

Potential Use of Raman Spectroscopy for Determination of Amylose Content in Maize Starch

David Lee Phillips,^{1,2} Jie Xing,³ Huijun Liu,³ Duo-Hai Pan,¹ and Harold Corke³

ABSTRACT

Cereal Chem. 76(5):821–823

An analytical method using Raman spectroscopy was developed for the determination of amylose concentration in maize starches. FT-Raman spectra of four maize starches with amylose content varying from 3.3 to 66% were obtained. A Raman band at $\approx 1657\text{ cm}^{-1}$ correlated linearly with amylose concentration in the four maize starches, and a calibration curve for Raman

band intensity versus amylose content was developed. The linear correlation of the I_{1657}/I_{900} integrated areas with amylose content was $r = 0.997$. The Raman-based calibration curve allows fast and nondestructive determination of the amylose content in maize starches with minimal sample preparation.

The amylose-to-amylopectin ratio (or amylose content) of starch affects many of its properties and influences cooking and textural qualities of whole grains, sheeting and gelling qualities of starch pastes, and the formation of resistant starch. Thus, the measurement of the amylose content of starch is an important quality control parameter in industrial starch processing. A variety of wet-chemistry-based methods have been developed to measure amylose content, and it is commonly measured using amperometric, potentiometric, or colorimetric determination of the iodine binding capacity of amylose to give amylose-iodine inclusion complexes (Matheson 1971, Banks et al 1974, Morrison and Laignelet 1983, Knutson 1986, Chrastil 1987, ISO 1987). However, these measurements are prone to interference as iodine forms complexes with long linear B chain sections of amylopectin that absorb at wavelengths similar to the amylose-iodine complexes. This type of interference varies significantly in rice (Takeda et al 1987). There is also significant variation in the iodine binding capacity of amylose from different commercial sources (Welsh and Blakeney 1992). Another problem in using amylose-iodine binding methods comes if amylose-only calibration curves are used, which may give noticeably different results than amylose-amylopectin mixture calibration curves (Blakeney et al 1994).

Although the iodine-amylose complex formation methods are convenient, they are not easily applied to starches from different botanical or commercial origins without additional corrections for amylopectin-iodine absorption. An alternative method that has fewer uncertainties uses specific precipitation of amylopectin by complex formation with concanavalin A lectin (Matheson and Welsh 1988, Yun and Matheson 1990, Gibson et al 1995).

Alternative spectroscopic methods for routine amylose determination have been sought, as they offer potential benefits in ease of sample preparation, speed, and applicability to on-line monitoring in the process control situation. Near-infrared (NIR) reflectance analysis of barley starch indicated minor spectral differences among waxy, normal, and high-amylose samples, but these were not applied to quantitative measurement (Czuchajowska et al 1994). NIR reflectance analysis of amylose content of milled whole grain rice gave a coefficient of determination on the validation set of $r^2 = 0.89$ (Delwiche et al 1996). NIR transmittance spectroscopy of amylose content also gave favorable results for unground brown or milled rice (Villareal et al 1994) and for whole grain maize samples (Campbell et al 1997). However, problems with NIR spectroscopy

include its sensitivity to sample preparation and presentation, and the indirect nature of the spectral correlations. Raman spectroscopy has been used as a quantitative analytical technique for some time in the polymer and pharmaceutical industries (Hendra et al 1991) and has increasingly become used as an analytical technique in food science and industry (Li-Chan 1996). Different compounds making contributions to a Raman spectrum can be discerned because each compound has a different vibrational spectrum. The intensity of Raman bands increases linearly with the amount of the compound in the sample (Long 1977, Hendra et al 1991). The Raman band positions and intensities can be very sensitive to the particular structure of the molecule and its surrounding environment. Even though amylose and amylopectin are very similar to one another, they differ significantly in their degree of polymerization ($\approx 1,000$ for amylose vs. $\approx 2,000,000$ for amylopectin in maize) and their degree of branching (amylose has very little branching, while amylopectin is highly branched). Our present Raman study has two objectives: 1) obtain high-quality Raman spectra of maize starches with varying amylose content to identify potential marker Raman bands for the determination of the amylose concentration, and 2) develop a Raman spectroscopic method to measure the amylose content of maize starches.

