes upon pectin–fatty acid esterification.
- Published data on FTIR spectra reveal KBr/NaCl-induced band shifts of polar groups.

GRAPHICAL ABSTRACT



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ABSTRACT

Chemically modified pectin derivatives obtained by partial esterification of its hydroxyl moieties with fatty acids (FA; oleic, linoleic and palmitic acids), as well as the initial apple peel pectin were comparatively characterised using diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. Characteristic changes observed in DRIFT spectra in going from pectin to its FA esters are related to the corresponding chemical modifications. Comparing the DRIFT spectra with some reported data on FTIR spectra of the same materials measured in KBr or NaCl matrices has revealed noticeable shifts of several polar functional groups both in pectin and in its FA-esterified products induced by the halide salts. The results obtained have implications for careful structural analyses of biopolymers with hydrophilic functional groups by means of different FTIR spectroscopic methodologies.

Introduction

The processes of chemical modification of pectins, plant cell-wall carboxylated (partly esterified, i.e. methylated or acetylated) polysaccharides with a wide range of useful biological and biotechnological properties [1–5], have received significant attention over the last years [6–11]. This is because pectins, already being versatile, upon targeted chemical modifications acquire novel properties and functions which may be applicable in diverse fields of technology.

Recently, a methodology for chemical functionalisation of pectin was proposed involving esterification of its hydroxyl moieties with different fatty acids (FA) [12,13]. The process was then further developed to exclude conventional heating by applying microwave irradiation of the dried pectin–FA mixtures in the presence of a catalyst (potassium carbonate) with further extraction and purification of the product [14]. The materials obtained were partly

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characterised with regard to their chemical structures and some physical properties [12–15], including antibacterial activity of some of the FA-modified materials and their applicability as promising coatings for polyethylene films for active packaging systems [15].

While Fourier transform infrared (FTIR) spectroscopy was among the techniques used earlier for partial structural characterisation of modified pectins (see, e.g. [12,15]), the FTIR spectra were obtained in pellets pressed in KBr or in NaCl matrices. In this communication, the diffuse reflectance methodology (DRIFT spectroscopy) was used, which allows FTIR spectra of the pristine materials to be obtained, avoiding the influence of the polar halide matrix on polar functional groups of pectin and its chemically modified derivatives. The DRIFT spectra obtained for the initial pectin (for comparison) and its products functionalised by non-saturated (oleic, linoleic acids) and saturated (palmitic acid) FAs are compared and discussed with regard to some representative functional groups.

Materials and methods

Materials

Pectin (from apple peel) was purchased from Fluka. It was a powder sample with high molecular weight (30,000–100,000 g/mol) and a high degree of esterification (70–75%) on a dry basis. The fatty acids and other chemicals were purchased from Sigma–Aldrich.

Synthesis of fatty acid anhydrides

The appropriate fatty acid (10 mmol) was dissolved in dichloromethane (2 ml), the solution was cooled in an ice-water bath and stirred vigorously under argon atmosphere. Dicyclohexylcarbodiimide (5 mmol), pre-dissolved in a minimum volume of dichloromethane, was added and stirring was continued at ice-water bath temperature for 2 h. The resulting white solid *N,N′*-dicyclohexylurea was removed by filtration and the solvent was evaporated in vacuo to give the final product (FA anhydride).

Synthesis of pectin derivatives

Pectin (30 mg) was manually ground with 30 mg of a fatty acid anhydride in an agate mortar in the presence of K_2CO_3 (0.1 equivalent) to obtain each of the different pectin-derived materials. Reactions were carried out in a domestic microwave oven; the mixture was irradiated (at 900 W) for two cycles of 3 min each (6 min total). After cooling down to room temperature, the obtained solid was dissolved in water, placed in a 250 ml separatory funnel and extracted with ethyl acetate in order to remove the unreacted fatty acid. Subsequently, the aqueous layer was neutralised by adding 0.5 M HCl solution in water and then dialysed (with a membrane cut-off 6000–8000) for 1 day in Milli-Q water. After lyophilisation, the final product was collected.

