

Quantitative Analysis of Benzyl Modification in Waxy Maize Starch by Fourier Transform (FT) Raman Spectroscopy

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ABSTRACT

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Waxy maize starch was chemically modified to varying benzylation levels and the degree of benzylation substitution was measured using a nuclear magnetic resonance (NMR) method. Fourier Transform (FT) Raman spectra of the chemically modified starches were acquired and aromatic C=C stretch Raman bands characteristic of the benzylation modification were used to derive a calibration curve for the Raman intensity of these

marker bands versus the degree of benzylation substitution. The best-fit linear regression to the plotted data gave a linear correlation coefficient of 0.997. The FT-Raman technique provides a fast, nondestructive method for the measurement of the degree of benzylation substitution of modified waxy maize starches and should be applicable for use with benzylation starches from other botanical sources.

Starch and chemically modified starches are widely used in food processing and a number of other industries. Chemical modification to introduce substituents to starch can be used to enlarge the range of physical properties of native starch to increase its usefulness in particular industrial applications. Starches that have been chemically modified typically display physicochemical properties (e.g., viscosity profiles, freeze-thaw stability) that are significantly different than their parent native starches (Rutenberg and Solarek 1984). The extent of chemical modification or degree of substitution of the modified starches is an important parameter and must be measured and controlled in order to optimize the modified starch for use in different applications.

Benzyl starch has been proposed for use as an acrylic resin additive, detergent component, adhesive and emulsifier for textile finishing, paper coatings, soil additives, and other applications (Hjerstad et al 1969; Hofreiter 1987; Lubecke and König 1989; Weaver and Otey 1982). Although benzyl starch does not have many widely used industrial applications, its potential use in various applications is a topic of current applied research and development (Braun and Stipp 1994; Cho and Lim 1998). Typically, wet chemistry methods and ultraviolet absorption spectrophotometry (Weaver and Otey 1982; Cho and Lim 1998) are used to determine the amount of benzyl modification, and nuclear magnetic resonance (NMR) spectroscopy also has been employed (Marsman et al 1990). These methods are destructive of the sample, usually need time-consuming periods of sample preparation, and are impractical for use in continuous monitoring in a quality control situation. The wet chemistry and ultraviolet spectrophotometry methods are also susceptible to interference from residual materials and impurities. A major reason for the long sample preparation time in some methods used currently is the difficulty of fully dissolving starch. Starch has a partially crystalline structure that has highly crystalline regions that are very resistant to hydrolysis or dissolution. Therefore, it is more convenient to choose an analytical technique that can directly use solid starch without any further sample preparation and can give a direct determination of the degree of benzylation.

The different substances that contribute to the Raman spectrum of a particular sample can usually be easily differentiated because each will be characterized by signature Raman bands. This can also

help minimize interference from residual compounds and impurities. The Raman band intensity depends linearly on the amount of substance present (Long 1977). The polymer, pharmaceutical, and food industries have been finding increasing applications for Raman spectroscopy as a quantitative analytical technique (Long 1977; Hendra et al 1991; Shope et al 1987; Davies et al 1990; Deely et al 1991; Jackson et al 1990; Jones and Wesley 1991; Sadeghi-Jorabchi et al 1991; Ozaki et al 1992; Nonaka et al 1993; Li-Chan 1996; Tseng et al 1994; Wang et al 1997; Phillips et al 1998, 1999a,b).

In this article, we present the development and application of a Raman spectroscopic method to measure the degree of benzylation of chemically modified waxy maize starches. Benzylation of starches attaches a benzyl functional group to the starch, and these aromatic moieties exhibit several characteristic Raman bands. We demonstrate that these Raman bands can be used to identify the benzyl modification and conveniently and quickly determine the degree of benzyl substitution.

