JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2009, 57, 6443–6448 6443
DOI:10.1021/jf900445w

Fourier Transform Infrared (FT-IR) Microspectroscopic Census of Single Starch Granules for Octenyl Succinate Ester Modification

Yanjie Bai, Yong-Cheng Shi,* and David L. Wetzel*

Microbeam Molecular Spectroscopy Laboratory and Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506

Fourier transform infrared (FT-IR) microspectroscopy was used to investigate reaction homogeneity of octenyl succinic anhydride modification on waxy maize starch and detect uniformity of blends of modified and native starches. For the first time, the level and uniformity of chemical substitution on individual starch granules were analyzed by FT-IR microspectroscopy. More than 100 starch granules of each sample were analyzed one by one by FT-IR microspectroscopy. In comparison to the native starch, modified starch had two additional bands at 1723 and 1563 cm^{-1}, indicative of ester formation in the modified starch. For the 3% modification level, the degree of substitution (DS) was low (0.019) and the distribution of the ester group was not uniform among starch granules. For the modified starch with DS of 0.073, 99% of individual starch granules had a large carbonyl band area, indicating that most granules were modified to a sufficient extent that the presence of their carbonyl ester classified them individually as being modified. However, the octenyl succinate concentration varied between granules, suggesting that the reaction was not uniform. When modified starch (DS = 0.073) was blended with native starch (3:7, w/w) to achieve a mixture with an average DS of 0.019, FT-IR microspectroscopy was able to detect heterogeneity of octenyl succinate in the blend and determine the ratio of the modified starch to the native starch granules.

KEYWORDS: FT-IR microspectroscopy; octenyl-succinic-anhydride-modified starch

INTRODUCTION

Starch occurs in higher plants as granules. A granule of waxy maize starch has a typical diameter of 15 μm (*1*). The reaction of solid starch granules with an organic acid anhydride in an aqueous slurry produces ester links with OH groups on glucose units. The modified starch that has hydrophobic groups replacing some OH sites has broad industrial uses as an emulsifying agent and additive to a large variety of products (*2*). One important derivative of this type of product is octenyl succinic anhydride (OSA)-modified starch, which is approved for use in foods by the U.S. Food and Drug Administration (FDA) and many other countries. The maximum level of the OSA treatment allowed for food applications is 3%. Heretofore, on a bulk scale, titration (*3*−*5*) or nuclear magnetic resonance (NMR) (*6*, *7*) has been used to determine the overall ester population relative to the native starch substrate. In addition, spectral features of carbohydrates are well-known from initially dispersive infrared spectroscopy (*8*, *9*) in a transmission mode applied to samples in a Nujol mull and, subsequently, with the KBr pellet technique. Conventional Fourier transform infrared (FT-IR) spectroscopy on a macro scale was reported by numerous workers (*10*−*13*). Formation of the octenyl succinate ester results in the appearance of the carbonyl band at 1724 cm^{-1} and asymmetric stretch of the carboxylate at 1572 cm^{-1} (*10*, *12*). In commercial production of chemically modified starch, assurance of homogeneity of the product and stoichiometry of the reaction at the desired level is of concern. In this study, for the first time, the spatial resolution achievable with image plane masking of FT-IR microspectroscopy enabled analysis of individual starch granules for the presence of a bound ester group.

In 1993, we reported mapping a single wheat aleurone cell and cell wall highlighting each with a different functional group map (*14*). Subsequently, the first imaging of a living cell undergoing mitosis was reported (*15*). Large-scale spectroscopic analysis of typically 100 μm oral muscosa or cervical cells by cancer researchers is now common practice (*16*). Analysis of individual starch granules is, therefore, a reasonable experiment to perform. The objectives of this study were to (i) investigate the reaction uniformity by analyzing individual granules to determine what fraction of granules did in fact contain ester modification sites and by a focal plane array mapping of granule groups with different levels of modification on the microscopic stage and (ii) develop and verify FT-IR microspectroscopy as a tool to determine the homogeneity of blends of highly modified starch and native starch. The spatial resolution of FT-IR microspectroscopy enables the microstructure to be revealed at the level of individual starch granules. This is achieved by reflection absorption measurement on an infrared reflecting glass substrate.

*To whom correspondence should be addressed. Telephone: 785-532-6771 (Y.-C.S.); 785-532-6005 (D.L.W.). Fax: 785-532-7010 (Y.-C.S.); 785-532-7010 (D.L.W.). E-mail: ycshi@ksu.edu (Y.-C.S.); dwetzel@ksu.edu (D.L.W.).