MATERIALS AND METHODS

Starch Samples and Amylose Content

Regular (Maize Cornflour 3401C, 22.4% amylose), Gelose 50 (47% amylose), Hi-Maize (66% amylose), and Mazaca 3401X (3.3% amylose) maize starches were supplied by Starch Australasia Ltd. (Lane Cove, Australia). Amylose content of the maize starches are means of four determinations using an amylose-amylopectin assay kit (Megazyme Pty Ltd, Bray, Ireland) based on the concanavalin-A lectin binding method. The average standard deviation of the mean was 0.54% amylose. The results are also fully consistent with those provided by the commercial supplier.

Method for Collection and Analysis of Raman Spectra

All of the Raman spectra of maize starches were obtained using the methods previously described in Phillips et al (1998, 1999) with an FT-Raman spectrometer (Bio-Rad, Cambridge, MA) equipped with 1,064-nm excitation. Samples of the maize starches were put into glass capillary tubes. FT-Raman spectra ($100\text{--}3,000\text{ cm}^{-1}$) were taken for the glass tube by itself and the glass tube with the maize starch sample. The Raman spectra of the maize starch samples were found by subtracting the FT-Raman spectra of the glass capillary tube from those of the maize sample in the glass capillary tube. A backscattering geometry was used to collect the

¹ Department of Chemistry, The University of Hong Kong, Pokfulam Rd, Hong Kong.

² Corresponding author. Fax: +852 2857-1586. E-mail: phillips@hku.hk

³ Department of Botany, The University of Hong Kong, Pokfulam Rd, Hong Kong.

Raman scattered signal and ≈ 100 mW of 1,064-nm laser light was used to excite the samples. The Raman signal was collected for ≈ 10 –30 min for each spectrum. It should be noted that this time would be much reduced if only a restricted spectral range were used in applying an analytical method. The integrated intensities of the region between 1,570 and 1,740 cm^{-1} , which includes the sharp 1,657 cm^{-1} amylose Raman band and the broad amylopectin Raman band $\approx 1,637$ cm^{-1} , and the region from 810 to 975 cm^{-1} that includes Raman bands associated with the C-C stretch modes were found for all four maize starch samples. The ratio of the 1,600 cm^{-1} region bands relative to the 810–975 cm^{-1} C-C stretch Raman bands were then obtained and plotted against amylose content.

RESULTS AND DISCUSSION

The Raman spectra for the waxy, regular, Gelose 50, and Hi-Maize starch samples are shown in Fig. 1. The Raman bands at ≈ 900 cm^{-1} were primarily due to C-C stretch vibrations, and the bands in the 2,800–3,000 cm^{-1} region were due to C-H vibrations. Although the FT-Raman spectra were very similar to one another, there were several Raman bands that showed discernible changes as the amylose concentration increased in the maize samples. The alpha C-C bands at ≈ 920 –960 cm^{-1} decreased a small amount in intensity as the amylose concentration increased. However, a new Raman band at $\approx 1,657$ cm^{-1} grew in intensity as the amylose concentration increased, clearly visible in the insets of Fig. 1 where the spectral region $\approx 1,570$ –1,740 cm^{-1} has been enlarged by a factor of 10. Inspection of the Raman spectrum for waxy maize ($\approx 3.3\%$ amylose and 96.7% amylopectin) in Fig. 1 showed a very small broad band at $\approx 1,637$ cm^{-1} that appears to be mainly due to amylopectin. As the amylose concentration increased, going from waxy maize to regular maize to Gelose 50 to Hi-Maize, a new Raman band appeared and increased in intensity at $\approx 1,657$ cm^{-1} .