MATERIALS AND METHODS

Starch Samples

Benzylation samples were prepared using waxy maize starch (Sigma-Aldrich, St. Louis, MO) following a method described in the literature (Weaver and Otey 1982; Braun and Stipp 1994; Cho and Lim 1998) with some modification. Anhydrous Na₂SO₄ (50 g) and NaOH (4 g) were dissolved in warm water (50°C, 75 mL). Waxy starch (50 g, dry weight) and varying amounts of benzyl chloride (2, 4, 6, 8, or 10 mL) were reacted for 12 hr at 65°C with stirring of the reaction mixture. Concentrated Na₂SO₄ in the solution prevented alkaline swelling of the starch granules during the reaction. The reaction mixture was neutralized by adding 1N HCl to achieve pH 6 to stop the reaction. The reaction mixture was then centrifuged for 5 min at 1,900 × g and washed with distilled water (six times) and a 95% ethanol solution (one time). The starch residue was then dried in an oven at 40°C.

Determination of the Degree of Substitution of Benzyl Modification

The degree of substitution of benzyl modification was determined using NMR spectroscopy and calibration with standard mixtures of benzyl alcohol and waxy maize starch. A starch sample (19–21 mg) was placed in an NMR tube with some D₂O solvent. This mixture was then shaken to disperse the starch sample, and pasted. After the sample cooled, NMR spectra were recorded on a Bruker Advanced DPX 300 Fourier-Transform spectrometer operating at 300 MHz for ¹H. The water signal was presaturated at 4.7 ppm to increase the signal-to-noise ratio of the spectra and 200 transients

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were acquired for each sample. The equatorial proton of starch at 5.3 ppm was used as an internal standard; this gives the degree of substitution (DS) = $A_{7.36 \text{ ppm}} / (5 \times A_{5.3 \text{ ppm}})$ due to solvent presaturation. NMR spectra measured under the same operating conditions of standard sample mixtures of known amounts of benzyl alcohol and waxy maize starch were used for absolute calibration.

Method for Collection and Analysis of Raman Spectra

The Raman apparatus and methods are similar to those previously described (Phillips et al 1998, 1999a,b). Raman spectra were obtained using a Fourier Transform (FT) Raman spectrometer (Bio-Rad, Cambridge, MA) with 1,064 nm (the wavelength of the light source, chosen to avoid fluorescence and provide high-quality spectra) excitation. Samples of the waxy maize starch samples were placed into glass capillary tubes. FT-Raman spectra were recorded for the glass tube by itself and the glass tube with the starch sample. The Raman spectrum of the maize starch sample was found by subtracting the spectrum of the glass tube from the spectrum of the glass tube plus starch sample. The Raman signal was collected using a backscattering geometry and 100 mW of 1,064 nm light (power reading measured at the source by the instrument) was used to excite the sample. The Raman signal was collected for 7 min (500 scans) for each spectrum with a resolution of 8 cm^{-1} .

RESULTS AND DISCUSSION

The FT-Raman spectra of waxy maize starch samples with differing amounts of benzylation (spectra A through F) are displayed in Fig. 1. A difference spectrum (G in Fig. 1) for the control spectrum subtracted from a benzylated starch spectrum was also prepared. It does not exhibit any characteristic C-Cl Raman bands, but is similar to the Raman spectrum of benzyl chloride above

