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Carbohydrate Polymers 56 (2004) 465–469

Carbohydrate Polymers

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Determination of the degree of esterification of pectinates with decyl and benzyl ester groups by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) and curve-fitting deconvolution method

Christos S. Pappas[a], Anna Malovikova[b], Zdenka Hromadkova[b], Petros A. Tarantilis[a], Anna Ebringerova[b], Moschos G. Polissiou[a,*]

[a]*Laboratory of Chemistry, Department of Science, Agricultural University of Athens, 75 Iera odos, 118 55 Athens, Greece*
[b]*Institute of Chemistry, Slovak Academic of Sciences, 845 38 Bratislava, Slovakia*

Received 13 June 2003; revised 18 February 2004; accepted 24 March 2004

Abstract

Pectinates with benzyl and decyl ester groups were prepared by alkylation of the tetrabutylammonium salt of pectic acid with benzyl and decyl bromides, respectively. The degree of esterification (DE) of the pectin derivatives was determined by diffuse reflectance infrared Fourier transform spectroscopy and the curve-fitting deconvolution method. A linear relationship between DE and the ratio of the peak area at 1745 cm^{-1} to the sum of the peak areas at 1745 and 1608 cm^{-1} was established with a high correlation coefficient 0.98. The deconvolution analysis using the curve-fitting method allowed the elimination of spectral interferences from pectin components and their degradation products. The limits of the method are given by DE 6 and 93%. The method was compared with chemical analysis and found to be equivalent in view of accuracy and repeatability (*t*-test, *F*-test). The method is applicable in analysis of natural or synthetic mixtures and/or crude pectin substances.

Keywords: Pectin; Esters; Diffuse reflectance infrared Fourier transform spectroscopy; Deconvolution; Degree of esterification

1. Introduction

Pectin is an acidic plant polysaccharide which is widely used in foods, pharmacy, and many other industries. This multifunctionality of pectin originates mainly from the degree of methyl esterification of carboxyl groups which influences the solubility, gelling, stabilizing, and emulsifying properties. The functional properties can be enhanced or even changed by introducing new functional groups onto the pectin polymer backbone (Crescenzi & Callegaro, 1993; Klavons & Bennet, 1995; Sinitsya, Copikova, Prutyanov, Skoblya, & Machovic, 2000; Morris et al., 2002). In a previous study (Hromadkova, Malovikova, Sasinkova, Alfoldi, & Ebringerova, 2000) esters of citrus pectin were prepared by transformation of the carboxyl group into a benzyl or C_n-alkyl ester group via reaction of its tetrabutylammonium salt with the corresponding benzyl and alkyl bromides. The water-soluble alkyl ester derivatives obtained exhibited excellent emulsifying properties (Malovikova et al., 2000).

Fourier transform infrared (FT-IR) spectroscopy is used as a method for monitoring chemical changes in pectic substances and, more specifically, changes in the ester content (Barros et al., 2002; Chatjigakis et al., 1998; Gnanasambandam & Proctor, 2000; Kacurakova & Wilson, 2001; Malovikova & Kohn, 1980; Monsoor, Kalapathy, & Proctor, 2001; Sereti, Stamatis, Pappas, Polissiou, & Kolisis, 2001). Main advantages of these methods are simplicity, speed, and ability to be non-destructive for the samples. Recently, similar methods for the determination of DE have been developed by various authors (Barros et al., 2002; Gnanasambandam & Proctor, 2000; Monsoor et al., 2001). Whereas, the method of Barros et al. (2002) was based on Attenuated Total Reflectance spectroscopy (ATR), the methods reported by Gnanasambandam and Proctor (2000) and Monsoor et al. (2001) were based on diffuse reflectance

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[*] Corresponding author. Tel.: +302105294241; fax: +302105294265.
E-mail addresses: mopol@aua.gr (M.G. Polissiou); ptara@aua.gr (P.A. Tarantilis).

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doi:10.1016/j.carbpol.2004.03.014

infrared Fourier transform spectroscopy (DRIFTS) technique. However, they have been applied on pure samples. So, they cannot be used in the case of mixtures or not purified pectin samples because there are many overlapping peaks in spectral region (1750–1600 cm^{-1}) which is used for the determination of DE. Consequently, this spectral region is necessary to be deconvoluted. In addition, the applicability of the above methods is limited by DE 28.5 and 93.0% in the first method and 26.2 and 76.2% in the second one.