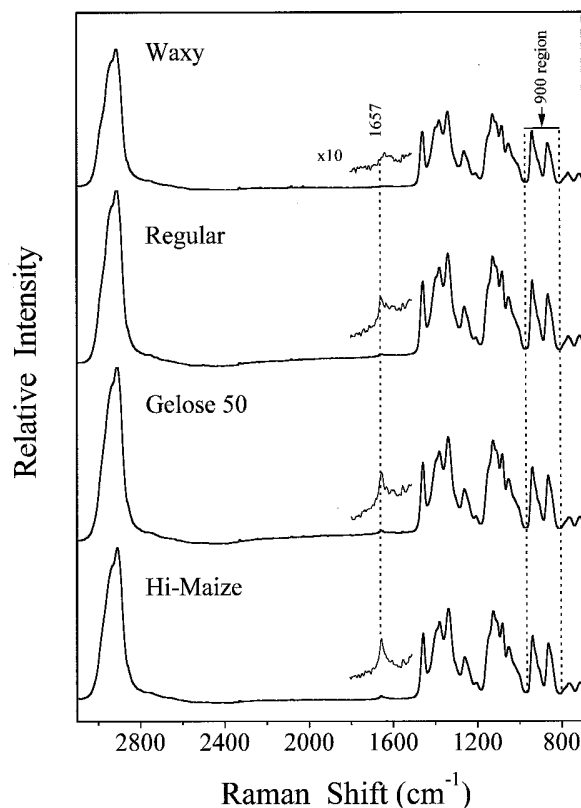


Fig. 1. Four representative FT-Raman spectra of maize starches: waxy, regular, Gelose 50, and Hi-Maize. Region 1,570–1,740 cm^{-1} enlarged by a factor of 10 in the inset for each spectrum to easily visualize the 1,657 cm^{-1} Raman band that correlates with amylose concentration.

The Raman band at $\approx 1,657$ cm^{-1} appeared to correlate with the amylose content of maize. The apparent amylopectin 1,637 cm^{-1} band gave a background contribution to the 1,657 cm^{-1} amylose Raman band. We have integrated the region between 1,570 and 1,740 cm^{-1} which includes the sharp 1,657 cm^{-1} amylose Raman band and the broad amylopectin Raman band at $\approx 1,637$ cm^{-1} as well as the region from 810 to 975 cm^{-1} associated with the C-C stretch Raman bands. The integrated areas of the C-C stretch Raman bands (810–975 cm^{-1}) were used as an internal standard between different maize samples. The ratio of the integrated areas of the Raman bands in the 1,570–1,740 cm^{-1} region versus the integrated areas of the 810–975 cm^{-1} region (represented by I_{1657}/I_{900}) was obtained for the spectra in Fig. 1. Figure 2 displays I_{1657}/I_{900} versus the percent amylose content. We fitted the data in Fig. 2 to a linear regression equation ($y = mx + b$ where $y = I_{1657}/I_{900}$, $x =$ amylose %, $m =$ slope, and $b =$ y-intercept) and the best fit linear regression parameters were $m = 0.0442 \pm 0.0025$, $b = 0.0212 \pm 0.0011$ with a correlation coefficient of $r = 0.997$. The correlation coefficient $r = 0.997$ clearly indicates that there is a strong linear correlation between the I_{1657}/I_{900} integrated area and the percent amylose content of the maize starch samples.

