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Food Chemistry 74 (2001) 233–238

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical nutritional and clinical methods section

Determination of polygalacturonic acid content in pectin extracts by diffuse reflectance Fourier transform infrared spectroscopy

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Received 23 August 2000; received in revised form 6 December 2000; accepted 6 December 2000

Abstract

A simple, rapid diffuse reflectance Fourier transform infrared spectroscopy (DRIFTS) method was developed for pectin measurement. Pectin calibration standards were prepared by blending polygalacturonic acid with potassium bromide to cover a range of polygalacturonic acid concentrations (10–98%). A linear relationship between pectin content and total carbonyl absorption band area was found ($R^2 = 0.982$). Linearity was up to 80% pectin, since there was no significant difference in peak areas of 80% and greater. Pectin contents of various commercial pectin samples were calculated from the linear fit equation. Accuracy of the DRIFTS method was determined by comparing the pectin contents to the values obtained by colorimetric and HPLC methods. For four different sources of samples, the pectin contents by FTIR method were 78.2, 89.4, 65.9 and 67.1%, which were comparable to values obtained by colorimetric analysis (79.1, 87.9, 64.7 and 57.7%) and HPLC (84.1, 91.4, 69.5 and 67.9%).

Keywords: Pectin; Method; DRIFTS; FTIR; Polygalacturonic acid

1. Introduction

Pectin is a linear polysaccharide consisting of a few hundred to one thousand saccharide units. The average molecular weight of pectins varies from 50,000 to 150,000 (Whistler & BeMiller, 1997). Pectin consists of D-galacturonic acid units with very small quantities of neutral sugars. The monomers are linked together by α 1-4 glycosidic linkages (Thakur, Singh, & Handa, 1997). The polygalacturonic acid is partly esterified with methyl groups and the free acid groups may be partly or fully neutralized with sodium, potassium, or ammonium ions. The textures of fruits and vegetables, and hence processing characteristics, are largely influenced by the pectin content. Many food processors and pectin ingredient suppliers need to determine pectin content to control the quality of their products.

Several chemical and instrumental methods are available for determination of pectin content in food products. Some of the methods, measuring pectin as galacturonic acid content, are quite sensitive, accurate, reproducible, rapid, and well-correlated to pectin content in many samples. Most of them require pectin extraction and hydrolysis prior to analysis (Carunchio, Girelli, Sinibali, & Tarola, 1988; Kintner & Van Buren, 1982; McComb & McCready, 1952). More recently, high-performance liquid chromatographic (HPLC) methods have been developed (Vazquez-Blanco, Vazquez-Oderiz, Lopez-Hernandez, Simal-Lozano, & Romero-Rodriguez, 1993) and replaced colorimetric methods (Dietz & Rouse, 1953; McComb & McCready, 1952). HPLC is faster, and more selective relative to colorimetric methods. Colorimetric methods are also sensitive to the presence of other uronic acids and sugars, which interfere with the galacturonic acid quantitation (Forni, Polesello, & Braga, 1987).

Infrared spectroscopy has been recognized as a powerful analytical technique in the food industry for many years (Van de Voort, 1992) and has been used to quantitate pectin uronic acid (Bociek & Welti, 1975). In this method, samples were dispersed in D_2O and measurements were made in IR transmission mode. However,

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0308-8146/01/$ - see front matter
PII: S0308-8146(01)00100-5

interference due to D_2O absorption in the IR region could result in underestimation of uronic acids (Casu, Scovenna, Cifonelli, & Perlin, 1978). Coimbra, Barros, Rutledge, and Delgadillo (1999) and Coimbra, Barros, Barros, Rutledge, and Delgadillo (1998) used multivariate analysis to determine uronic acid in cell-wall polysaccharide extracts.

Fourier transform diffuse reflectance techniques, using the mid infrared region, are useful for analysis of various food products. The mid-infrared adsorption (4000–400 cm^{-1}) is an extremely useful tool as it involves the fundamental adsorption of chemical groups. It provides qualitative information on functional groups and may be used to identify and quantify specific compounds. By spectra subtraction, components of mixtures have been identified, and quantitative analysis performed to determine free fatty acid (FFA) content in palm olein, based on the absorption of FFA at 1728–1662 cm^{-1} (Man & Setiowaty, 1999). Lanser, List, Holloway, and Mounts (1991) estimated FFA in mixtures of oleic acid and soybean oil samples by using the absorption band between 2000 and 1600 cm^{-1}. All these applications involved measurements of absorption in the transmission mode.

