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Determination of the Degree of Succinylation in Diverse Modified Starches by Raman Spectroscopy

David Lee Phillips,[†] Jie Xing,[‡] Chan Kong Chong,[†] Huijun Liu,[‡] and Harold Corke*[,‡]

Departments of Chemistry and Botany, The University of Hong Kong, Pokfulam Road, Hong Kong

A method using Raman spectroscopy was recently developed for the determination of the degree of substitution of succinate in waxy maize starch. In this paper it is demonstrated that the method can be generalized to a wide range of starches of different amylose contents and botanical origins. Raman calibration sets were used to form regression equations for five types of succinylated starches, that is, waxy, regular, and two high-amylose maize samples (47 and 66% amylose, respectively) and wheat. The derived calibration curves can be used to find the degree of substitution in samples with unknown levels of succinylation. The Raman calibration lines had linear correlation coefficients of 0.995 or better and enable the fast and nondestructive determination of the degree of substitution of succinate for different types of starches with minimal sample preparation. Also discussed is the potential utility of Raman spectroscopy to simultaneously determine the degree of substitution of succinate and amylose content, using previously determined calibration curves developed for the amylose content of maize starches.

Keywords: *Raman spectroscopy; succinylated starches; amylose content*

INTRODUCTION

Chemically modified starches generally have physicochemical properties that differ significantly from those of the parent starch. These differences in modified starches can increase their usefulness in many applications in food manufacturing and other industrial processes (Rutenberg and Solarek, 1984). Succinylation of starches is a commercially used chemical modification, and the degree of succinylation is usually determined using wet chemistry methods or NMR spectroscopy (Wurzburg, 1964; Assempour et al., 1994). Unfortunately, these techniques are destructive of the sample, typically require time-consuming sample preparation, and are not easily amenable for use in a quality control situation requiring continuous monitoring. The wet chemistry determination techniques may also be prone to interference from residual substances, such as residual succinic anhydride or starch impurities.

The problem of dissolving starch is largely responsible for the protracted sample preparation for the wet chemistry and NMR methods used to determine the degree of succinylation of starches. The partially crystalline structure of typical starches contains portions that are more resistant toward hydrolysis or dissolution. To avoid dissolving the starch, it would be better to use an analytical method that could directly use solid starch and that could also give a direct measure of the amount of succinylation of the modified starch.

Because different substances have different Raman vibrational spectra, their individual contributions to a Raman spectrum can usually be readily distinguished. Raman bands in a Raman spectrum depend linearly on the amount of compound contributing to them (Long, 1977; Hendra et al., 1991). Raman spectroscopy has long been used as a quantitative analytical method in the pharmaceutical and polymer industries (Hendra et al., 1991) but has only recently been finding applications in the food industry (Li-Chan, 1996).

Previous work established analytical Raman methods for acetylated starches (Phillips et al., 1998, 1999a). We have recently developed a Raman spectroscopic method to determine the degree of succinylation in waxy maize starch (Phillips et al., 1999c). We used purified succinylated waxy maize starch samples and Raman spectra to make a calibration curve for the degree of succinylation versus the intensity ratio of the C═O stretch Raman band to a C−C stretch Raman band. The ratio of the C═O stretch to the C−C stretch Raman bands from an unknown waxy maize sample can be compared to the calibration curve to find the degree of succinylation in the unknown sample. Because the Raman band position and relative intensity can be sensitive to the specific structure of the molecule and/or its surrounding environment, the C═O stretch and C−C stretch Raman bands used to prepare the calibration curve may vary noticeably between different types of starches. It is possible that the Raman method we have developed for waxy maize starches would need different calibration curves for the degree of succinylation of different types of starches. In this paper, we present the additional development of Raman spectroscopy as an analytical method for the measurement of the amount of succinylation for a number of different starches (waxy maize, regular maize, two types of high-amylose maize, and wheat). The aim is to show the generality of Raman spectroscopy for the measurement of the degree of succinylation in starch and to show the extent of variation in the molecular structure around the C═O and C−C bonds in starches from different biological sources. We also show that Raman spectroscopy can

* Author to whom correspondence should be addressed (e-mail harold@hku.hk; fax +852 2858-3477).