DRIFT measurements

Spectra were obtained directly from powdered dry materials. DRIFT measurements were performed on a Nicolet 6700 FTIR spectrometer (Thermo Electron Corporation, USA) by placing ca. 2 mg of the dry powdered biomass in a Micro sampling cup (Spectra-Tech Inc., USA) without using potassium bromide (KBr) in sample preparation, and mounting the sampling cup onto the DRIFT accessory sample holder of the spectrometer. Spectra were collected with a total of 100 scans (resolution 4 cm^{-1}) against a KBr background. All the spectroscopic data were well reproducible.

Results and discussion

The FTIR spectroscopy technique is highly sensitive to the structure and conformation of the functional groups in the substance under study and, which is yet more important, to all intermolecular and intramolecular interactions therein that can modify the bonding lengths and energies. In particular, when using the conventional transmission measurements of samples pressed in pellets with KBr (or in NaCl discs), the resulting spectra may be altered (involving some KBr/NaCl-induced band shifting) as a result of the influence of the polar salt matrix on polar functional groups as well as on the system of H-bonds. This may be especially significant in biopolymers where the H-bonding system often defines the structure of the macromolecules and their ensembles [16,17]. In order to avoid such influence, the DRIFT methodology may be applied which shows undisturbed positions of vibrational peaks and can allow fine structural features of biopolymers to be revealed (see, e.g. [17] and references reported therein).

In this study, we compare DRIFT spectra of the initial pectin and its chemically modified derivatives (partly esterified at its OH-moieties with fatty acids [14,15]; Fig. 1), that were measured without using KBr or NaCl matrices.

The DRIFT spectra obtained are presented in Fig. 2. An overall comparison of the spectra of the pristine pectin (Fig. 2d) and its FA-esterified products (Fig. 2a–c) shows the following main changes observable upon the chemical modification of the initial pectin. As could be expected, the relative intensity of the O—H stretching region (very broad asymmetrical envelope about 2500–3600 cm^{-1}) changed in shape and reduced in intensity as compared to the fingerprint region (under 2000 cm^{-1}), reflecting the esterification of part of OH groups of the galacturonide chain (see Fig. 1). The generally weak composite absorption (overlapping with the broad O—H envelope) related to the C—H stretching vibrations (around 2940 cm^{-1}) is increased, in line with the increased regions of ester stretching C=O vibrations (strong peaks at ca. 1750 cm^{-1}) and the region of skeletal stretching C—O and C—C modes of the pyranoid rings and glycosidic bonds (950–1150 cm^{-1}). This region also changed owing to a combination of new C—C and C—O moieties in the introduced FA residues (see Fig. 1).

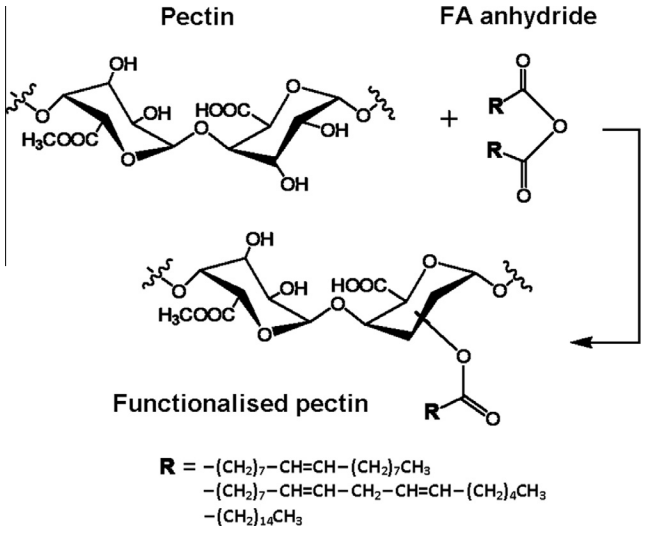

Fig. 1. Scheme of pectin functionalisation by fatty acids using fatty acid anhydrides (where **R** represents residues of: $-(CH_2)_7-CH=CH-(CH_2)_7CH_3$, oleic acid; $-(CH_2)_7-CH=CH-CH_2-CH=CH-(CH_2)_4CH_3$, linoleic acid; and $-(CH_2)_{14}CH_3$, palmitic acid).