Diffuse reflectance Fourier transform infrared spectroscopy (DRIFTS), which involves measurements in reflection mode, can also be used with solid samples without any sampling preparation. Gnanasambandam and Proctor (2000) used DRIFTS to determine the degree of pectin esterification. They found that absorbance of the ester carbonyl (COO-R) groups increased with increase in degree of esterification and the band area was linearly related to degree of esterification. Bands occurring between 1760–1745 cm^{-1} and between 1640 and 1620 cm^{-1} indicate the ester carbonyl groups and carboxylic ion (COO-), respectively (Gnanasambandam & Proctor, 1999).

The objective of the current investigation was to develop a rapid DRIFTS method for determination of pectin content as polygalacturonic acid.

2. Materials and methods

2.1. Pectin standard curve by DRIFTS

A set of 10 calibration pectin standards was prepared by blending polygalacturonic acid (98% polygalacturonic acid, No. P-3889, Sigma Chemical Co., St. Louis, MO) with potassium bromide (KBr) to obtain pectin standards with polygalacturonic acid content of 10, 20, 30, 40, 50, 60, 70, 80 and 98%, respectively (W/W). DRIFTS spectra of pectin standards were obtained using a Nicolet Model 410 FTIR instrument (Nicolet Analytical Instruments, Madison, WI). A baseline diffuse reflectance kit (containing slide-mounted optical accessory and macro sampling cups; Spectra-tech, Shelton, CT) was used to obtain DRIFT spectra. Spectra were collected by co-adding 100 scans at a resolution of 4 cm^{-1}. A background spectrum was recorded using a blank disk (for correcting absorption due to CO_2 and moisture in air) before each standard spectra was collected. Total carbonyl absorption peak areas at 1650 and 1750 cm^{-1} from free (COO-) and esterified (COO-R) carboxyl groups, were obtained using a software package (OMNIC FTIR Software, v4.1, Nicolet Analytical Instruments, Madison, WI). Peak area was measured as area above the baseline between 1840 and 1550 cm^{-1}. The area under the curve was used to calculate total carbonyl peak area. Five replications of each standard were obtained and used to develop a calibration curve for pectin content as polygalacturonic acid.

2.2. Pectin analysis

Two citrus pectin samples (Sigma Chemical Co., St. Louis, MO, lot No. 96H0580, and 30F0108, respectively), commercial food grade pectin (Danisco Ingredients Inc., USA), and soy hull pectin prepared by the methods of Gnanasambandam and Proctor (1999) were used to calculate polygalacturonic acid content by the following analyses.

2.2.1. Determination of pectin content by DRIFTS methods

DRIFTS spectra and total carbonyl absorption peak area at 1650 and 1750 cm^{-1}, from free (COO-) and esterified (COO-R) carboxyl groups of pectin samples, were obtained using the method described earlier for pectin standards. The polygalacturonic acid contents of these samples were calculated from the line fit equation developed from DRIFTS analysis and the values were compared with those obtained by the colorimetric and HPLC methods.

2.2.2. Determination of pectin by colorimetric method

Pectin content of the samples was determined colorimetrically using *m*-hydroxydiphenyl (Kintner & Van Buren, 1982).

2.2.3. Determination of pectin as galacturonic acid by HPLC

The galacturonic acid content of the test samples was determined using a HPLC method (Vazquez-Blanco et al, 1993). Thirty milligrams of pectin were dispersed in 12 ml of 0.1 N NaOH and stirred 2 h for complete saponification. The solution was then acidified with 0.1 N HCl to pH 4.2. Enzymatic hydrolysis was carried out by incubating the pectin solution with 150 mg of pectinase from *Rhizopus* sp. (EC 3.2.1.15, Sigma Chemical Co., St. Louis, MO) and 25 mg of cellulase from *Trichoderma viride* (EC 3.2.1.4, Sigma) at 55°C for 20 h. The

solution was filtered through a Whatman 41 paper. One milliliter of solution was refiltered through a 0.45-μm membrane (Whatman) prior to chromatography.

A Spectra System AS 1000, HPLC (Spectra Physics, Fremont, CA) equipped with a spherisorb ODS2 250×4.6 mm C18 column (particle size, 5 μm), a 15×3.2 mm guard column (Waters Corporation, Milford, MA), and a forward optical scanning detector (Spectra Focus, Spectra Physics, Fremont, CA) was used to determine galacturonic acid content in samples. D-Galacturonic acid monohydrate (Sigma) was used as standard. An autosampler (Spectra Physics AS 1000) was used to inject 20 μl of the sample. The mobile phase was HPLC-grade water, acidified with sulfuric acid to pH 2.2. Flow rate was 0.6 ml/min, and the detection wavelength was 200 nm.

