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Determination of pectin degree of esterification by diffuse reflectance Fourier transform infrared spectroscopy

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Abstract

A diffuse reflectance Fourier transform infrared spectroscopy (DRIFTS) method was developed to measure the degree of esterification (DE) of commercial pectin samples. The relationship between infrared spectroscopy data and titrimetrically determined DE values was investigated. The ester carbonyl band area (C=O) occurring at a mean frequency of 1756 cm^{-1} had the highest correlation ($R^2 = 0.822$) with the mean DE of the bands observed. The DE values of pectin samples calculated from the line fit equation were comparable to those obtained from the titrimetric method. Mean DE values obtained were within 3.23% for DE 71.6, 2.9% for DE 62, and less than 1% for DE 55.3 of values obtained by titrimetric method. Spectral variations due to sample source have to be considered in developing prediction equations using FTIR. DRIFTS can be a rapid, alternative method to titrimetric analysis of pectin DE.

Keywords: Pectins; DE; FTIR

1. Introduction

Pectin is a polymer of D-galacturonic acid. The galacturonic acid molecule has a carboxyl group on C5, some of which are esterified to form methyl esters. Most of the functional uses of pectins are directly or indirectly related to the extent to which the carboxyl groups are esterified. Measurement of the degree of esterification (DE) is a routine analytical procedure in pectin analysis. DE may be reported as the percent of the total number of carboxyl groups esterified or as the percent of methoxyl content of total pectin (Walter, 1991).

Several methods are reported in literature for the analysis of DE of pectins. A titrimetric method proposed by Food Chemical Codex (FCC, 1981) is a commonly used procedure for DE determinations. This procedure involves titration of a pectin suspension with sodium hydroxide before and after saponification of the suspended pectin. The first endpoint indicates the unesterified carboxyl groups and the second endpoint, volume following saponification, shows the total number of carboxyl groups. The difference between the two readings indicates the percent of esterified carboxyl groups.

An alternative method to measure DE is to de-esterify the pectins and estimate methanol as an indicator of DE. Wood and Siddiqui (1971) used a colorimetric procedure for the analysis of methanol content of pectin esters that is extensively reported in the literature. An enzymatic procedure using alcohol oxidase has also been proposed by Klovans and Bennett (1986). Another method using gas chromatography to quantify methanol after pectin deesterification is credited to Walter, Sherman and Lee (1983). The ability of pectins to reduce copper has also been used to quantify methoxyl content of pectins (Keijbets & Pilnik, 1974). However, starch interfered with the analysis. Titrimetric procedures and methanol quantitation after pectin deesterification are time consuming techniques and require much technical expertise. Hence, rapid methods for pectin DE determination for research and for use in quality assurance are necessary.

Infrared spectroscopy has been proposed as a useful technique for quantitative determination of pectin uronic acids (Bociek & Welti, 1975; Casu, Scovenna,

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Cifonelli, & Perlin, 1978). Sampling for dispersive infrared spectroscopy (DIR) involved dispersion of samples in D_2O. The D_2O makes sampling cumbersome and may absorb in IR regions that interfere with sample analysis, resulting in underestimation of uronic acids (Casu et al.). Therefore, using solid powdered samples without D_2O may be more convenient; however, quantitative DIR analysis using solid samples has a limitation because of the scattering effects and band variations due to differing degrees of intermolecular order. This can be, to some extent, reduced by using powdered samples in the form of mulls and pellets in a medium of high refractive index such as Nujol or KBr. However, these agents make sampling inconvenient and interfere with the estimation of certain groups such as aliphatic C–H and C–C vibrations as in the case of Nujol (Kincaid, 1986). With the advent of Fourier transform infrared spectroscopy (FTIR), the diffuse reflectance technique has become a useful means for sample analysis in mid-infrared region. The sample response at all wavelengths is measured at once, thus making the technique very rapid with a highly improved signal to noise ratio relative to DIR (Wehling, 1994). Furthermore, diffuse reflectance Fourier transform infrared spectroscopy (DRIFTS) can also be used with direct solid samples without involving laborious sample preparation procedures as required in the case of D_2O-DIR. DRIFTS can be used for identification of a sample by examining and matching the spectra with that of a known standard.