The aim of this study was to develop a FT-IR method in the DRIFTS mode using the curve-fitting deconvolution method for the determination of DE of pectinates having decyl and benzyl groups instead of methyl groups with a higher breadth of DE values.

2. Experimental

2.1. Materials

Pectin standards with a known degree of methylesterification (31, 68 and 93%) were purchased from SIGMA (Germany) and the Na salt of polygalacturonic acid was from SERVA (Germany). The starting material for the esterification experiments was citrus pectin (CP) from Pectinfabrik (Denmark) containing ~10% of neutral sugars. The sample was purified and totally de-esterified in suspension of 60% EtOH with 0.5 M KOH (Malovikova & Kohn, 1980). The obtained potassium pectate was converted into the tetrabutylammonium pectate (TBAP) by means of cation exchanger Amberlite IRN 77 (TBA^+ form). None of the pectin samples under investigation contained protein as estimated by elementary analysis.

2.1.1. Preparation of standard samples

Subsequent pectin standards with a known degree of methylesterification (6, 9, 16, 22, 44, 50, 57, 62 and 80%) were prepared by mixing corresponding amounts of the four commercial pectin standards.

2.1.2. Preparation of pectin decyl and benzyl esters

Pectin esters were prepared by alkylation of TBAP with benzyl bromide (samples G1–G5) and decyl bromide (samples G6–G10) in DMSO according to Crescenzi and Callegaro (1993) after a slight modification already described (Hromadkova et al., 2000). The non-esterified carboxyl groups of the pectin esters were present in the sodium salt form.

2.2. Methods

DE was determined by the chemical method based on precipitation of the insoluble copper pectates and pectinates (Tibensky, Rosik, & Zitko, 1963). For the spectroscopic measurements the standard samples and the pectin esters (G-1 to G-10) were freeze-dried from an aqueous solution. The measurements were triplicate. Spectra were collected with a Nicolet Magna 750 FT-IR spectrophotometer (Madison, Wisconsin, USA) equipped with a Deuterated TriGlycine Sulfate (DTGS) detector at 4 cm^{-1} resolution, 100 interferograms with 'signal-averaged' to obtain a high signal to noise ratio and Happ-Genzel apodization. The spectra were recorded using a Spectra Tech DRIFTS accessory and rationed against a KBr background.

Table 1
Wavenumbers of the major peaks of the spectra in the region 1800–1500 cm^{-1} (Abbott et al., 1987; Nakanasy, 1962; Sene et al., 1994)

Wavenumbers (cm^{-1})	Assignments
1742–1748	νC=O of alkyl esters
1665–1680	νC=O of aromatic ketones, νC=O/COOH conjugated with double bonds
1645–1656	Amide I (protein)
1629–1637	Water bending
1606–1610	ν_{as}C=O ($-COO^-$)
1594–1601	Aromatic ring stretching
1541–1559	Amide II (protein)

The FT-IR spectra were analyzed according to previous works (Chatjigakis et al., 1998; Gnanasambandam & Proctor, 2000). The FT-IR spectra were smoothed and their baselines were corrected automatically using the built-in software of the spectrophotometer (OMNIC 3.1). Thus, the root-mean squared (RMS) noise of every spectrum was calculated. By means of the second derivative in the spectral region 1800–1500 cm^{-1}, four major peaks for the standards and seven for the ester samples were resolved and assigned to structures which might exist in pectin preparations (Table 1). Using the wavenumber of the previous peaks as a guide, the above mentioned spectral region was deconvoluted by the curve-fitting method with the Levenberg-Marquadt algorithm and the peaks at 1745 cm^{-1} (esterified carboxyl, −COOR) and 1600 cm^{-1} (ionized carboxyl, $-COO^-$) were adjusted and the area measured with the Lorentzian function. The curve-fitting method analysis was performed using the PEAKSOLVE software (Ver. 1.05) of the Galactic Industries Corporation (PeakSolve Guide, 1991).