2.3. Statistical analysis

A linear regression model was developed with one independent (X) and one response variable (Y), and described as $Y = \beta_0 + \beta_1 X + \epsilon$, where $Y =$ FTIR carbonyl peak area, $X =$ concentration of pectin standards, and ϵ is random error term. Results of five replicates were used to calculate correlation coefficient (R^2) and regression line. Student's 't' test was used to analyze data. Least significance difference (LSD) values were used to differentiate mean values, and significance, as defined at $P < 0.05$ (SAS Institute, 1994).

3. Results and discussion

3.1. Pectin standard curve for DRIFTS

The diffuse reflectance FTIR spectra of pectin standards are presented in Fig. 1. According to Whiffen (1957), the 1000–1250 cm^{-1} interval is not very useful for carbohydrates and their derivatives. However, bands in the 1000–2000 region are independent of pectin source and may be used to identify galacturonic acid (Filippov & Shamshurina, 1972; Wellner, Kacurakova, Malovikova, Wilson, & Belton, 1998). The broad and strong area of absorption between 3600 and 2400 cm^{-1} was from O–H stretching due to adsorbed moisture in the pectin samples. Bands around 3000–2800 cm^{-1} refer to C–H absorption and due to stretching vibration of methyl (CH_3) group of the methyl ester. The absorption band between 1100 and 1200 cm^{-1} was from ether (R–O–R) and C–C bond in the ring structure of pectin molecules. The carbonyl absorption bands shown at 1650 and 1750 cm^{-1} were from free (COO-) and esterified (COO-R) carboxyl groups, respectively. It was observed that the total carbonyl absorption band area increased as the polygalacturonic acid content increased (Fig. 1). The mean values and standard deviations of carbonyl absorption band area of pectins with varying polygalacturonic acid contents are presented in Table 1. A linear increase in carbonyl absorption band area was

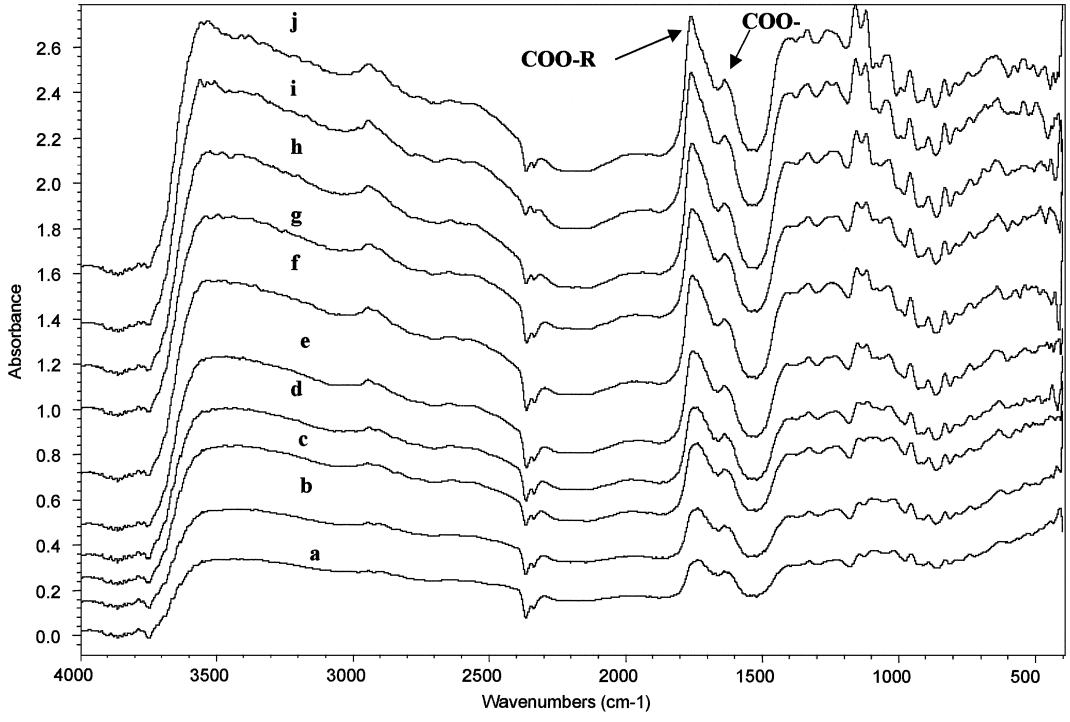

Fig. 1. FTIR spectra of the 4000–400 cm^{-1} region of polygalacturonic acid diluted with KBr to (a) 10, (b) 20, (c) 30, (d) 40, (e) 50, (f) 60, (g) 70, (h) 80, (I) 90, and(j) 98%.