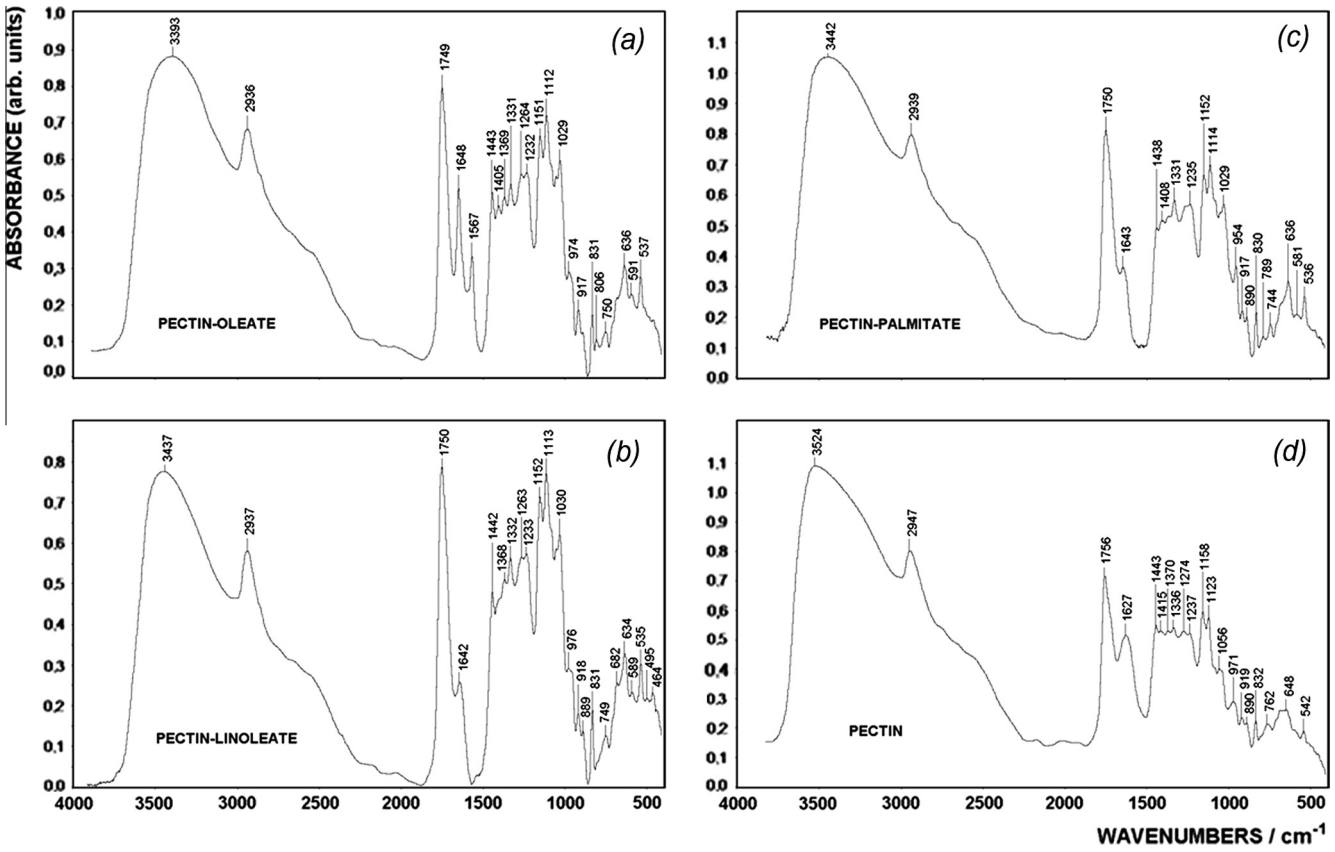

Fig. 2. Diffuse reflectance infrared Fourier transform (DRIFT) spectra of pectin esterified with oleic (a), linoleic (b) and palmitic acid (c), as well as of the initial pectin (d).

Table 1
Positions of the maxima of some representative vibrational bands in pectin and its FA-modified derivatives (see also Fig. 2).

