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Distribution of Octenylsuccinic Substituents in Modified A and B Polymorph Starch Granules

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ABSTRACT: The octenylsuccinic (OS) substituent distribution in octenylsuccinic anhydride (OSA)-modified normal maize and potato starches with different degrees of subsititution (DS) was studied using confocal laser scanning microscopy (CLSM) and surface gelatinization. The remaining non-gelatinized portions of starch granules after removal of surface-gelatinized starch (remaining granules) were studied with light microscopy, scanning electron microscopy, Fourier transform infrared spectroscopy (FTIR), and the level of succinylation. Results showed that greater proportions of the OS groups were present at the periphery than at the core of the granules. However, the granular interior of OS maize starch has higher fluorescent intensity than that of OS potato starch, as shown by CLSM. The DS of OS maize starch degraded less than that of OS potato starch under the same degree of gelatinization. In addition, the characteristic peaks of the remaining OS maize granules in the FTIR were more protruding than that of the OS potato granules after 50% chemical surface gelatinization. The results implied that maize starch displayed much more homogeneous OSA reaction pattern when compared to potato starch. With the special architectures (pinholes and channels) of maize, it is easier to change the location of OS groups than with potato starch by changing reaction conditions or starch pretreatments.

KEYWORDS: *Granular starch, octenylsuccinic anhydride-modified starch, substituent distribution*

INTRODUCTION

Octenylsuccinate (OS) starch is usually prepared by an alkaline-catalyzed reaction of octenylsuccinic anhydride (OSA) with granular starch in an aqueous suspension. OS starch contains both hydrophilic and hydrophobic groups and displays useful emulsifying, thermal, nutritional, and rheological properties.[1] In recent years, the materials, optimized reaction parameters, OS group distributions, physiochemical properties, and industrial applications of OS starch have been studied extensively.[2−7] Shogren et al.[8] suggested that OS groups on the immediate surface of the OS starch granules were about 3−4 times that of the bulk, as observed by OsO_4 staining and backscattered electron imaging. Because of the poor penetration of the big oily droplets of OSA into starch granules in an aqueous suspension, the reaction sites are mostly limited to the granular surface. The surface of OS maize starch is enriched with OS groups by a factor of 2−4 over that of the whole granule.[9]

Starch granules from various botanical sources differ in shape, size, and morphology. Most starch granules consist of amylose and amylopectin molecules. Starch granules possess different types of crystallinity, displaying A-, B-, and C-type X-ray patterns, depending upon their amylopectin branch chain length.[10] Starch with shorter chains displays A-type X-ray pattern, and starch with longer chains displays B-type X-ray pattern.[11] The amylopectin structure of A-type starch has more short A chains (DP 6−12) than that of the B-type starch. The short A chain is likely attached to a B chain with the branch linkage located in the crystalline region. The branch linkages present in the crystalline region and the short double helices derived from the short A chains provide the "weak points", which are more susceptible to acid hydrolysis.[12] Surface pinholes and serpentine-like channels throughout are commonly found in A-type starch granules, such as maize, but are not found in B-type starch, such as potato.[10] Recent studies have indicated that the amylose content is higher at the periphery than at the core of both maize[13] and potato starch.[14] Amylose molecules are randomly interspersed among amylopectin molecules, and the associated amylose and amylopectin contribute to the internal structures of starch. The A-type starch granules have loosely packed internal structures, which are easily hydrolyzed and more sensitive to chemical reactions, whereas the B-type granules possess a solid internal structure.[10]

The reaction sites within starch granules could be affected by the structure of the starch granule and reagents. Azemi and Wootton[15] reported that hydroxypropylated waxy, normal, and high-amylose maize starches differed with regard to their distribution of substituent groups on starch polymer molecules. Huber and BeMiller[16] hypothesized that channels and cavities have a significant role in directing the location of the reaction on a granule basis. Hydroxypropyl waxy maize starch and $POCl_3$-modified sorghum starch granules, which possess channels leading to their interiors, exhibited similar patterns of infiltration by reagents, while $POCl_3$-modified potato starch granules, which do not possess channels, reagent-diffused inward through exterior granule surfaces.

It is well-known that a high concentration of $CaCl_2$ and LiCl solutions can gelatinize starch granules from the periphery.[17] The cations of the salts interact with the hydroxyl groups of starch molecules, releasing heat that can melt nearby starch crystalline regions. The surface-gelatinized starch can be separated from starch, and then the inner fraction of the starch

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can be further analyzed. Huang et al.[18] investigated the properties of remaining granules obtained from acetylated waxy and normal potato starch samples (modified with acetic anhydride) after chemical surface gelatinization. Kuakpetoon et al.[19] found that there were more carboxyl groups at the periphery than at the core of oxidized corn starches by chemical surface gelatinization.