observed as the pectin content, expressed as polygalacturonic acid, increased (Fig. 1). The linear relationship between polygalacturonic acid content of pectin and carbonyl absorption band area revealed a high correlation ($R^2 = 0.982$) (Fig. 2). The fitted model was represented by the equation: $Y = 18.80 + 0.69X$, where Y is FTIR peak area and X is pectin content. A higher correlation coefficient ($R^2 = 0.994$) was observed between pectin content and peak area when the upper limit of pectin content was 90%, and even higher ($R^2 = 0.998$) for 80% pectin. There were no significant differences observed between peak areas for pectin contents of 80, 90, and 98% but all other pectin concentrations were significantly different from each other (Table 1). This may be due to the limitation of the instrument at higher absorbance, which results in deviation from Beers's law.

Table 1
Mean values, and standard deviations (S.D.) of DRIFTS carbonyl peak area of pectin standards of varying pectin content

Pectin content (%)	FTIR carbonyl peak area[a]
10	23.40a (0.51)
20	30.06b (0.63)
30	40.57c (1.12)
40	47.28d (0.72)
50	55.09e (0.25)
60	62.63f (0.76)
70	68.99g (1.65)
80	77.20h (1.72)
90	80.43h (0.63)
98	80.71h (0.73)

[a] Values with different letters are significantly ($P < 0.05$) different from each other.

3.2. Pectin analysis

The diffuse reflectance FTIR spectra of pectin samples are shown in Fig. 3. The total FTIR peak area of the carbonyl absorption band (COO-R and COO-) was used to calculate the total pectin content of pectin samples from the line fit equation obtained from Fig. 2. Pectin contents obtained by the DRIFTS methods were compared to those obtained by the colorimetric and HPLC methods. Mean pectin contents and standard deviations of test pectin samples, determined by FTIR carbonyl area, colorimetric methods, and HPLC methods, are presented in Table 2. Mean values obtained from FTIR spectra were comparable to the colorimetric and HPLC methods. There were no significant differences in the values obtained from the FTIR and HPLC methods. The standard deviation values for the FTIR

Table 2
Mean values, and standard deviations (S.D.) of pectin content as galacturonic acid of pectin samples by different analytical methods[a]

Methods	Citrus Pectin I (Sigma, Lot No. 96H0580) (%)	Citrus Pectin II (Sigma, Lot No. 30F0108) (%)	Commercial Pectin (Danisco) (%)	Pectin from Soy Hull (%)
FTIR	78.2a (2.03)	89.4a (1.68)	65.9a (0.79)	67.1a (1.46)
Colorimetric	79.1a (1.71)	87.9a (1.49)	64.7a (3.12)	57.7b (5.12)
HPLC	84.1a (3.93)	91.4a (3.19)	69.5a (1.32)	67.9a (1.01)

[a] Values with different letters in each column are significantly ($P < 0.05$) different from each other.

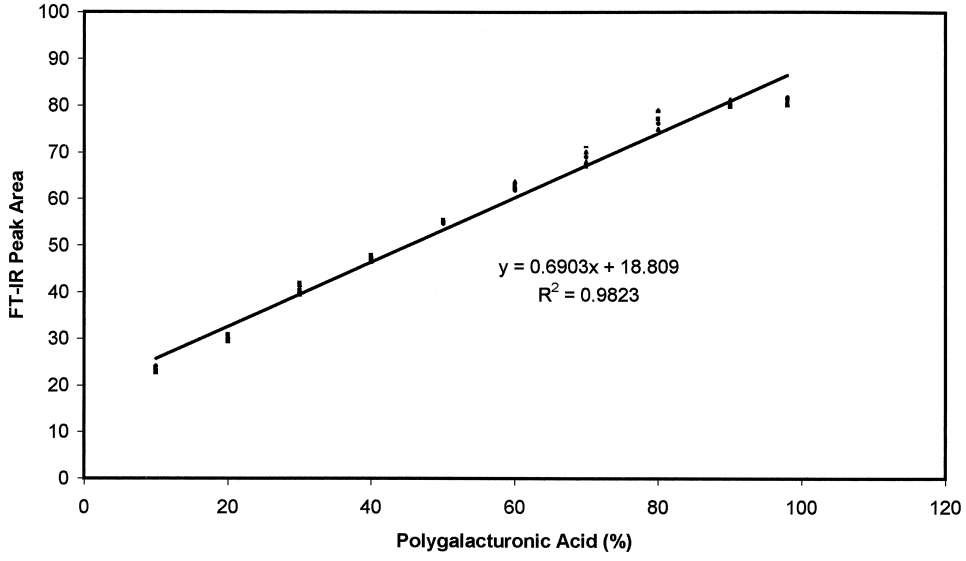

Fig. 2. Regression line of polygalacturonic acid content of standards with DRIFTS procedure. Five observations were made for each standard.