[†] Department of Chemistry.

[‡] Department of Botany.

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simultaneously measure the degree of succinylation and indicate the approximate amylose content in starch.

MATERIALS AND METHODS

Materials. Waxy maize (3.3% amylose), regular maize (22.4% amylose), Gelose 50 (47% amylose) maize, Hi-Maize (66% amylose) (all from Starch Australasia Ltd., Lane Cove, Australia), and wheat starch (30% amylose) (Sigma Chemical Co., St. Louis, MO) (Phillips et al., 1999a) were succinylated using the method of Wolff et al. (1951) with small modifications. For each sample, 100 g of starch was dispersed in 225 mL of distilled water and stirred for 60 min at 25 °C. The pH was adjusted to 8.0 with 3.0% NaOH solution. Succinic anhydride (various amounts were added to achieve different degrees of substitution) was added dropwise to the stirred slurry while a pH of 8.0−8.4 was maintained using 3.0% NaOH. The reaction was allowed to continue for 10 min after the addition of succinic anhydride was completed. The sample slurry was then adjusted to a pH of 4.5 using 0.5 M HC1, centrifuged for 3 min at 2000 rpm, washed free of acid twice with distilled water and once with 95% ethanol, and oven-dried at 40 °C.

Determination of the Level of Succinylation. The level of succinylation of the prepared starch samples was determined using the titrimetric method of Wurzburg (1964). A standard KOH solution was added to a suspension of succinylated starch and then placed for 24 h on a shaker. The excess alkali was titrated with standard HC1 and retitrated 2 h later to account for any further alkali that may leach from the starch.

Method for the Collection and Analysis of Raman Spectra. The Raman spectra of succinylated and control starches were obtained using a Fourier transform Raman (FT-Raman) spectrometer (Bio-Rad) using 1064 nm cw excitation and 100 mW power. The samples were put into a glass capillary tube, and FT-Raman spectra were taken for an empty glass tube and a glass tube containing the starch sample. A 180° backscattering geometry was used for sample excitation and collection of the scattered light, and 1200 scans per sample were summed with 8 cm^{-1} resolution (20 min data collection time). The Raman spectrum of each starch sample was found by subtracting the Raman spectrum of the empty glass tube from the Raman spectrum of the glass tube containing the starch sample. The integrated areas of the ~1730 cm^{-1} C═O stretch Raman band and the 870−970 cm^{-1} region C−C stretch Raman bands were obtained. The integrated areas of the 870−970 cm^{-1} C−C stretch Raman bands were used as an internal standard. The ratio of the 1730 cm^{-1} C═O stretch Raman band area to the 870−970 cm^{-1} region C−C stretch Raman bands area was calculated for each spectrum, and this ratio was then plotted versus the degree of substitution of succinate determined from the titrimetric method in order to obtain a calibration curve.

RESULTS AND DISCUSSION

The four types of maize starches with various amylose contents and the wheat starch samples with different degrees of succinylation gave very similar Raman spectra (Figure 1), showing that the 1730 cm^{-1} Raman band due to the C═O stretch of the succinyl functional group (which is not present in the unmodified starch control samples) increases in intensity as the amount of succinylation increases. For each of the Raman spectra, the ratio of the 1730 cm^{-1} C═O stretch Raman band intensity to the ~941 cm^{-1} C−C stretch Raman bands intensity was determined. For each of the starch samples, the amount of succinylation was found using the Wurzburg titrimetric method. Best fit linear regression parameters were obtained (data in Table 1) for the calibration curves of the Raman intensity ratio (I_{1730}/I_{941}) versus the degree of succinylation (Table 2), which