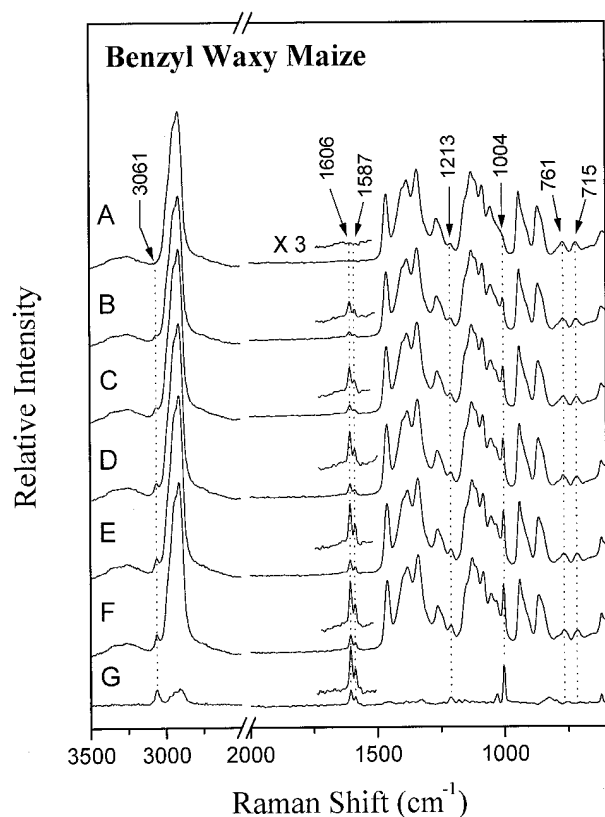


Fig. 1. Fourier Transform (FT) Raman spectra of a control nonbenzylated waxy maize starch (A) and five benzylated waxy maize starch samples (B–F) with differing degrees of benzylation. G is a difference spectrum of F – A. Aromatic C=C stretch Raman bands (1,606 and 1,587 cm^{-1}) are expanded ($\times 3$) in the inset for each spectrum.

1,000 cm^{-1} , which is consistent with the presence of a benzyl functional group. The difference spectrum displays enough vibrational bands to be useful for identification of the benzylation of starches. Several different Raman bands can be used as marker bands for the benzylation modification of starch. We have chosen the aromatic C=C stretch bands at $\approx 1,606$ and $1,587 \text{ cm}^{-1}$ as our marker Raman bands because they are intense and have little overlap with the starch bands. The 761 and 715 cm^{-1} doublet was used as an internal standard between different samples. The ratio of the intensities of the 1,606 plus 1,587 cm^{-1} bands relative to the 761 plus 715 cm^{-1} doublet was determined for the Raman spectra of the control and benzylated waxy maize starch samples (spectra A through F in Fig. 1). The degree of benzyl substitution measured using NMR spectra for each sample and the ratio of the (1,606 cm^{-1} , 1,587 cm^{-1})/(761 cm^{-1} , 715 cm^{-1}) intensities are shown in Tables I and II for the waxy maize starch samples whose FT-Raman spectra are given in Fig. 1.

Figure 2 shows a plot of the ratio of the (1,606 cm^{-1} , 1,587 cm^{-1})/(761 cm^{-1} , 715 cm^{-1}) intensities versus the degree of benzyl substitution of the waxy maize starch samples, with a linear regression fit $y = mx + b$ (where y = the ratio of the [1,606 cm^{-1} , 1,587 cm^{-1}]/[761 cm^{-1} , 715 cm^{-1}] intensities, x = the degree of benzyl

TABLE I
Data for Calibration Set of Benzyl Substituted Waxy Maize Starch^a

Starch Sample	Degree of Benzyl Substitution	Intensity Ratio
Pure waxy maize	0.0003	0.0014 \pm 0.0014
Sample 1	0.0111	0.0566 \pm 0.0081
Sample 2	0.0181	0.0853 \pm 0.0116
Sample 3	0.0210	0.105 \pm 0.0059
Sample 4	0.0241	0.124 \pm 0.0067
Sample 5	0.0268	0.143 \pm 0.0024

^a Calibration set: Intensity ratio $I_{(1606 \text{ cm}^{-1}, 1587 \text{ cm}^{-1})} / I_{(761 \text{ cm}^{-1}, 715 \text{ cm}^{-1})}$ vs. degree of benzyl substitution of waxy maize starch samples.