The objective of this study was to develop a DRIFTS method for rapid determination of degree of esterification of commercial pectins by collecting FTIR spectra of commercial pectin samples of known DE and correlating the spectral features of pectins with their DE values to develop line fit equations for calculation of DE from the FTIR spectra. The equations were verified by calculating the DE values of test pectin samples using the line fit equation and comparing them with DE values obtained by titrimetric determination.

2. Materials and methods

2.1. Pectin samples

The pectin samples used in the FTIR analysis for developing a linear regression equation included pectins of DE 28.5%, and DE 93.0% (Sigma Chemical Co. St. Louis, MO) and DE 55.3, 69.5, and 74.5%, (Danisco Ingredients USA Inc., New Century, KS). Test pectin samples for DE calculation from the linear fit equations using FTIR spectra were supplied by Danisco Ingredients (Grindsted pectins, Danisco Ingredients USA Inc., New Century, KS).

2.2. FTIR analysis

Pectin samples of DE 93.0, 74.5, 69.5, 55.3, and 28.5% were placed in a macro sampling cup accessory for diffuse reflectance analysis (Baseline Diffuse Reflectance kit, Model 0002-495, SpectraTech, Inc., Shelton, CT). The samples were pressed to ensure an even surface. Diffuse reflectance FTIR spectra of pectin samples were obtained using a Nicolet Model 410 FTIR instrument (Nicolet Analytical Instruments, Madison, WI). The spectral values of the samples were means obtained by co-adding 100 scans at a resolution of 4 cm^{-1}. FTIR spectral parameters including band frequency, band intensity, and band area of the samples were obtained using a software package (OMNIC FT-IR Software, v 4.1, Nicolet Analytical Instruments, Madison, WI). Ten replications of the FTIR analysis were performed. Each replication included sampling, acquisition of the spectra, and measurement of FTIR spectral parameters including band frequency, band intensity, and band area. The data were analyzed using JMP IN statistical software (JMP IN version 3.2.1, SAS Institute Inc). Analysis of variance was conducted to observe the mean and standard deviation of spectral band frequency, band intensity, and band area (JMP IN, SAS, 1997).

2.3. Determination of DE by titrimetric method

DE of the commercial samples was determined by the titrimetric method of Food Chemical Codex (FCC, 1981). Linear regresison (line fit) analysis was conducted to obtain the relationship (R^2) between mean titrimetric DE values and FTIR spectral band frequency, intensity, and band area.

2.4. Determination of DE values of test pectin samples

Test pectin samples were obtained from Danisco Ingredients (Grindsted pectins, Danisco Ingredients USA Inc., New Century, KS). The DE values of these pectins were calculated from the line fit equations developed from FTIR analysis, and the values were compared with those obtained by the titrimetric method. Four replications of this experiment were performed, and the data were analyzed to obtain mean DE values and standard deviation for each method of analysis.

3. Results and discussion

3.1. FTIR spectra of commercial pectin samples

The FTIR spectra of pectin samples of nominal DE 28.5, 55.3, 69.5, 74.5, and 93.0% are presented in Fig. 1. The frequencies and nature of the bands of surface