Published on Web 06/24/2009

MATERIALS AND METHODS

Sample Preparation. Waxy maize starch (AMOICA TF) was obtained from the National Starch and Chemical Company (Bridgewater, NJ). Waxy maize starch was reacted with different levels of octenyl succinic anhydride (Gulf Bayport Chemicals L.P., Pasadena, TX) (3, 9, 15, and 25%, based on starch weight), producing a series of octenyl-succinate-modified waxy maize starches that had degree of substitution (DS) values of 0.019, 0.056, 0.073, and 0.11, respectively. DS was determined by a titration method (*3*, *12*). The modified starch was prepared as follows. Waxy maize starch (100 g, dry weight) was suspended in 150 mL of water at room temperature, and the slurry was mixed homogeneously by an overhead stirrer. The pH of the slurry was adjusted to 7.5 with 3% (w/w) NaOH solution. Different levels of OSA were added by a buret in different time intervals from 30 s to over 4 h. The pH of the starch slurry was controlled at 7.5 by a pH controller (model 501-3400, Barnant Co., Barrington, IL). After the pH was stable for 30 min and there was no further consumption of NaOH, the reaction was terminated by adjusting pH to 6.0 with 1.0 M HCl. Starch was recovered by filtration, washed by 300 mL of water, dried at 40 °C in an oven for 48 h, and ground on an analytical mill. In addition, combining 3.00 g of modified starch (DS = 0.073) and 7.00 g of native starch resulted in a blend with an average DS of 0.019. Starch slurries of 1 wt % were prepared by suspending 0.1 g of starch in 10 mL water. A drop of starch slurry was placed on an infrared reflecting glass microscope slide (Tienta Sciences, Indianapolis, IN), oven-dried at 40 °C for 4 h and then 105 °C for 2 h. The dried starch granules viewed under a low-power microscope were flattened with a miniature (7 mm in diameter) stainless-steel roller until they became transparent to visible backlighting.

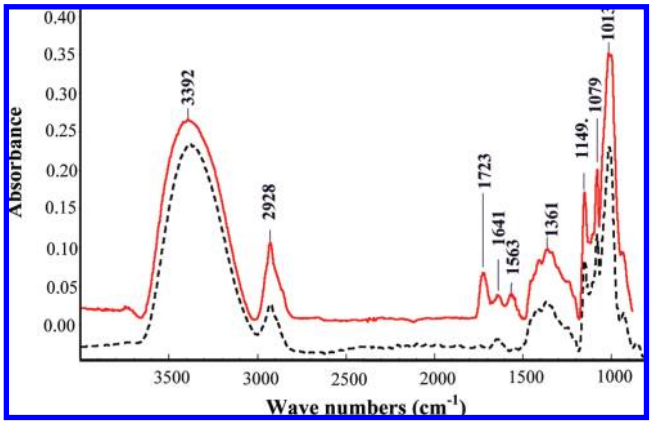

Figure 1. FT-IR spectra of native (- - -) and 25% OSA-modified waxy maize starch (DS = 0.11) (red —).

Instrumentation and Procedure. A Perkin-Elmer (Shelton, CT) Spectrum Spotlight 300 imaging system that was equipped with a single liquid-nitrogen-cooled mercury cadmium telluride (MCT) detector allowed single-image plane masking. The instrument was also equipped with a 16 element linear array of MCT detectors imaged at the focal plane. In the latter arrangement, the pixel size was determined by projecting the detector element through one of two optical systems onto the focal plane. This produced nominal pixel sizes of either $6.2 \times 6.2\ \mu$m or $25 \times 25\ \mu$m. The infrared microscope was equipped with front surface optic Schwarzschild mirror lenses before and after the microscope stage. The same optical path was used for both viewing the specimen with visible light by way of a video camera and interrogation of the sample with infrared rays coming from the beam splitter of the FT-IR spectrometer. From the visible illumination optics, the target granule or group of granules was selected for either obtaining a single spectrum or mapping. The standard procedure for the preparation of tissue specimens for microspectroscopy involves microtoming frozen sections 2−8 μm thick for transmission measurement. Because starch granules have a nearly spherical shape with an average diameter of 15 μm, in the case of waxy maize, it is not possible to obtain a good spectrum without reducing the thickness and avoiding the lens effect that a spherical transmitting object produces. The latter causes a severe loss in signal because the lens function directs a great deal of the light from a direct transmission pathway with the resulting loss of signal. The signal-to-noise ratio for the experiment is poor. In addition, when the specimen is too thick, absorbance is too high and linearity is lost on the absorbance scale as bands approach saturation. After the starch granules were flattened by rolling with a small stainless-steel roller, their diameters were increased to approximately 50 μm. Those that appeared transparent under the microscope were tested for infrared transmission and the production of a good spectrum in which the absorbance was sufficiently low enough to avoid distortion of the major peaks of interest.