TABLE II
Data for Comparison Set of Benzyl Substituted Waxy Maize Starch^a

Sample	DS _{Raman}	DS _{NMR}	Relative Error (%) ^b
Unknown 1	0.0124 \pm 0.0004	0.0129	-4.5
Unknown 2	0.0111 \pm 0.0007	0.0115	-3.8
Unknown 3	0.0168 \pm 0.0013	0.0164	2.2
Unknown 4	0.0249 \pm 0.0010	0.0240	3.6
Unknown 5	0.0250 \pm 0.0002	0.0254	-1.7

^a Comparison set: Degree of substitution by Raman and nuclear magnetic resonance (NMR) methods (DS_{Raman} and DS_{NMR}, respectively) and relative error.

^b Relative error = $(\text{DS}_{\text{Raman}} - \text{DS}_{\text{NMR}}) / \text{DS}_{\text{NMR}}$.

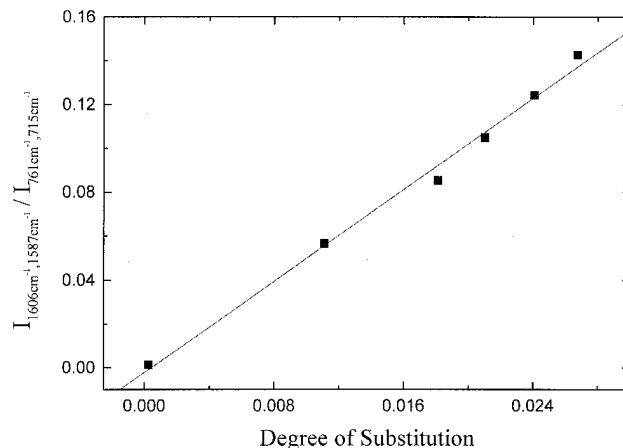


Fig. 2. Ratio of intensities of the Raman 1,606 plus 1,587 cm^{-1} bands to the 761 plus 715 cm^{-1} bands vs. degree of benzylation determined by nuclear magnetic resonance for waxy maize starch samples whose spectra are shown in Fig. 1. Line is a best-fit linear regression to the plotted data.

substitution found from the NMR measurements, $m = 5.21156 \pm 0.21815$, and $b = -0.00214 \pm 0.00417$). The linear regression gave a correlation coefficient of 0.997. This shows that the intensity of the 1,606 and 1,587 cm^{-1} Raman bands had a very good linear relationship with the degree of benzyl substitution for modified starch samples. We also note that zero intensity for the Raman intensity is within the uncertainty of the linear regression y -intercept; this implies that the 1,587 and 1,606 cm^{-1} Raman band intensities are mostly due to benzylation of the waxy maize starch. For the experimental conditions given here, the limit of detection is ≈ 0.0024 for the 1,587 plus 1,606 cm^{-1} Raman bands (using three times the standard deviation of the y -intercept divided by the slope of the best fit linear regression line). The Raman calibration curves can be used to quickly determine the degree of benzyl substitution for samples with unknown levels of benzyl substitution. We determined the degree of benzyl substitution for a set of waxy maize samples with unknown levels of substitution using the Raman calibration curve for confirmation purposes. The actual degree of substitution was found using the standard NMR method for unknown waxy maize samples; these results are shown in Table II. Examination of Table II indicates that the relative errors between the values determined from the standard NMR method and the values found from the Raman calibration curve are on the order of 5% or less. This demonstrates that the Raman calibration curve can be used with confidence for finding the degree of benzyl substitution with an accuracy similar to that of the standard NMR method.

The Raman method demonstrated in this article has several advantages compared to other methods for the measurement of the level of benzylation of modified starches once a calibration curve has been determined. First, the Raman method needs almost no sample preparation for the solid starch samples. This makes the Raman method much more convenient and potentially faster than currently used methods. Second, the Raman technique given here is nondestructive of the starch sample, which can easily be used for other tests immediately, whereas wet chemistry methods are destructive of the sample. The Raman technique also has the potential to be developed for a quality control situation. Other possible advantages of the Raman method include potential simultaneous measurement of multiple starch modifications if there are appropriate Raman marker bands and calibration curves, and lack of interference from impurities or residues (which, if present, could usually be identified by their fingerprint Raman spectra).

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