3. Results and discussion

Esters of citrus pectin were prepared by transformation of the carboxyl group into a benzyl or decyl ester via reaction of the tetrabutylammonium pectate (TBAP) with the corresponding benzyl and decyl bromides (Hromadkova et al., 2000). The DE of the obtained pectinates determined by the copper method ranged from 9 to 72% (Table 2).

Typical FT-IR spectra of methylesterified pectin standards and selected benzyl and decyl esters are presented in Figs. 1–3. For DE analysis, the bands at

Table 2
The DE of the pectinates obtained by the chemical method and DRIFTS technique using the curve-fitting deconvolution method

Pectinate			DE (%)		
Sample	Ester	Chemical method[a]	DRIFTS[a]	$F_{\text{experimental}}$[b]	$t_{\text{experimental}}$[b]
G-1	Benzyl	9 ± 1	11 ± 2	4	1.265
G-2	Benzyl	28 ± 1	34 ± 2	4	9.487
G-3	Benzyl	40 ± 1	42 ± 3	9	0.894
G-4	Benzyl	52 ± 1	52 ± 2	4	0.000
G-5	Benzyl	72 ± 3	73 ± 3	1	0.333
G-6	Decyl	15 ± 1	19 ± 2	4	2.530
G-7	Decyl	10 ± 1	13 ± 2	4	1.897
G-8	Decyl	15 ± 1	15 ± 3	9	0.000
G-9	Decyl	33 ± 3	33 ± 3	1	0.000
G-10	Decyl	39 ± 3	41 ± 3	1	0.667

[a] $n = 3$.
[b] Confidence level 95%.

1745–1750 and 1616–1634 cm^{-1} are determinative as they have been assigned to stretching vibrations of the esterified and ionised carboxyl groups of the pectin molecules, respectively (Filippov & Kohn, 1974; Pappas, Tarantilis, & Polissiou, 2003). The use of the second derivative and the deconvolution analysis by the curve-fitting method in this region, revealed the existence of four major peaks for the pectin standards (Fig. 4) and seven for all derivatives shown for sample G-4 in Fig. 5. The above peaks were further analyzed for their exact shape and area with various peak functions such as Gaussian, Voight, a mixture of Gaussian and Lorentzian, log–normal, Pearson VII and Lorentzian. The correlation coefficient was the highest (0.99) in every analysis using the Lorentzian function.

As shown in Table 1, not only acidic carbohydrates but also phenolics and protein have specific absorption bands in the spectral region 1700–1500 cm^{-1}, similarly as pyron-type structures containing double bond and conjugated carbonyl/carboxyl groups (Nakanasy, 1962) and lignin (Abbott, Tjarks, & Bagby, 1987; Sene, McCann, Wilson, & Grinter, 1994). As the pectin standards and the citrus pectate before esterification were free of protein and lignin (based on the absence of the typical stretching vibration of the aromatic ring at ~1505 cm^{-1}), the peak at ~1650 cm^{-1} seen in the spectra of pectin standards (Fig. 1) very probably originated from 4-deoxy-D-hex-4-enopyranuronic acid (HexA) residues which had been identified by NMR spectroscopy in a previous contribution (Malovikova et al., 2000). HexA is known to be formed by β-eliminative degradation of uronic acid-containing carbohydrates (Kiss, 1974). The higher number of peaks in the studied pectin ester samples can be explained by the presence of secondary reaction products introduced from the reaction medium. It is to be mentioned that degradation reactions of the minor neutral carbohydrate pectin components might contribute to formation of unsaturated structures as well. However, in the case of the benzyl esters, the FT-IR spectra contain also bands corresponding to the typical stretching vibrations of the aromatic ring at ~1595 and 1510 cm^{-1} (Fig. 2). The last band is absent in the FT-IR spectra of the decyl esters, as shown for sample G-9 in Fig. 3.