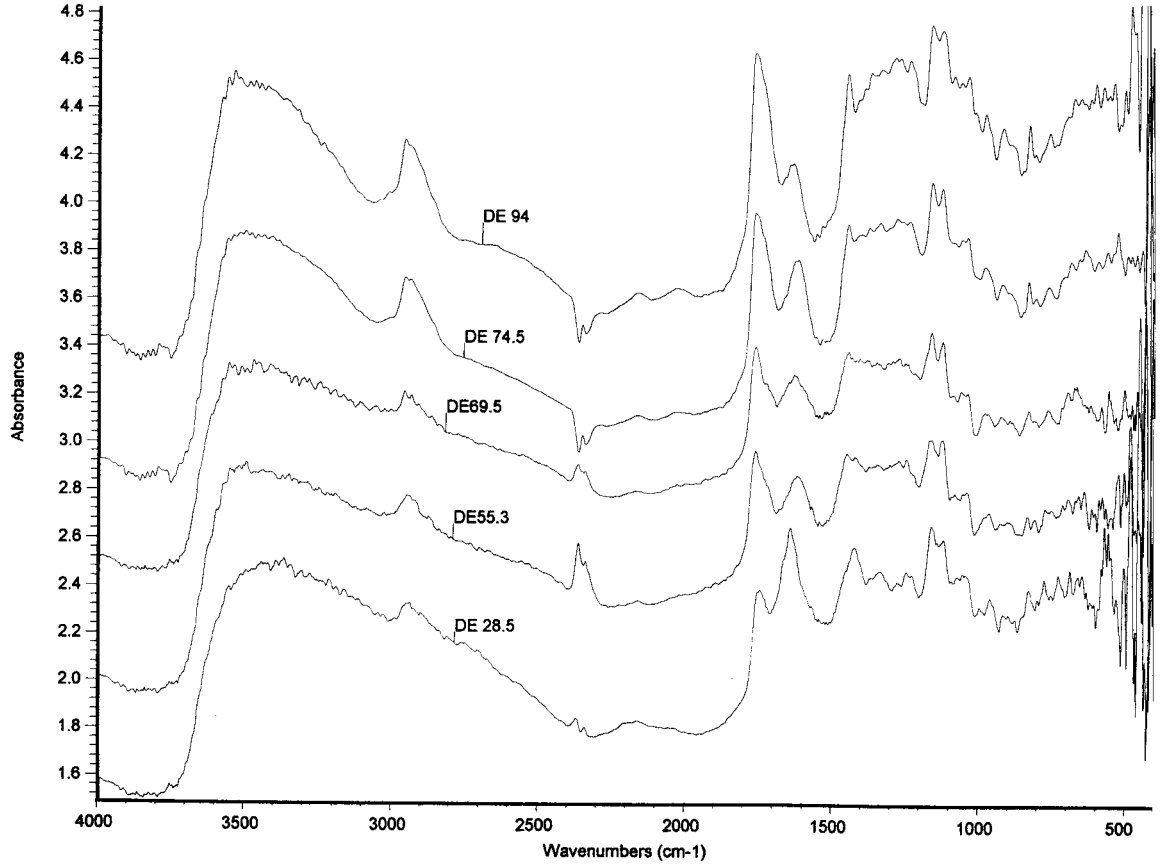

Fig. 1. Diffuse reflectance Fourier transform infrared spectra of commercial pectin samples: 1, 2, 3, 4, and 5 are pectin samples of degree of esterification 93.0, 74.5, 69.5, 55.3, and 28.5%, respectively.

functional groups of pectins are presented in Table 1. The broad, strong area of absorption between 3600 and 2500 cm^{-1} refers to O–H stretching absorption due to inter- and intramolecular hydrogen bonds. The O–H stretching vibrations occur within a broad range of frequencies and indicate several features of a compound, including "free" hydroxyl groups stretching bands which occurs in samples in vapor phase and bonded O–H bands of carboxylic acid (Silverstein, Bassler & Morril, 1991). In the case of pectin samples, absorption in the O–H region is due to the inter and intramolecular hydrogen bonding of the galacturonic acid polymer. Finer bands appearing at the longer end of the O–H region indicate overtones and combination of tones.

Bands around 2950 cm^{-1} (3000–2800 cm^{-1}) refer to C–H absorption. These include CH, CH_2, and CH_3 stretching and bending vibrations. Typically, two moderately intense bands are observed in the C–H region of aliphatic compounds. In pectin samples, the C–H stretching and bending vibrations are seen, usually, as a band superimposed upon the broader O–H band that ranges from 2500 to 3600 cm^{-1}. This was observed with

Table 1
Frequencies and intensities of functional groups present on commercial pectin samples analyzed by Diffuse reflectance Fourier transform infrared spectroscopy