Prior to conducting a census of modification sites found in individual starch granules, starches prepared with various degrees of modification were imaged with a nominal pixel size of $6.2 \times 6.2\ \mu$m in an area of $150 \times 100\ \mu$m. Individual spectra were extracted from the large number of spectra obtained in the mapping procedure. The pixels from the resulting image were selected on the basis of their absorption characteristics of a particular band of interest. A functional group image of the baseline-corrected carbonyl absorption band area at 1723 cm^{-1} revealed the levels of modification within the sample. High intensity indicated a high level of ester formation. To avoid absorption band intensity variations because of differences in thickness of the specimen at that point, the band area of the carbonyl was divided by a representative area of the major carbohydrate band. Averaging the band area ratios from a number of spectra produces the spectroscopic response for starch at a particular level of modification.

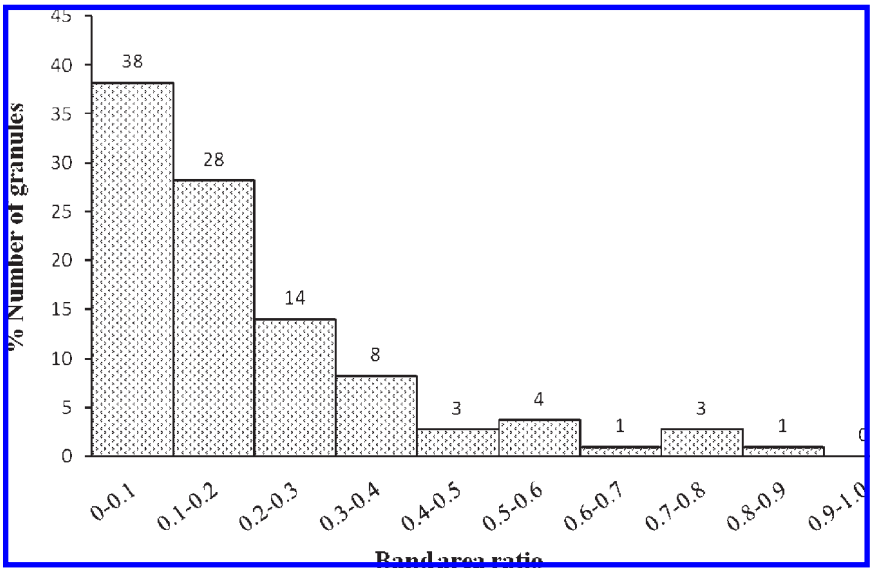

Figure 2. Band area ratio (carbonyl/carbohydrate) distribution of native waxy maize starch.

To conduct a census of three different starch specimens, a single MCT detector was used with single-image plane masking of $15 \times 15\ \mu m$; this was suitable for producing good spectra in less than 2 min without the necessity of co-adding an excessive number of scans. In one field of view, typically there were 30−40 starch granules. Granules that, after flattening, appeared transparent from visible light received a preliminary test of 10 scans. Only granules that produced a clear spectrum with a smooth baseline were selected to be analyzed with the co-addition of 128 scans to record their spectrum. The carbonyl contribution, if any, appeared in the resulting single-granule spectrum. This procedure was applied to more than 100 individual granules from each of three different waxy maize starch lots. Baseline data were collected by interrogating more than 100 individual native waxy maize starch granules. In a second experiment, more than 100 individual 15% OSA-modified starch granules were interrogated. A third group of granules interrogated was composed of a blend that contained three parts by weight of 15% modified granules to seven parts of native starch granules. This latter mixed group was calculated to produce a 3% overall octenyl succinate ester modification. In all cases, spectroscopic resolution was set at 8 cm^{-1}. The co-addition of 128 scans was used for both the 16 element "pushbroom" mapping procedures and the single detector experiments on individual granules.