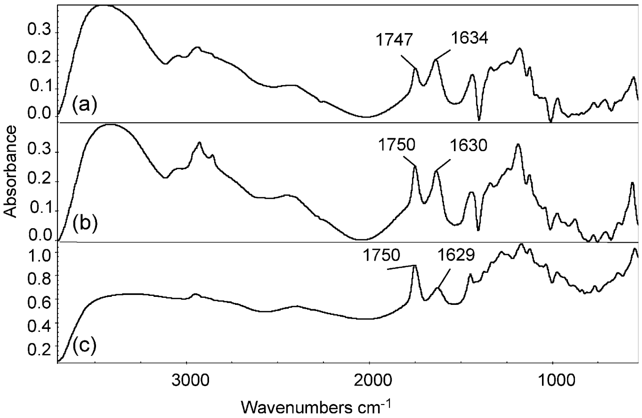

Fig. 1. FT-IR spectra of standard methylesterified pectins (a) DE 31%, (b) DE 50%, and (c) DE 85%.

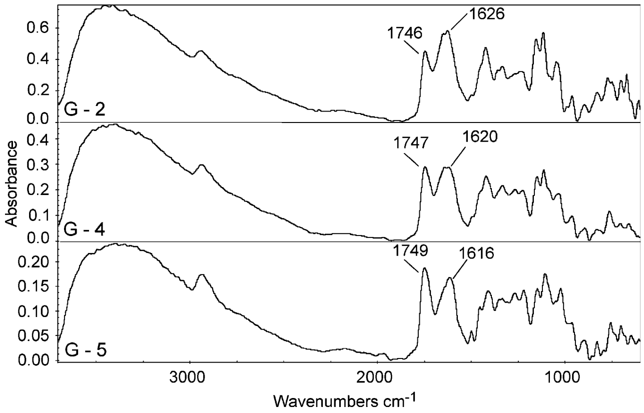

Fig. 2. FT-IR spectra of benzyl pectin esters G-2, G-4, and G-5.

For the spectroscopic DE analysis of the benzyl and decyl pectin esters, the well separated vibration bands at 1608 and 1745 cm^{-1} were used. There was no carbonyl

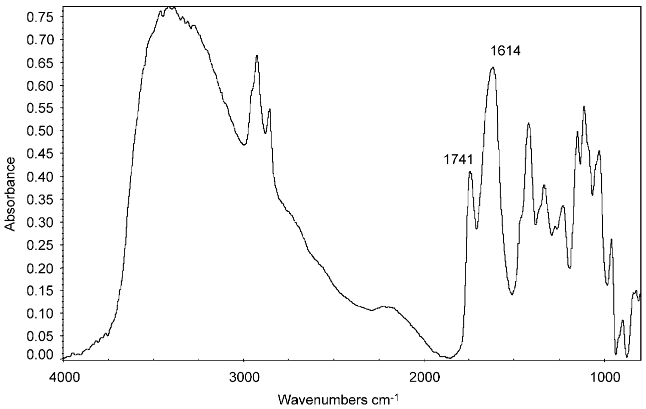

Fig. 3. FT-IR spectrum of decyl pectin ester G-9.

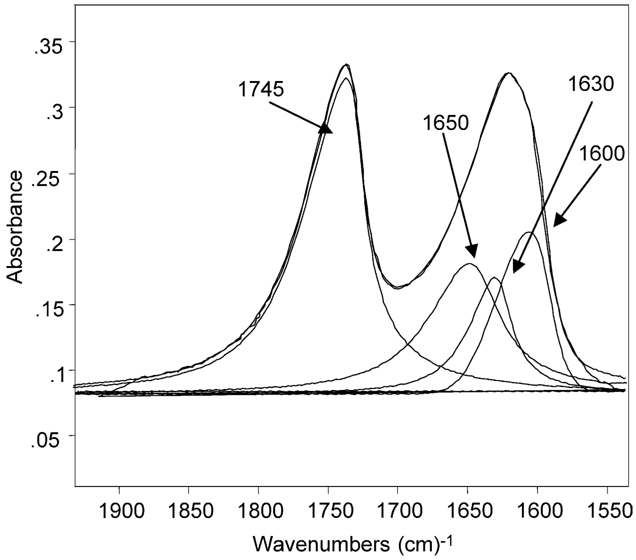

Fig. 4. Deconvoluted spectral region from 1800 to 1500 cm^{-1} of standard pectin sample DE 68%.

absorption band of the protonised carboxyl at 1730 cm^{-1}, which might interfere with the ester group vibration, indicating the absence of this form in the pectin esters.