Because the integrated Raman band areas for the 1,570–1,740 cm^{-1} region has contributions from both the $\approx 1,637$ cm^{-1} amylopectin band and the $\approx 1,657$ cm^{-1} amylose band, the integrated area of the 1,570–1,740 cm^{-1} region can be expressed as a sum of linear functions: $I_{1657} = m_1(x) + b_1 + m_2(1-x) + b_2$ where $x =$ amylose % (where amylopectin % = $1 - x$), $m_1 =$ slope for amylose Raman band intensity alone, $m_2 =$ slope for amylopectin Raman band intensity alone, $b_1 =$ y-intercept for amylose Raman band intensity alone, and $b_2 =$ y-intercept for amylopectin Raman band intensity alone. This function can be rearranged to give an effective linear function $I_{1657} = mx + b$ where $m = m_1 - m_2$ and $b = b_1 + b_2 + m_2$. Dividing through by the internal standard to scale the I_{1657} integrated areas between different maize Raman spectra leads to the effective linear function ($y = mx + b$ where $y = I_{1657}/I_{900}$, $x =$ amylose %, $m =$ slope, and $b =$ y-intercept) used to fit the plot of I_{1657}/I_{900} versus the amylose % in Fig. 2. Inspection of the Raman spectra in Fig. 1 and the integrated area ratios in Fig. 2 shows that when I_{1657} has mostly contributions from amylopectin (as is the case for waxy maize) then the integrated area is much smaller than when I_{1657} has mostly contributions from amylose (as is the case for Hi-Maize). Specifically, the ratio of I_{1657}/I_{900} was 0.0217 for waxy, 0.0324 for regular, 0.0421 for Gelose 50, and 0.0499 for Hi-Maize. This implies that m_1 is substantially greater than m_2 and that most of the changes observed in the I_{1657}/I_{900} ratio in Fig. 2 are due to changes in the 1,657 cm^{-1} amylose Raman band area with a smaller and more slowly varying background contribution from

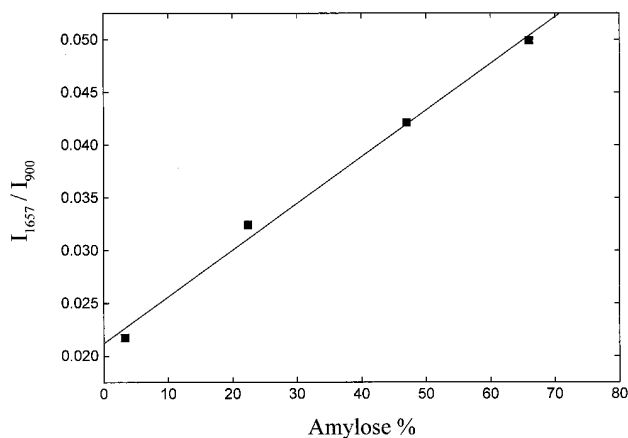


Fig. 2. Plot of ratio of 1,570–1,740 cm^{-1} region intensity to the 900 cm^{-1} region internal standard intensity (I_{1657}/I_{900}) vs. amylose content. Line is best linear fit, linear regression parameters are given in text.

the 1,637 cm^{-1} amylopectin Raman band. We can estimate m_1 and m_2 from our effective linear regression parameters if we assume that only the amylopectin and amylose bands contribute to the intensity of I_{1657} ($b_1 = b_2 = 0$). This appears to be reasonable and gives $m_2 = 0.02118$ and $m_1 = 0.06542$. The effective linear regression calibration curve we use to determine the amylose concentration in maize starches functions well for overlapped amylose and amylopectin Raman bands only when there is a substantial difference in the relative sensitivity of amylose and amylopectin bands (the slopes m_1 and m_2 are different). This points out a possible drawback in making this Raman spectroscopic method generally applicable to starches from different botanical sources. The Raman bands of amylose ($\approx 1,657 \text{ cm}^{-1}$) and amylopectin ($\approx 1,637 \text{ cm}^{-1}$) may be sensitive to changes in the degree of polymerization or degree of branching which do change significantly for some botanical sources of starch (for example, potato as compared with maize). If this is the case, then different calibration curves for the determination of amylose concentration from different sources should be used. Degree of polymerization may also vary among genotypes of the same species or among samples of the same genotype produced in the same environment. An analytical method developed for routine analysis should be robust enough to cope with these differences. This report considers only four maize samples of widely varying amylose content. The method needs to be challenged with more diverse samples, and work on this is in progress.

An advantage of NIR methods is that they can be used on whole grain (milled or unmilled) samples. This factor should be considered in choice of spectroscopic method. The Raman spectroscopic method for the determination of amylose concentration in maize starches that we report here has several advantages when compared with standard wet-chemistry methods. The Raman spectroscopic method requires minimal sample preparation and can be faster than current wet-chemistry methods. The Raman method is nondestructive, while the wet-chemistry methods are destructive of the sample. It can potentially be used on either starch or ground meal. More importantly, as the Raman spectroscopic method is improved to obtain higher signal-to-noise spectra in shorter times, there is the possibility of using it as a quality control technique for fast analysis of amylose content of starches and even in situ monitoring of dynamic processes.