Sample	Band assignment	Wavenumbers (cm^{-1})		References
		DRIFT (this work)	FTIR[a]	
Pectin	ν(C═O)[b]	1756	1747	[12]
	ν(COOH)[c]	1627(broad)	1649	
	ν(C—O—C)[d]	1158	1149	
Pectin-oleate	ν(C═O)[b]	1749	1739	[12]
	ν(COOH)[c]	1648	1636	
	ν(C—O—C)[d]	1151	1146	
Pectin-linoleate	ν(C═O)[b]	1750	1739	[12]
	ν(COOH)[c]	1642	1639	
	ν(C—O—C)[d]	1152	1146	
Pectin-palmitate	ν(C═O)[b]	1750	1749, 1723–1705	[15]
	ν(COOH)[c]	1643	1635	
	ν(C—O—C)[d]	1152	1133	

[a] Spectra measured in NaCl discs [12] or in KBr pellets [15].
[b] Ester moieties.
[c] C═O of the carboxylic moiety.
[d] Glycosidic bond.

In is noteworthy that both for the initial pectin and for its FA-esterified products, our DRIFT data show very similar strong and relatively symmetric stretching C═O peaks of the ester moieties, the maximum only slightly changing from 1756 cm^{-1} in pectin to 1749–1750 cm^{-1} in the modified products (see Fig. 2 and Table 1). This shows that the ester moieties, both in methyl esters of the pectin and in its FA esters, show essentially the same typical DRIFT maxima. Comparing these data with those reported in the literature (see Table 1) for FTIR spectra measured in pressed KBr pellets or in NaCl discs, it can be seen that pressing the samples with the polar halides resulted in ester ν(C═O) band downshifting by 10 ± 1 cm^{-1}, which is well over the spectral resolution of the FTIR spectrometers. Moreover, in FTIR spectra measured in KBr pellets [15], some splitting of the ester ν(C═O) bands were observed. This may be reasonably explained by the fact that grinding and pressing the materials with KBr may significantly influence part of the ester ν(C═O) moieties (which then undergo a noticeable downshift), while some part of the ester ν(C═O) moieties remain virtually unaffected (giving a maximum at about 1749 cm^{-1}; see Table 1).

Comparing the DRIFT maxima of some other polar groups, it may be noted that they also undergo slight changes in going from pectin to its FA esters (see Fig. 2 and Table 1). The broadened band of the stretching C═O vibration of pectin non-esterified carboxylic groups (with a maximum at 1627 cm^{-1}, showing their significant

involvement in intermolecular H-bonding), which can be strongly influenced by the surrounding polar C—O and O—H moieties of the pectin backbone, in the pectin FA esters is observed at 1642–1648 cm^{-1}. However, in FTIR spectra of the FA esters measured with KBr or NaCl, they appear at wavenumbers yet lower by 3–12 cm^{-1} (see Table 1).

The same general trend is observed for the stretching C—O—C vibration of the glycosidic moiety, which is observed in DRIFT spectra at 1158 cm^{-1} (in pectin) and at 1151–1152 cm^{-1} (in its FA esters). In FTIR spectra measured with KBr or NaCl, the band is downshifted by 9 cm^{-1} in the pectin and by 5–19 cm^{-1} in its FA esters (see Table 1).

It should also be noted that in pectin-oleate (see Fig. 2a), besides the stretching C=O moiety of pectin non-esterified carboxylic groups involved in intermolecular H-bonding (at 1648 cm^{-1}), there appears another band at 1567 cm^{-1} (absent in the DRIFT spectra of other samples) which may also be assigned to the same mode (although it is usually more common for ionised carboxylic groups featuring the strong antisymmetric stretching mode of $—COO^-$). Note that such a band was observed in FTIR spectra of some samples in pectin-palmitate esters (measured in NaCl discs) in [13], although its appearance was not specially commented. Thus, its presence in DRIFT spectra (measured without KBr or NaCl) may show that part of non-esterified carboxylic groups in the modified pectin are ionised.

Conclusions

It has been shown using DRIFT spectroscopy that partial esterification of pectin O—H moieties with fatty acids brings about some noticeable relevant spectroscopic changes related to differences in the structures of the initial pectin and its derivatives. Comparison of the DRIFT spectroscopic results obtained in this work with the literature data on FTIR spectra measured in KBr or NaCl matrices reveals some characteristic shifting of vibrational bands related to several polar functional groups induced by grinding and pressing with the halide salts. The results obtained have implications for a more careful structural analysis of biopolymers, particularly those having hydrophilic (polar) functional groups, by means of different FTIR spectroscopic methodologies.

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