Data Processing. Spectrum Spotlight Software, version 5.3.1 (Perkin-Elmer, Shelton, CT), was used for the calculation of band areas and ratios of band areas. Localized baseline adjustment was performed in all cases. This was accomplished by specifying the points on the frequency scale for the determination of the baseline. Two additional points within the baseline frequency region defined the part of the band to be integrated. In this case, the band between 1764 and 1687 cm^{-1} originated from the carbonyl group and its area was integrated. The carbohydrate band area was integrated between 187 and 954 cm^{-1}, which resulted from the C−O stretching vibration in starch. To correct the variation in thickness within the specimen, the baseline-corrected area of the carbonyl band was ratioed to the carbohydrate band area. The band area ratio was calculated as (band area of carbonyl/band area of carbohydrate) × 100. Functional group images were produced from the mapping procedures to highlight the carbonyl concentration at 1723 cm^{-1}. A false color scheme was used in the z axis of the images, where red indicated the strongest absorbance and blue indicated the lowest value. For each spectrum obtained from individual starch granules in the census procedure, the baseline-adjusted band area ratio of carbonyl to starch was determined.

Statistical Analysis. The band area ratio data of the samples was analyzed by the SAS program (version 9.1.3, SAS Institute, Inc., Cary, NC). A boxplot of each sample was obtained.

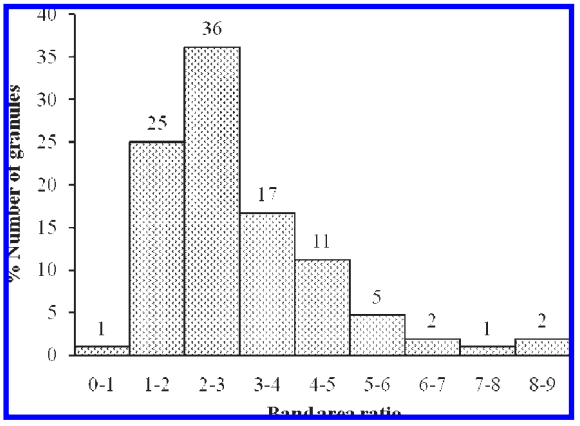

Figure 3. Band area ratio (carbonyl/carbohydrate) distribution of 15% OSA-modified starch (DS = 0.073).

RESULTS AND DISCUSSION

FT-IR Spectra of Native and OSA-Modified Waxy Maize Starches. Figure 1 shows the FT-IR spectra of native and OSA-modified starches. The band at 3392 cm^{-1} was assigned to the OH stretch of starch. The 2928 cm^{-1} CH stretching vibrational band was enhanced in the modified starch by contribution from the carbon chain associated with the octenyl succinic group. In comparison to the native starch, modified starch had two

Table 1. Statistical Analysis of the Band Area Ratio of Starch Samples

sample	mean	median	75% quartile (Q3)	25% quartile (Q1)	maximum value	minimum value
native waxy maize starch	0.18	0.14	0.23	0.04	0.90	0
3% OSA-modified starch	0.44	0.32	0.48	0.18	2.50	0
15% OSA-modified starch	2.99	2.63	3.66	1.98	9.48	0.05

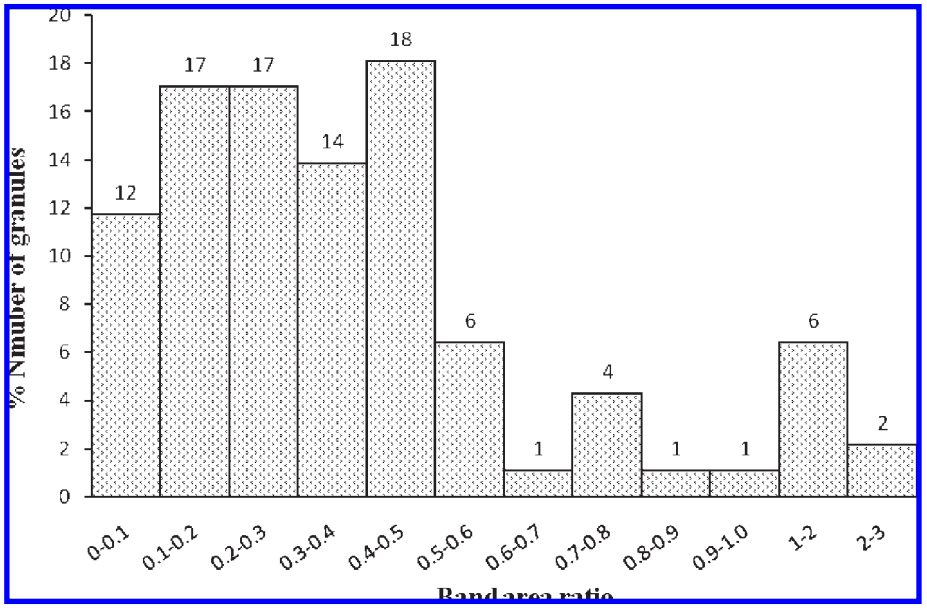

Figure 4. Band area ratio (carbonyl/carbohydrate) distribution of 3% OSA-modified starch (DS = 0.019). Note: the number of starch granules in each band area ratio was divided by the total number of starch granules and rounded to whole numbers, as shown in the bar graph. In this particular case, the results gave an apparent loss of 1% starch granules when the data were rounded to whole numbers.