ACKNOWLEDGMENTS

We thank Starch Australasia Ltd., Australia, for supply of maize starches. Financial support was received from Hong Kong Research Grant Council awards to DLP and HC.

LITERATURE CITED

Banks, W., Greenwood, C. T., and Muir, D. D. 1974. The characterization of starch and its components. 6. A critical comparison of the esti-

mation of amylose-content by colorimetric determination and potentiometric titration of the iodine-complex. *Starch/Staerke* 26:73-78.

Blakeney, A. B., Welsh, L. A., and Martin, M. 1994. Analytical methods for wheat starch amylose. Pages 275-278 in: *Proceedings of the 44th Australian Cereal Chemistry Conference*. J. F. Panozzo and P. G. Downie, eds. RACI: Melbourne.

Campbell, M. R., Brumm, T. J., and Glover, D. V. 1997. Whole grain amylose analysis in maize using near-infrared transmittance spectroscopy. *Cereal Chem.* 74:300-303.

Czuchajowska, Z., Szczodrak, J., and Pomeranz, Y. 1994. Characterization and estimation of barley polysaccharides by near-infrared spectroscopy. I. Barleys, starches, and β -D-glucans. *Cereal Chem.* 69:413-418.

Chrastil, J. 1987. Improved colorimetric determination of amylose in starches or flours. *Carbohydr. Res.* 159:154-158.

Delwiche, S. R., McKenzie, K. S., and Webb, B. D. 1996. Quality characteristics in rice by near-infrared reflectance analysis of whole-grain milled samples. *Cereal Chem.* 73:257-263.

Gibson, T. S., McCleary, B. V., and Solah, V. A. 1995. Con A revisited—A simplified assay to measure amylose in cereal starches. Pages 162-165 in: *Proceedings of the 45th Australian Cereal Chemistry Conference*. Y. A. Williams and C. W. Wrigley, eds. RACI: Melbourne.

Hendra, P. J., Jones, C. H., and Warnes, G. M. 1995. *Fourier Transform Raman Spectroscopy, Instrumentation and Chemical Applications*. Ellis Horwood: Chichester, England.

ISO. 1987. 1987E. Rice—Determination of amylose content. 6647. International Organisation for Standardisation: Geneva

Knutson, C. A. 1986. A simplified colorimetric procedure for determination of amylose in maize starches. *Cereal Chem.* 63:89-92.

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

Long, D. A. 1977. *Raman Spectroscopy*. McGraw-Hill: London.

Matheson, N. K. 1971. Amylose changes in the starch of developing wheat grains *Phytochemistry* 10:3213-3219.

Matheson, N. K., and Welsh, L. A. 1988. Estimation and fractionation of the essentially unbranched (amylose) and branched (amylopectin) components of starches with concanavalin A. *Carbohydr. Res.* 180:301-314.

Morrison, W. R., and Laignelet, B. 1983. An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. *J. Cereal Sci.* 1:9-20.

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

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

Takeda, Y., Hizukuri, S., and Juliano, B. O. 1987. Structures of rice amylopectins with low and high affinities for iodine. *Carbohydr. Res.* 168:79-88.

Villareal, C. P., de la Cruz, N. M., and Juliano, B. O. 1994. Rice amylose analysis by near-infrared transmittance spectroscopy. *Cereal Chem.* 71:292-296.

Welsh, L. A., and Blakeney, A. B. 1992. Choosing an amylose standard for cereal starch analysis. Pages 347-350 in: *Proceedings of the 42nd Australian Cereal Chemistry Conference*. V. J. Humphrey-Taylor, ed. RACI: Melbourne.

Yun, S.-H., and Matheson, N. K. 1990. Estimation of amylose content of starches after precipitation of amylopectin by concanavalin-A. *Starch/Staerke* 42:302-305.

[Received December 28, 1998. Accepted July 15, 1999.]