Frequency (wave number, cm^{-1})	Functional groups	Intensity
3600–2500	O–H stretching	Broad, strong
3000–2800	C–H stretching, symmetric, asymmetric	Sharp, occasionally double overlapping with O–H
1760–1745	C=O, esterified	Strong
1640–1620	COO− asymmetric stretching	Strong
1400	COO− symmetric stretching	Weak
1380	C–H bending	Weak
1300–1000	C=O Stretching	Weak

all pectin samples studied. In the case of esterified pectins, an O–CH_3 stretching band would be expected between 2950 and 2750 cm^{-1} due to methyl esters of galacturonic acid. However, due to a large O–H stretching response occurring in a broad region (3600–2500 cm^{-1}), the O–CH_3 activity is masked and not a reliable indicator of methoxylation. Stronger bands occurring between 1760–1745 cm^{-1}, and between 1640 and 1620 cm^{-1} indicate the ester carbonyl (C=O) groups and carboxylate ion stretching band (COO–), respectively. It was observed that the ester carbonyl groups increased in their intensity and band area as the DE increased, while the intensity of the carboxylate stretching band decreased (Fig. 1). Since O–CH_3 stretching bands are not useful for quantitative pectin analysis, the bands representing ester carbonyl (1760–1745 cm^{-1}) and free carboxylate groups (1640–1620 cm^{-1}) would be important in the identification and quantitation of pectin samples. Carboxylate groups show two bands, an asymmetrical stretching band near 1650–1550 cm^{-1}, and a weaker symmetric stretching band near 1400 cm^{-1}. In pectin samples, the weaker symmetric COO– stretching is followed by moderately intense absorption patterns between 1300 and 800 cm^{-1}, collectively referred to as the ''finger print'' region that is unique to a compound. These bands are usually difficult to interpret. Other bands of lesser importance in pectin samples are C–H bending, occurring at 1380 cm^{-1}, and C–O stretching occurring at 1300–1000 cm^{-1}.

3.2. Relationship between FTIR response and titrimetric DE values

The frequency, intensity, and band area of ester C=O stretching, and carboxyl ion (COO–) stretching vibrations and their correlation with titrimetric DE values (R^2) are presented in Table 2. The mean frequency of C=O was 1756 cm^{-1}, which is close to the range for ester carbonyls reported in the literature (1750–1730 cm^{-1}) (Silverstein et al., 1991). The highest R^2 values were observed for the mean band area of the ester carbonyl (C = O) group. Although a decrease in the COO– band area was observed with increased DE, their correlation with titrimetric DE values ($R^2 = -0.643$) was not as strong as that of the carbonyl C=O band. This is because COO– stretching occurs at two different spectral regions (an asymmetrical stretching band near 1650–1550 cm^{-1}, and weaker symmetric stretching bands near 1400 cm^{-1}), as opposed to ester carbonyl (C=O) which occurs as a single major band around 1750 cm^{-1}. Hence, the band occurring at 1620 cm^{-1} in pectin samples does not quantitatively represent the free carboxyl groups (COO–) that participate in methyl esterification.

Table 2
Mean values for FTIR band frequency, intensity, and band area of surface functional groups of commercial pectin samples and their corresponding correlation coefficient values with titrimetric DE values[a]

Functional groups	FTIR parameter	R^2 values[b]
C=O	Mean frequency, 1756 cm^{-1}	0.610
	Mean band height, 2.71	0.444
	Mean band area, 25.82	0.822
COO–	Mean frequency, 1630 cm^{-1}	0.001
	Mean band height, 2.62	0.400
	Mean band area, 25.82	0.643

[a] Number of replications = 10.

[b] R^2 values are calculated based on the linear fit between DE values obtained by titrimetric method and FTIR parameters. The DE values of pectin samples were 28.5, 55.2, 69.5, 74.5, and 93.0%.

3.3. Relationship between titrimetric DE values and FTIR carbonyl band area

Mean values and standard deviations of ester carbonyl band area of pectins of varying DE are presented in Table 3. A linear increase in carbonyl ester area was observed as the DE of the samples increased. It was also observed that the standard deviation values were smaller for lower DE samples. A students *t*-test of the mean values performed indicated significant differences in the mean comparisons (Student '*t*' = 2.014). The linear relationship between titrimetric DE values and ester carbonyl area revealed a high positive correlation (0.822) (Fig. 2), and the linear fit was represented by the equation:

$$\text{Ester carbonyl area} = -4.0013 + 0.46472\text{DE}$$

3.4. DE values of test pectin samples from FTIR carbonyl ester area

The line fit equation obtained from Fig. 2 was used to calculate the DE of test pectin samples and was

Table 3
Mean values of FTIR ester carbonyl (C=O) peak area of commercial pectins of varying degrees of esterification (DE)[a]

DE	Ester carbonyl band area[b]
28.5	11.91a
55.3	19.75b
69.5	24.36c
74.5	30.30d
93.0	42.76e

[a] FTIR analysis: Spectra from co-adding 100 scans at a resolution of 4 cm^{-1} (diffuse reflectance FTIR from Nicolet Model 410 FTIR instrument, Nicolet Analytical Instruments, Madison, WI).