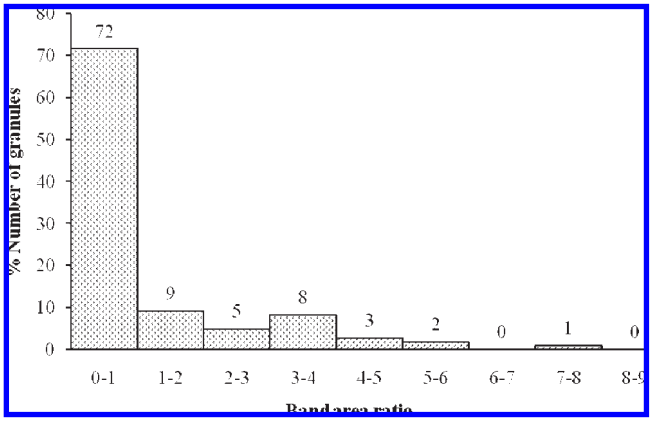

Figure 5. Band area ratio (carbonyl/carbohydrate) distribution of a starch blend (DS = 0.019) of 15% OSA-modified (DS = 0.073) and native starches.

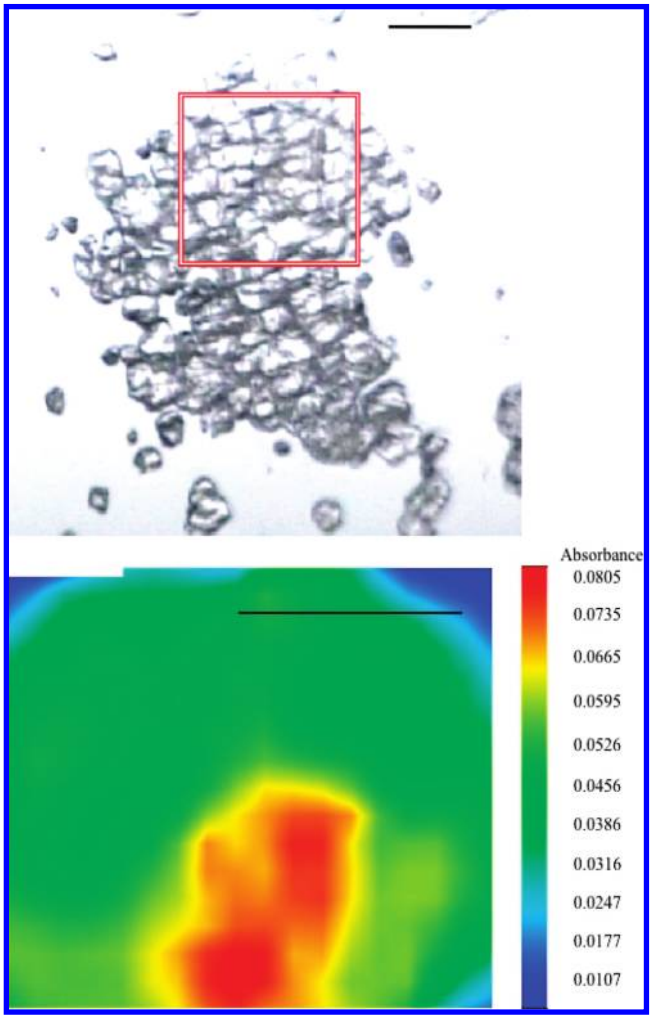

Figure 6. Photomicrograph of 9% OSA-modified starch (DS = 0.056) (top) and its single-band carbonyl functional map (1723 cm^{-1}) (bottom). The bar in each picture is 50 μm.

additional bands at 1723 and 1563 cm^{-1}. The band occurring at 1723 cm^{-1} originated from the carbonyl group as evidence of the formation of an ester. A carboxylate band occurring at 1563 cm^{-1} was also indicative of ester formation in the modified starch (*9*). For determination of the carbonyl band area at 1723 cm^{-1}, contributions from the H−O−H deformation band at 1641 cm^{-1} were deliberately avoided. This band was present because of the