Although the synthesis and physicochemical characteristics of OS starch have been studied extensively,[2−7] little work has been reported on the OS substituent distribution differences between A- and B-type crystalline starches. Huber and BeMiller[20] have reported the architecture and permeability of maize starch granules. They have shown that pores on the surface of starch granules, internal cavities at the granule hilum, and channels connecting the two could significantly influence the granule reactions to chemicals. The structures of A- and B-type starch granules are quite different, which may result in the differences of OS substituent distribution in the modified starch. In addition, the distribution of OS groups consequently influences the paste stability.[9] Such knowledge about the differences of OS substituent distribution in A- and B-type starch granules would be helpful in understanding the physical behavior of starch chemical modification as well as designing modified starches with improved functional properties.

MATERIALS AND METHODS

Materials. Maize starch and potato starch were purchased from Dacheng Company (Changchun, China). OSA was obtained from Nanjing Golden Chemical Co., Ltd. (Nanjing, China). Other chemicals were commercial products of analytical reagent grade.

Measurement of Droplet Size Distribution of OSA Dispersed in Water. OS starch is usually prepared by an alkaline-catalyzed reaction of OSA with granular starch suspended in water. Extremely dispersed or droplets of OSA may be presented in the mixture, which may significantly effect the degree of subsititution (DS) or OS substitute distribution of OS starch. However, there is no report about the droplet size distribution of OSA in the reaction. We measured the droplet size distribution of OSA dispersed in water with different stirring speeds.

The OSA was dispersed in distilled water (250 mL) under different stripping speeds (100, 1000, and 10 000 revolutions/min) for 5 min. Particle size distributions of the samples were measured by an integrated laser light scattering instrument (Mastersizer 2000, Malvern Instruments, Ltd., Worcestershire, U.K.). Relative refractive index and absorption were set to 1.467 and 0.001, respectively. The average particle size (volume−surface average diameter, $d_{3,2}$) and volume percentage (%) were recorded.

Preparation of OS Starch. The starch [100.0 g, dry starch basis (dsb)] was suspended in distilled water (35%, w/w) with agitation. The pH was adjusted to 8.0−9.0 by adding 3% NaOH using a pH controller. A weighed quantity of OSA (1, 3, and 7% of the dsb, respectively) was added slowly within 2 h while maintaining the pH at 8.0−9.0. The reaction was allowed to proceed for 3 h in total at 35 °C. After reaction, the reaction mixture was neutralized to pH 6.5 with diluted HCl. The mixture was centrifuged and washed twice with distilled water and twice with ethanol. The OS starch was oven-dried at 40 °C for 24 h and then passed through a 100-mesh nylon sieve.[4]

Determination of the Degree of Substitution. OS starch (2.5 g, dsb) was accurately weighed and suspended by stirring for 30 min in 12.5 mL of HCl−isopropyl alcohol solution (2.5 M). Isopropyl alcohol solution (50 mL, 90%, v/v) was added and stirred for an additional 10 min. The suspension was filtered through a glass filter, and the residue was washed with 90% isopropyl alcohol solution until no Cl^- could be detected (using 0.1 M $AgNO_3$ solution). The starch was redispersed in distilled water (300 mL) and heated in a boiling water bath for 20 min with stirring. The starch dispersion was titrated with standard NaOH solution (0.1 M), using phenolphthalein as an end-point indicator. A blank was simultaneously titrated with native starch as a control.[4] The DS was calculated using eq 1

$$\mathrm{DS} = \frac{0.162(AM)/W}{1 - [0.209(AM)/W]} \quad (1)$$

where A is the titration volume of NaOH solution (mL), M is the molarity of NaOH solution, and W is the dry weight (g) of the OS starch.

Confocal Laser Scanning Microscopy (CLSM). The OS starches were dye-stained for CLSM and suspended in 30 mL of water. The pH of the suspensions was adjusted to 8.0, and then 1% methylene blue (MB^+) solution was added to each sample. The mixture was incubated in a water bath with a constant temperature vibrator at room temperature for 3 h, and the granules were washed with methanol to remove the residual dye.