[b] Mean comparisons for the ester carbonyl band areas using Student's *t* test, $t = 2.014$. Mean values with different superscripts are significantly different, $t = 2.014$.

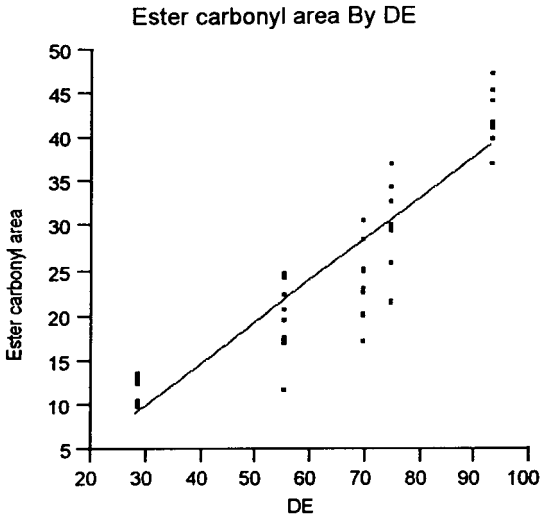

Fig. 2. Linear fit for carbonyl ester band area and degree of esterification calculated by titrimetric method: $R^2 = 0.822$; number of observations = 10.

compared to that obtained by the titrimetric method. Mean DE values and standard deviation of test pectin samples determined by the titrimetric method as well as from the FTIR ester carbonyl area are presented in Table 4. Mean values obtained from FTIR data were comparable to those of the titrimetric method. Of the pectin samples tested, the mean values of samples with lower DE (55.28 vs. 55.3) were closer compared to the two methods than the samples with higher DE values (66.34 vs. 62.00, and 67.09 vs. 71.6). A higher spread (standard deviation) of ester carbonyl band areas of higher DE samples might explain this difference in precision. The standard deviation values for the FTIR ester carbonyl method were smaller compared to those obtained for the titrimetric method. The mean values obtained from the FTIR ester carbonyl method were within 3.23% for DE 71.6, 2.9% for DE 62, and less than 1% for DE 55.3, of values obtained by the titrimetric method.

Table 4
Mean values, standard deviation (SD) and coefficient of variation (CV) values of degree of esterification (DE) of test pectin samples calculated by titrimetric method and from the FTIR ester carbonyl band area using linear fit equation[a]

Sample DE by titrimetric method		Sample DE from FTIR data[a]
	55.3	55.28
SD	1.63	3.25
CV	2.95	5.88
	62.0	66.34
SD	1.63	3.48
CV	7.44	5.25
	71.6	67.09
SD	6.72	4.82
CV	9.39	7.19

[a] Linear fit equation used in the determination of sample DE from FTIR data: ester carbonyl area = −4.0013 + 0.46472DE.

To summarize, pectin samples used in this study were obtained from different sources (DE 28.5 and DE 93.0 from Sigma Chemical Co., and DE 55.3, DE 69.5, and DE 74.5 from Danisco USA, Inc). Differences in sample source/origin and processing variation might account for part of the variation in DE analysis. This is because even a small difference in the structure and constitution of a molecule results in significant changes in the absorption peaks. Differences in the type and content of pectin side chain constituents, due to differences in sample source or processing (extent of amidation), will affect absorption of certain group frequencies. Hence, samples of different DE from the same source/origin can be expected to have lower FTIR spectral variations and may have an improved linear fit, thus lowering the standard deviation for means calculated from a linear fit equation. Changes in the sample source might result in minor variations in spectral response, requiring the use of appropriate linear fit developed from standards of known DE. This might render the procedure prone to batch to batch variation. A linear relationship between ester carbonyl area and the degree of esterification exists independent of the ester moieties (viz. methyl, acetyl), total galacturonic acid content, or the content of free carboxyl groups, thus making DRIFTS a useful, rapid, alternative method for DE analysis of pectins within a large batch of samples.

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