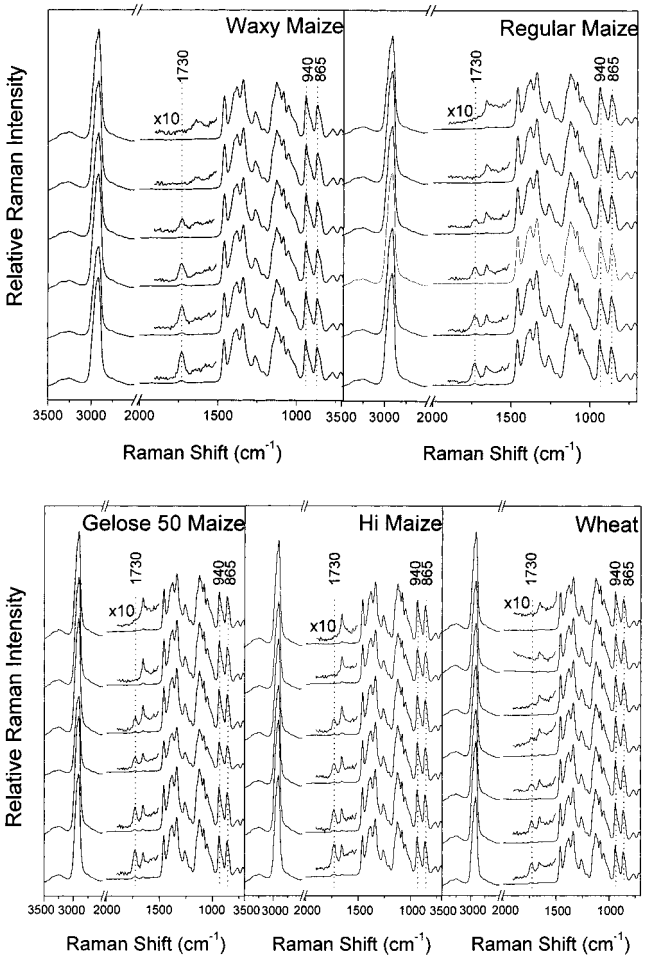

Figure 1. FT-Raman spectra of (A) waxy maize, (B) regular maize, (C) Gelose 50 maize, (D) Hi-Maize, and (E) wheat starches, with various degrees of substitution of succinate and a control (native) starch sample. The expanded region around 1600−1800 cm^{-1} shows the Raman signature bands for succinate at 1730 cm^{-1} and amylose at 1657 cm^{-1}.

were plotted (Figure 2). The calibration curve for wheat starch was similar to those for the maize starches. Examination of the Raman spectra in Figure 1 and the calibration plots in Figure 2 shows that the 1730 cm^{-1} Raman band increases in intensity as the degree of succinylation increases. The calibration curves have linear regression correlation coefficients of 0.995 or better and their slopes varied from 0.5562 to 0.6233. Our results clearly indicate that the Raman intensity ratios of the 1730 cm^{-1} to 941 cm^{-1} bands versus the degree of substitution of succinylation are highly linear, and the calibration curves presented can be used with confidence to obtain the degree of substitution of succinylation of different types of starches.

Examination of the linear regression parameters (Table 2) for the maize starches with different amylose contents showed that the slopes varied (from 0.556 to 0.623). This variation in the slopes does not appear to correlate with amylose content and is likely due to some other environmental factor influencing the relative intensities of the C═O stretch and C−C stretch bands. The variation in the slopes of the Raman calibration curves suggests that one should use a calibration curve specific for the particular type of starch under study.

Inspection of the Raman spectra (Figure 1) shows a small Raman band ~1657 cm^{-1} that correlates with the amylose content of the modified starch. We have re-

Table 1. Weight Percent and Degree of Substitution of Succinate in Modified Starches and Ratio of Raman Intensity of the ~1730 cm^{-1} C═O Stretch and the ~941 cm^{-1} C—C Stretch Bands (I_{1730}/I_{941})