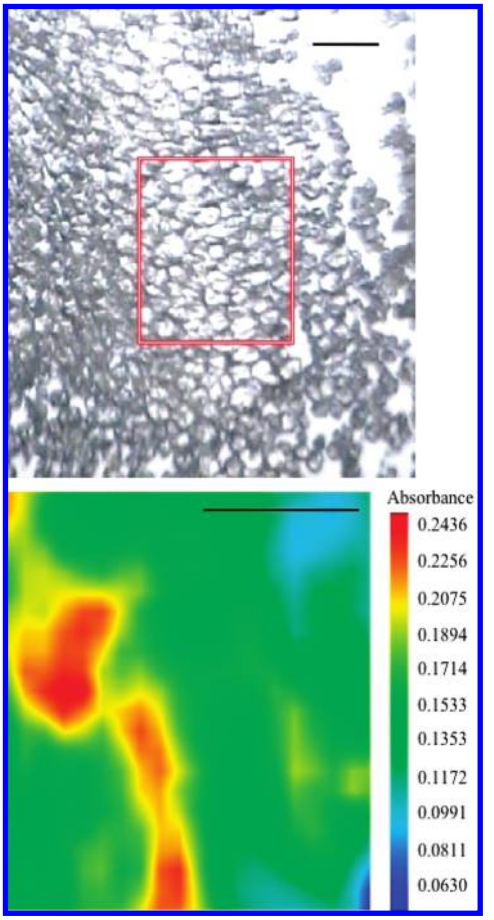

Figure 7. Photomicrograph of 25% OSA-modified starch (DS = 0.11) (top) and its single-band carbonyl functional map (1723 cm^{-1}) (bottom). The bar in each picture is 50 μm.

residual bound water. The ensemble of peaks that characterized starch vibrations had a maximum at 1013 cm^{-1} accompanied with lesser bands at 1149 and 1079 cm^{-1}.

Homogeneity via Census of Individual Starch Granules. *Individual Granules of Native Starch.* For native waxy maize starch granules, the carbonyl/carbohydrate band area ratio in **Figure 2** included 80% below 0.3, 95% below 0.6, and none of the granules above 0.9. All starch granules were regarded as native.

Individual Granules of 15% OSA-Modified Starch (DS = 0.073). Individual granules of modified starch were analyzed by FT-IR microspectroscopy. Spectra from 15% OSA-modified starch granules were obtained with a high signal-to-noise ratio. The absorbance of each functional group was within the acceptable limit to avoid nonlinearity. The band area ratios were divided into nine groups (**Figure 3**). Of the individual starch granules analyzed, 99% had carbonyl/carbohydrate band area ratios higher than 1 and distributed from 1 to 9. These results indicate that 99% of the experimental waxy maize starch granules were modified to a sufficient extent that the presence of their carbonyl ester classified them individually as being modified. However, the octenyl succinate concentration varied from granule to granule, indicating that the reaction was not uniform. Previous work using osmium staining with OSA-modified waxy maize starch of DS of 0.03−0.11 suggested that OSA groups are located throughout the starch granule, but analysis of debranched modified starch with pullulanase indicated heterogeneity in OSA distribution at the branch level (*17*).

Individual Granules of 3% OSA-Modified Starch (DS = 0.019). When starch granules with only 3% modification