A calibration curve was established for the series of pectin standards as the function of DE in dependence on peak area ratio (A) between the band at 1745 cm^{-1} and the sum of the bands at 1745 and 1608 cm^{-1} after deconvolution analysis (Chatjigakis et al., 1998; Pappas et al., 2003). The linear dependence obtained from the correlation data is shown in Fig. 6 and expressed by the equation:

$$\mathrm{DE}\,(\%) = (0 \pm 3) + (89 \pm 5)A; \qquad r = 0.98, \qquad n = 3.$$

On the base of this calibration curve and the above equation, the DE of each ester sample was calculated. Using the curve-fitting deconvolution method data, the DE of the pectinates were found to range from 11 ± 2 to 73 ± 3% (Table 2).

The proposed method of DE determination was compared with the chemical method in view of repeatability and accuracy using F-test and t-test, respectively, for level of confidence 95%. The experimental values of the F-test were between 1 and 4 while the corresponding theoretical value is 19. Consequently, the two methods are equivalent as for their repeatability. The experimental values of the t-test ranged between 0.000 and 2.530 (except in the case of G-2 which was 9.487) while the corresponding theoretical value is 2.776. This means that the two methods are equally in view of accuracy.

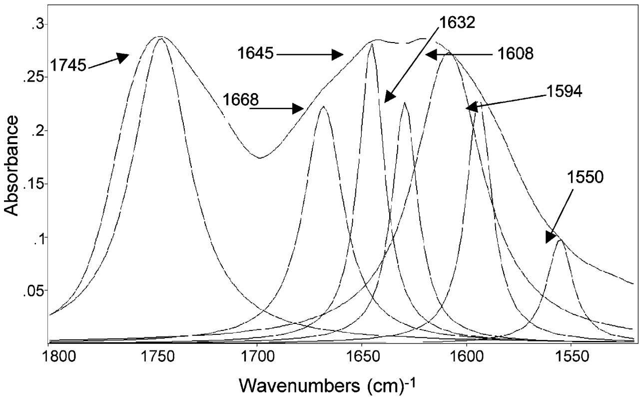

Fig. 5. Deconvoluted spectral region from 1800 to 1500 cm^{-1} of sample G-4.

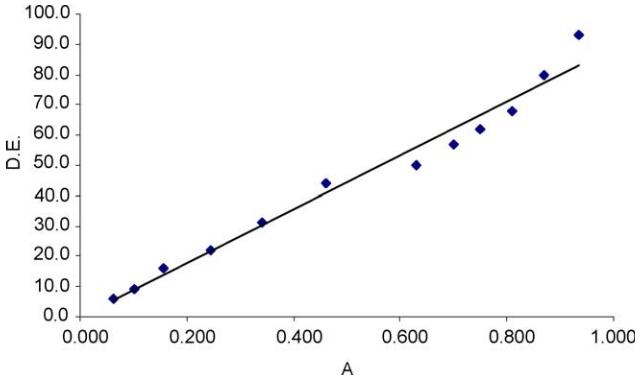

Fig. 6. Calibration curve based on the deconvolution analysis data of the FT-IR spectra of standard pectins. A = Area (1745 cm^{-1})/Area (1608 cm^{-1} + 1745 cm^{-1}).

4. Conclusions

The DE of pectinates prepared from citrus pectin by introduction of benzyl and decyl ester groups was determined using the chemical copper method and DRIFTS technique coupled with the curve-fitting deconvolution method. The results indicated that the proposed method showed the same repeatability and accuracy when compared to the equivalent chemical method. However, a significant advantage of the proposed method is its higher efficiency in comparison to other published FT-IR methods because it allows to separate the absorption bands corresponding to free and esterified carboxyl groups for DE determination from interfering vibrations of contaminating components of pectin. It can be, therefore, applied on mixtures containing pectin esters as well as on non-purified samples. In comparison to chemical methods, it is by far more rapid and, moreover, non-destructive.

Acknowledgements

This work was financially supported by the Hellenic Ministry of Development, General Secretariat of Research and Technology (program code number 2013555) as well as by the Slovak Grant Agency VEGA (project No 2/3162/23) and the Agency for Support of Science and Technique, Slovakia (project APVT-51-015802).

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