A TCS SP5 CLSM fitted with an argon ion laser (Leica, Wetzlar, Germany) was used for the detection of the fluorescence signal from the dye-stained starch granules. The details of the Leica objective lens used were 40×/1.25 oil. The excitation wavelength was 514 nm with 56 capacity. During the image acquisition, each line was scanned 4 times, and the average was calculated to reduce the noise.[4]

Chemical Surface Gelatinization. Chemical surface gelatinization was conducted according to the method by Koch and Jane,[17] with some modifications. Starch samples (10 g) were suspended in a $CaCl_2$ (4 M) solution (75 mL) and stirred with a magnetic stirrer at room temperature for different periods of time to achieve different levels of surface gelatinization. The reaction was stopped by quickly adding 750 mL of cold (4 °C) distilled water. Samples were centrifuged at 5000*g* for 20 min, washed twice with distilled water, washed once with ethanol, and dried at 40 °C overnight.

The remaining non-gelatinized portions of starch granules were separated from the gelatinized starch by resuspending the surface-gelatinized starch in distilled water (30 mL) and blending with a commercial blender (JYL-C051, Joyoung Company, Ltd., Guangzhou, China) for about 20 min. The mixture was centrifuged at 3000*g* for 10 min. The procedure was repeated 3−5 times with distilled water (200 mL) until the supernatant was clear.

Fourier Transform Infrared Spectroscopy (FTIR). During the esterification, hydroxyl groups of starch molecules were substituted by carbonyl groups of OSA that can be confirmed by FTIR. Changes in the chemical structure of starch were analyzed by FTIR (Vector 33, Bruker Optics, Ettlingen, Germany) according to the method by Song et al.[4] OS starches were dried at 105 °C for 12 h before analysis to avoid interference from water. Samples were prepared by finely grinding starch with KBr in a ratio of 1:150 (w/w) and scanned over the wavenumber range from 400 to 4000 cm^{-1}.

Light Microscopy. Light microscopy was performed using an Olympus BX-51 light microscope (Tokyo, Japan) with normal light and polarized light. One drop of starch suspension (before centrifugation) was placed on the microscope slide before covering with a coverslip, and the images were recorded at 500× magnification.[21]

Scanning Electron Microscopy (SEM). Scanning electron micrographs were obtained with a scanning electron microscope (TM 3000, Hitachi, Tokyo, Japan). Starch samples were mounted on an aluminum stub using double-sided tape, coated with a thin film of gold (10 nm), and then examined at an accelerating voltage of 10 kV.

Statistical Analysis. All experiments were performed in triplicate, and results are expressed as their means ± standard deviation (SD). When necessary, the number of repetitions is noted in the text. The significance of the differences between groups was tested using *t*-test analysis. A probability level (p value) of <0.05 was considered to be statistically significant, unless stated otherwise. Statistical analysis was performed by the data analysis tool pack of SPSS 13.0.

RESULTS AND DISCUSSION

OS Substituent Distribution in the Starch Revealed by CLSM. CLSM is increasingly being applied in testing the internal structure of starch granules, such as channels, growth