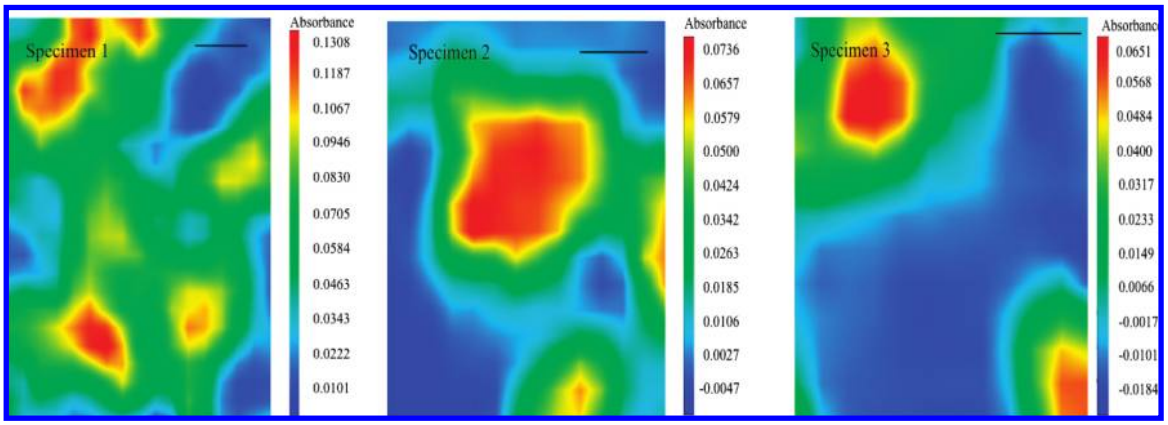

Figure 8. Single-band chemical maps (1723 cm^{-1}) of a starch blend (DS = 0.056) of 25% OSA-modified (DS = 0.11) and native starches. The bar in each picture is 20 μm.

(DS = 0.019) were analyzed by FT-IR microspectroscopy, the DS of each granule, as anticipated, was low and the distribution of the ester group in 3% OSA-modified starch (DS = 0.019) was apparently non-uniform. Of all of the granules examined, 92% showed the band area ratio below 1 and the rest of the granules had the band area ratio from 1 to 3 (**Figure 4**). In comparison to the native starch (**Figure 2**), the modified starch had fewer granules with the band area ratios between 0 and 0.3 but more in the range of 0.3−1 (**Figure 4**) and a larger proportion of granules having band ratios greater than 0.18 (**Table 1**).

Individual Granules of a Mixed Population of Modified (DS = 0.073) and Native Starches. A blend (average DS = 0.019) was prepared by mixing 70% native starch and 30% modified starch (DS = 0.073). Among the 110 granules of the mixture analyzed, 72% of the starch granules had band area ratios below 1 and 28% of the starch granules had band area ratios from 1 to 8 (**Figure 5**). When the starch granules with band area ratios below 1 were further analyzed, the shape of the distribution was similar to that of native starch. The experimental census of 110 individual granules nearly matched the calculated formulation of 30% modified starch used to produce the synthetic mixture. Results of this novel single granule analysis suggest that FT-IR microspectroscopy is a useful tool for detecting heterogeneity of starch blends containing octenyl-succinate-modified waxy maize and native starch. The process can be used to verify a suspected starch product that is blended with highly modified and native starches.

Focal Plane Array Imaging of Groups of Granules. *OSA-Modified Starches (DS = 0.056 and 0.11)*. The waxy maize starch was reacted with 9 and 25% OSA to achieve a DS of 0.056 and 0.11, respectively. The bulk area of modified starch granules (DS = 0.056) imaged by FT-IR microspectroscopy was observed from the carbonyl functional group map, and a false color scheme was produced (**Figure 6**). Color in the carbonyl functional group map reflected absorbance and level of substitution. Red indicated the strongest absorbance and highest level of modification, whereas blue indicated the lowest value. The carbonyl absorption at 1723 cm^{-1} was noted throughout the specimen, indicating that the starch granules were reacted with the octenyl succinate groups. However, the octenyl succinate quantitative information may have been affected by the sample thickness because starch bands for comparison were not available with a specimen this thick.

With another modified starch specimen (DS = 0.11) that was mapped within the selected area of the specimen, carbonyl absorption was evident among the starch granules (**Figure 7**). This result suggested that the majority of starch granules were reacted to form ester links, although the carbonyl content differed from granule to granule.

Blend of OSA-Modified (DS = 0.11) and Native Starches. A precision blend of octenyl-succinate-modified starch (DS = 0.11) and native waxy maize starch was prepared to achieve an average DS of 0.056. Some spots having very low carbonyl absorption values (0−0.01) are shown in blue in **Figure 8**. These spots were assumed to be native starch. The FT-IR microspectropic techniques used were able to detect the native starch and also differentiated starch blends from uniformly modified starch lots. The time of mapping a 100 × 200 μm specimen was 20 min or less, which was much shorter than required for analyzing individual starch granules one by one. The mapping procedure is an effective and reasonably accurate method to detect the two components and their distribution within starch blends.

In conclusion, for the first time, the level and reaction uniformity of chemical substitution on individual starch granules were analyzed by FT-IR microspectroscopy. When waxy maize starch was reacted with 15% OSA, most starch granules were esterified, although the octenyl succinate content varied from granule to granule. For the 3% modification level, the degree of substitution was low and the distribution of the ester group was not uniform among starch granules. FT-IR microspectroscopy was also useful for detecting heterogeneity of octenyl succinate in a blend of modified (DS = 0.073) and native starches (3:7, w/w), and the technique may be applied to other chemically modified starches. Continued research is being conducted to determine the distribution of chemical modification sites within a single starch granule.