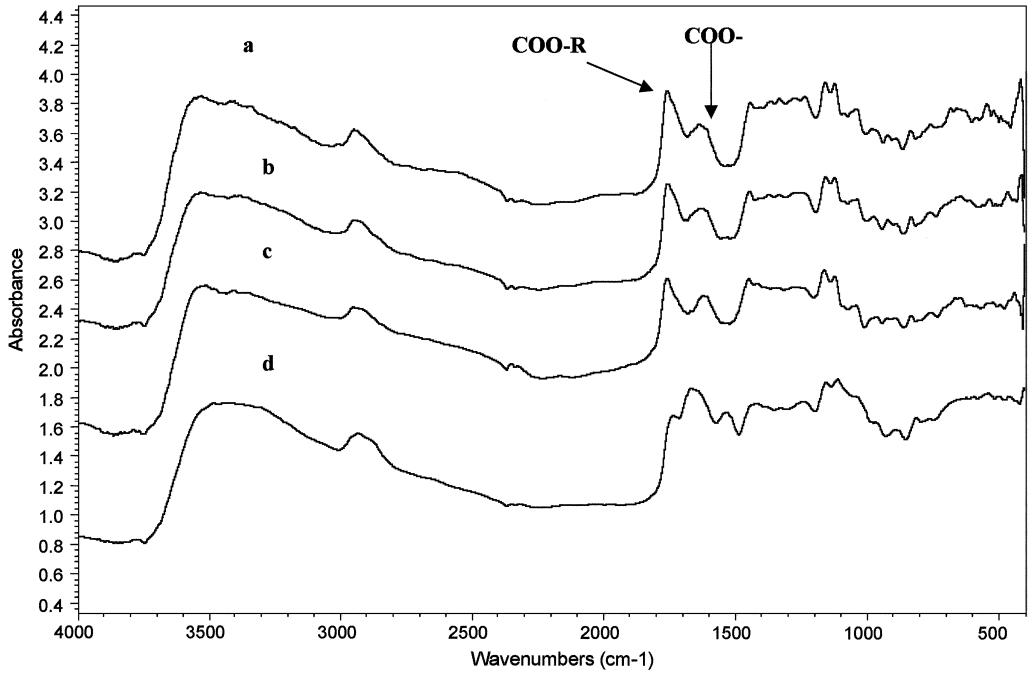

Fig. 3. FTIR spectra of the 4000–400 cm^{-1} region for (a) citrus pectin I (Sigma, Lot No. 96H0580), (b) citrus pectin II (Sigma, Lot No. 30F0108), (c) commercial food grade pectin (Danisco, USA), and (d) soy hull pectin.

method were comparable to those obtained by the colorimetric method and HPLC method. The highest standard deviation value (5.12) was obtained by the colorimetric method. The colorimetric method also showed a significantly lower pectin content (57.7%) for soy hull pectin than the FTIR (67.1%) and HPLC (67.9%) methods. This may be due to the incomplete digestion of pectin materials or the presence of interfering materials in soy hull pectin. Both the analytical grade pectins showed comparable results in all the methods examined.

Differences in sample sources and compositional differences may affect determination. Both the HPLC and the colorimetric method require complete digestion of the pectin materials by acid and enzymes to liberate galacturonic acid for determination. Thus, impurities, or presence of enzyme inhibitors, may interfere with the digestion process, resulting in lower pectin values. However, FTIR is independent of pectin sources and production practices and hence can be used to identify and quantify pectin in variety of samples. The values for slope, intercept, fitted line, and the random scattering in the residual plot (figure not shown), indicate a straight line model. Additionally, regression analysis showed a linear relationship between total carbonyl area and pectin content.

Diffuse reflectance FTIR can be used as an alternative method for determining pectin content in commercial pectin samples and pectin extracts. Advantages of the FTIR method are: it requires no reagents, no sample preparation is required, and it is rapid and cost-effective. Since this is a surface reflectance measurement, for accurate determination the sample should be homogeneous with pectin distributed uniformly. Application of this method to complex food products may require prior pectin extraction steps, as carbonyl groups in proteins and lipids can interfere with pectin determination.

Acknowledgements

This project was funded by the USDA (NRI Competitive Grants Program, Project #1822). Published with the approval of the Director, Arkansas Agricultural Experiment Station, manuscript #00048.

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