sample	wt %, succinate[a]	degree of substitution, succinate[a]	ratio of I_{1730}/I_{941}
waxy maize			
pure (A)	0.00	0.0000	0.000
sample 1 (B)	1.91 ± 0.22	0.0257 ± 0.003	0.0142 ± 0.0017
sample 2 (C)	2.93 ± 0.07	0.0398 ± 0.0010	0.0191 ± 0.0019
sample 3 (D)	3.50 ± 0.21	0.0477 ± 0.0030	0.0250 ± 0.0050
sample 4 (E)	4.40 ± 0.15	0.0605 ± 0.0022	0.0312 ± 0.0057
regular maize			
pure (A)	0.00	0.0000	0.000
sample 1 (B)	1.07± 0.12	0.0143 ± 0.0016	0.0096 ± 0.0008
sample 2 (C)	1.47 ± 0.01	0.0196 ± 0.0002	0.0136 ± 0.0028
sample 3 (D)	1.92 ± 0.16	0.0257 ± 0.0022	0.0159 ± 0.0021
sample 4 (E)	2.34 ± 0.10	0.0315 ± 0.0014	0.0188 ± 0.0035
Gelose 50 maize			
pure (A)	0.00	0.0000	0.000
sample 1 (B)	1.73 ± 0.40	0.0232 ± 0.0055	0.0105 ± 0.0016
sample 2 (C)	2.13 ± 0.17	0.0286 ± 0.0023	0.0139 ± 0.0028
sample 3 (D)	2.87 ± 0.37	0.0390 ± 0.0051	0.0207 ± 0.0024
sample 4 (E)	3.99 ± 0.22	0.0547 ± 0.0031	0.0278 ± 0.0011
Hi-Maize			
pure (A)	0.00	0.0000	0.000
sample 1 (B)	1.77 ± 0.37	0.0238 ± 0.0051	0.0109 ± 0.0033
sample 2 (C)	2.17 ± 0.11	0.0292 ± 0.0015	0.0155 ± 0.0011
sample 3 (D)	3.08 ± 0.03	0.0419 ± 0.0005	0.0232 ± 0.0038
sample 4 (E)	4.24 ± 0.01	0.0582 ± 0.0002	0.0313 ± 0.0010
wheat			
pure (A)	0.000	0.0000	0.000
sample 1 (B)	0.374 ± 0.203	0.0050 ± 0.0027	0.0024 ± 0.0023
sample 2 (C)	1.058 ± 0.267	0.0141 ± 0.0036	0.0069 ± 0.0011
sample 3 (D)	1.345 ± 0.328	0.0180 ± 0.0044	0.0099 ± 0.0018
sample 4 (E)	1.667 ± 0.070	0.0223 ± 0.0010	0.0126 ± 0.0029
sample 5 (F)	2.189 ± 0.330	0.0295 ± 0.0045	0.0160 ± 0.0017

[a] Determined with the titration method described under Materials and Methods.

Table 2. Parameters for Linear Regression Analysis of the Calibration Lines for the Raman Intensity Ratio versus the Weight Percent and Degree of Substitution of Succinate

sample	B (± SD)	A (± SD)	r
Parameters for Calibration Line of Weight Percent Succinate versus Raman Intensity Ratio			
waxy maize	0.00766 ± 0.00039	−0.00218 ± 0.00131	0.996
regular maize	0.00757 ± 0.00035	0.00158 ± 0.00063	0.997
Gelose 50 maize	0.00855 ± 0.00042	−0.00494 ± 0.00123	0.996
Hi-Maize	0.00840 ± 0.00027	−0.00355 ± 0.00086	0.998
wheat	0.00807 ± 0.00031	−0.00124 ± 0.00056	0.997
Parameters for Calibration Line of Degree of Substitution of Succinate versus Raman Intensity Ratio			
waxy maize	0.5562 ± 0.0304	−0.00201 ± 0.00141	0.996
regular maize	0.5611 ± 0.0278	0.00163 ± 0.00067	0.996
Gelose 50 maize	0.6233 ± 0.0353	−0.00470 ± 0.00140	0.995
Hi-Maize	0.6109 ± 0.0234	−0.00333 ± 0.00102	0.998
wheat	0.5994 ± 0.0238	−0.00119 ± 0.00058	0.997

[a] $Y = A + B \times X$, where Y = ratio of the Raman intensity of the 1730 cm^{-1} C═O stretch and 941 cm^{-1} C—C stretch bands, B = slope of the linear regression, A = intercept of the linear regression, and X = weight percent of succinate or degree of substitution of succinate as appropriate. $N = 5$ for maize samples and $N = 6$ for wheat sample.