ACKNOWLEDGMENT

We thank Hicran Koc and Lauren Brewer, who facilitated use of the instrument and software, Yoonsung Jung for statistical analysis, and the microbeam Microbeam Molecular Spectroscopy Laboratory and the Kansas Agricultural Experiment Station for support.

LITERATURE CITED

(1) Wurzburg, O. Introduction. In *Modified Starches: Properties and Uses*; Wurzburg, O., Ed.; CRC Press: Boca Raton, FL, 1986; pp 4−15.
(2) Trubiano, P. Succinate and substituted succinate derivatives of starch. In *Modified Starches: Properties and Uses*; Wurzburg, O., Ed.; CRC Press: Boca Raton, FL, 1986; pp 131−147.
(3) Bao, J. S.; Xing, J.; Phillips, D. L.; Corke, H. Physical properties of octenyl succinic anhydride modified rice, wheat, and potato starches. *J. Agric. Food Chem.* **2003**, *51*, 2283–2287.
(4) Bhosale, R.; Singhal, R. Process optimization for the synthesis of octenyl succinyl derivative of waxy corn and amaranth starches. *Carbohydr. Polym.* **2006**, *66*, 521–527.

(5) He, G. Q.; Song, X. Y.; Ruan, H.; Chen, F. Octenyl succinic anhydride modified early indica rice starches differing in amylose content. *J. Agric. Food Chem.* **2006**, *54*, 2775–2779.
(6) Shih, F. F.; Daigle, K. W. Gelatinization and pasting properties of rice starch modified with 2-octen-1-ylsuccinic anhydride. *Nahrung* **2003**, *47*, 64–67.
(7) Čížová, A; Koschella, A.; Heinze, T.; Ebringerová, A.; Sroková, I. Octenylsuccinate derivatives of carboxymethyl starch—Synthesis and properties. *Starch/Staerke* **2007**, *59*, 482–492.
(8) Mathlouthi, M.; Koenig, J. Vibrational spectra of carbohydrates. *Adv. Carbohydr. Chem. Biochem.* **1986**, *44*, 7–89.
(9) Kacurakova, M.; Wilson, R. H. Development in mid-infrared FT-IR spectroscopy of selected carbohydrates. *Carbohydr. Polym.* **2001**, *44*, 291–303.
(10) Fang, J.; Fowler, P.; Sayers, C.; Williams, P. The chemical modification of a range of starches under aqueous reaction conditions. *Carbohydr. Polym.* **2004**, *55*, 283–289.
(11) Chi, H.; Xu, K.; Xue, D. H.; Song, C. L.; Zhang, W. D.; Wang, P. X. Synthesis of dodecenyl succinic anhydride (DDSA) corn starch. *Food Res. Int.* **2007**, *40*, 232–238.
(12) Song, X.; He, G.; Ruan, H.; Chen, Q. Preparation and properties of octenyl succinic anhydride modified early *Indica* rice starch. *Starch/Staerke* **2006**, *58*, 109–117.
(13) He, J.; Liu, J.; Zhang, G. Slowly digestible waxy maize starch prepared by octenyl succinic anhydride esterification and heat–moisture treatment: Glycemic response and mechanism. *Biomacromolecules* **2008**, *9*, 175–184.
(14) Wetzel, D. L.; Reffner, J. A. Using spatially resolved Fourier transform infrared microbeam spectroscopy to examine the microstructure of wheat kernels. *Cereal Foods World* **1993**, *38*, 9–20.
(15) Jamin, N.; Dumas, P.; Moncuit, J.; Fridman, W.; Teilland, J.; Carr, G.; Williams, G. Highly resolved chemical imaging of living cells by using synchrotron infrared microspectrscopy. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4837–4840.
(16) Matthäus, C.; Miljković, M.; Romeo, M.; Boydston-White, S.; Diem, M. Raman and infrared micro-spectral imaging of mitotic cells. *Appl. Spectrosc.* **2006**, *60*, 1–8 (cover article).
(17) Shogren, R. L.; Viswanathan, A.; Felker, F.; Gross, R. A. Distribution of octenyl succinate groups in octenyl succinic anhydride modified waxy maize starch. *Starch/Staerke* **2000**, *52*, 196–204.

Received February 8, 2009. Revised manuscript received May 25, 2009. Accepted June 09, 2009. Contribution 09-176-J from the Kansas Agricultural Experiment Station is gratefully acknowledged.