cently developed a Raman spectroscopic method to determine the amylose content of maize starches (Phillips et al., 1999b), and we can use the calibration curve from that study to estimate the amylose content of the succinylated maize starches, assuming that the modified starches have the same amylose content calibration curve as the parent starches. It is not yet certain whether the modified starch amylose calibration curve is in fact comparable to the parent starch calibration

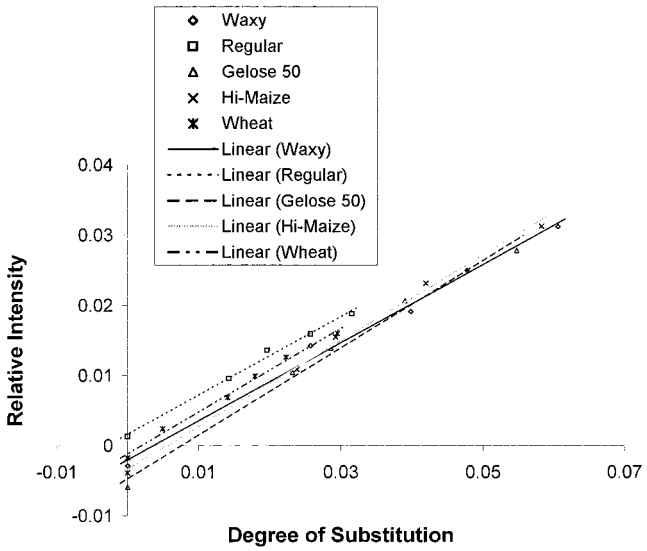

Figure 2. Plot of the ratio of the 1730 cm^{-1} C═O stretch succinate Raman band to the 941 cm^{-1} C—C stretch starch Raman bands versus the degree of substitution of succinate determined using a titrimetric method. The lines are best linear fits (linear regression parameters are given in Table 2).

curves, so we have not yet attempted a quantitative analysis of the spectra in Figure 1 for amylose content. There are some interesting qualitative features in the Raman spectra of Figure 1. The broad band around 1630 cm^{-1}, mainly due to amylopectin in the waxy maize Raman spectra, becomes noticeably smaller with increasing succinylation. The sharper Raman band around 1657 cm^{-1} in the spectra of nonwaxy samples usually becomes smaller with increasing succinylation. These changes in the amylose/amylopectin Raman bands suggest that succinylation causes changes in the amylose/amylopectin environment. We plan to study these changes further and to develop more robust Raman amylose content calibration curves for succinylated starches. The appearance of the amylose/amylopectin Raman bands and the C═O stretch succinylation Raman band illustrates one of the potential advantages of using Raman or other spectroscopic methods (such as infrared or NMR), which are very sensitive to the structures of the molecules: one can determine the degree of modification and the amylose content simultaneously provided that the signature Raman band of the chemical modification is well resolved from the signature amylose 1657 cm^{-1} Raman band. Similarly, one can simultaneously determine several different chemical modifications of the same starch as long as all of the signature Raman bands are well resolved from one another and good Raman calibration curves exist for each of the chemical modifications. Due to the interaction of amylose content with the physical properties of chemically modified starches [e.g., Liu et al. (1999)], future development of starches with highly specific properties may be aided by the ability to simultaneous measure amylose and chemical modification. We also note that the Raman method is less susceptible to interference from residual compounds or starch impurities than the titrimetric methods, because new bands would develop in addition to the masking or interfering band and enable easy detection of the impurity (Phillips et al., 1999a). The use of the Raman method in quality assurance or continuous monitoring is feasible. The limit of detection or signal-to-noise ratio

is proportional to the square root of the data collection time. Adequate data could be collected within 2 min, rather than the 20 min used in the present study.

It is interesting to compare the present results for succinylated starches with those for acetylated starches (Phillips et al., 1998, 1999a). The more compact acetyl modification gave very similar calibration curves for the four types of maize, wheat, and also potato starches with the slopes of the calibration curves within their mutual standard deviations. The larger succinyl group gives somewhat larger differences than the acetyl group in the slopes for the calibration curve. This indicates that the four types of maize have very similar molecular environments around the C=O bonds added by acetylation but larger changes around the C=O bonds by succinylation. One possible reason for this difference could be that the larger succinate group (with two C=O bonds and not just one as for acetate) is more sterically demanding so that its second C=O bond interacts with the rest of the starch molecular environment more strongly. A second possible explanation, which is not mutually exclusive, is that the acetylation and succinylation occur at somewhat different sites in the starches (such as more branching points relative to linear chain points). Further work is needed to more fully understand why the slopes for succinylation vary so much for the different types of maize starches. We also note that in this study, the starch succinate ester was acidified to pH 4.5, and some degree of pH-dependent cross-linking may have occurred, potentially affecting the calibration of the method. However, in practice the modification would be done under consistent conditions, and this is not likely to bias the results.

In conclusion, we have presented calibration curves for 1730 to 941 cm^{-1} Raman band intensity ratios versus level of succinylation for five types of starches. These calibration curves all display a very high degree of linearity and are suitable for the quantitative analytical determination of the degree of succinylation of starch samples. There is greater variation in the slopes of the Raman calibration curves for succinyl substitution than for acetyl substitution of the four types of maize starches, suggesting that the C=O bonds from succinylation experience more perturbation of their molecular environment is seen with acetyl substitution. We have also discussed the potential for Raman spectroscopy to simultaneously determine the degree of succinate substitution and amylose content for maize starches by using the appropriate marker Raman band areas and suitable calibration curves.

LITERATURE CITED

Assempour, H.; Koenig, M. F.; Huang, S. J. Synthesis and characterization of dodecenyl succinate derivatives of saccharides. In *Polymers from Agricultural Coproducts*; ACS Symposium Series 575; American Chemical Society: Washington, DC, 1994; pp 68−81.

Hendra, P. J.; Jones, C. H.; Warnes, G. M. *Fourier Transform Raman Spectroscopy, Instrumentation and Chemical Applications*; Ellis Horwood: Chichester, U.K., 1991; 212 pp.

Li-Chan, E. C. Y. The applications of Raman spectroscopy in food science. *Trends Food Sci. Technol.* **1996**, *7*, 361−370.

Liu, H. J.; Corke, H.; Ramsden, L. Functional properties and enzymatic digestibility of cationic and cross-linked cationic *ae*, *wx* and normal maize starch. *J. Agric. Food Chem.* **1999**, *47*, 2523−2528.

Long, D. A. *Raman Spectroscopy*; McGraw-Hill: London, U.K., 1977; 276 pp.

Phillips, D. L.; Pan, D. H.; Liu, H. J.; Corke, H. Raman spectroscopic determination of the level of acetylation in modified wheat starch. *Anal. Lett.* **1998**, *31*, 2105−2114.

Phillips, D. L.; Liu, H. J.; Pan, D. H.; Corke, H. General application of Raman spectroscopy for the determination of level of acetylation in modified starches. *Cereal Chem.* **1999a**, *76*, 439−443.

Phillips, D. L.; Xing, J.; Liu, H. J.; Pan, D. H.; Corke, H. Potential use of Raman spectroscopy for determination of amylose content in maize starch. *Cereal Chem.* **1999b**, *76*, 821−823.

Phillips, D. L.; Xing, J.; Liu, H. J.; Chong, C. K.; Corke, H. Raman spectroscopic determination of the degree of succinate in modified waxy maize starches. *Anal. Lett.* **1999c**, *32*, 2703−2711.

Rutenberg, M. W.; Solarek, D. Starch derivatives: production and uses. In *Starch: Chemistry and Technology*; Whistler, R. L., BeMiller, J. N., Paschall, E. F., Eds.; Academic Press: London, U.K., 1984; pp 312−388.

Wolff, I. A.; Olds, D. W.; Hilbert, G. E. Acetylation of starch, amylose, and amylopectin. *J. Am. Chem. Soc.* **1951**, *73*, 346−349.

Wurzburg, O. B. Starch derivatives and modification. In *Methods in Carbohydrate Chemistry*, 4th ed.; Whistler, R. L., Ed.; Academic Press: New York, 1964; pp 286−288.

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