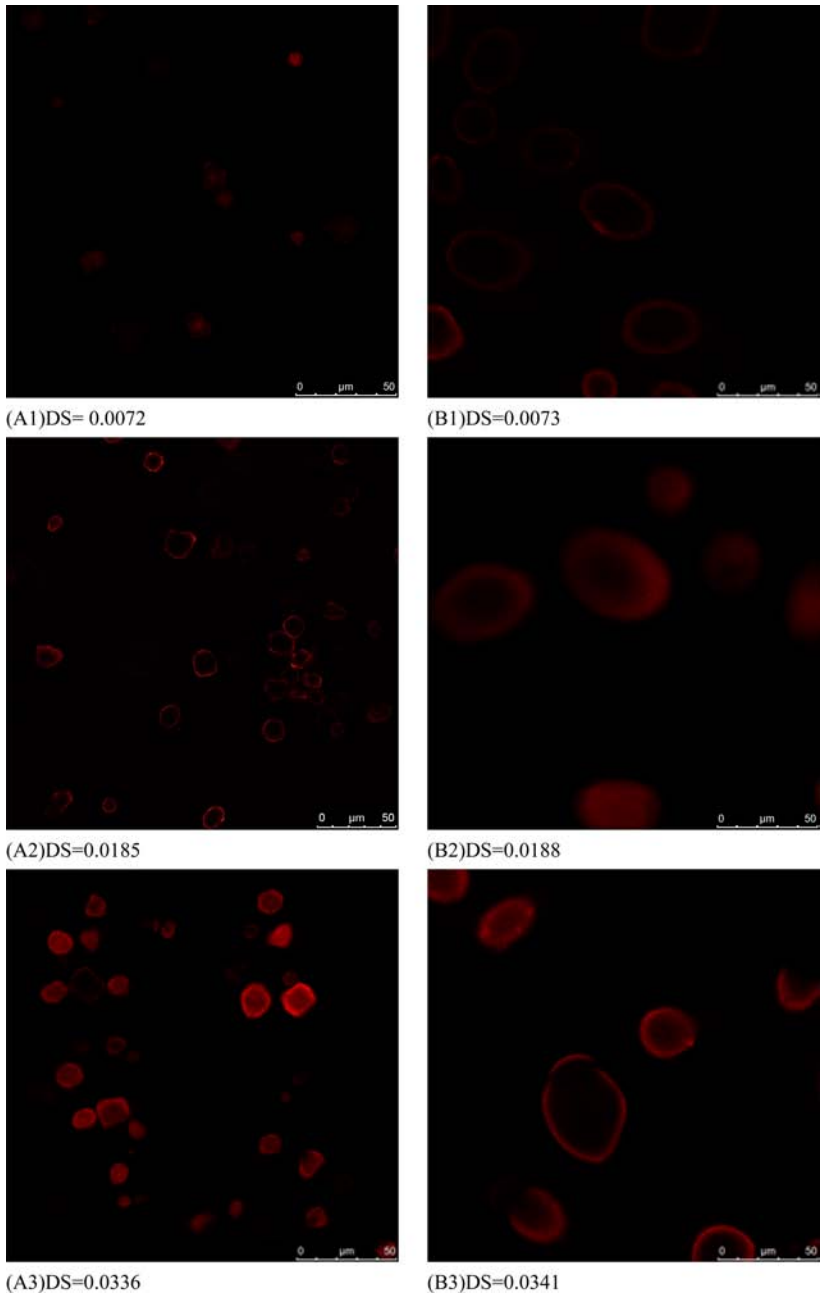

Figure 1. CLSM optical sections of native and OS starches with different DS were prepared by different amounts of OSA used (1, 3, and 7% of the dsb, respectively) in the reaction: (A1) OS maize starch, DS = 0.0072; (B1) OS potato starch, DS = 0.0073; (A2) OS maize starch, DS = 0.0185; (B2) OS potato starch, DS = 0.0188; (A3) OS maize starch, DS = 0.0336; and (B3) OS potato starch, DS = 0.0341.

rings, equatorial grooves, and the distribution of amylose, protein, and phosphate.[22−26] In this study, OS substituent distribution in the starch granules was investigated using CLSM after being stained by MB^+ dye. MB^+ is one of the fluorescence dyes that highlight anionic substances, which has previously been used for specific labeling of anionic head groups of sodium dodecyl sulfate.[27] In addition, MB^+ specifically stains $-COO^-$ of OS groups; thus, the OS substituent distribution in starch granules could be clearly revealed.[4]

The CLSM optical sections of OS starch granules are shown in Figure 1. The native starch granules did not show any fluorescence (photo not shown), while the OS starch granules had fairly uniform bright fluorescence, especially on the surface. OS starch showed stronger fluorescence intensity as the DS increased (Figure 1). In comparison to the OS potato starch, the interior of OS maize starch granules, especially for the sample with higher DS, showed stronger fluorescence intensity.

Maize starch has some pinholes on the surface and serpentine-like channels inside of the granules, whereas potato starch does not have these structural features. During enzyme digestion, enzymes (size of ∼5 nm for α-amylase and 8−10 nm for amyloglucosidase) tend to migrate inside the starch granule through susceptible sites, e.g., surface pores (0.1−0.3 μm), cavities, and channels (0.07−0.1 μm), and return to the surface after all material is consumed.[28]

To evaluate the droplet size of OSA during the reaction, the average particle size of OSA with different stirring speeds was determined and the result is shown in Figure 2. The particle

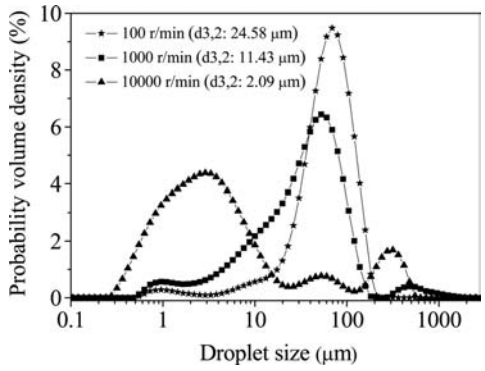

Figure 2. Droplet size distributions of OSA dispersed in water with different stirring speeds.

size of OSA showed a bi- or trimodal profile, and the average particle size decreased with the increasing stirring speed. The $d_{3,2}$ value of OSA decreased from 24.58 to 2.09 μm when the stirring speed increased from 100 to 10 000 revolutions/min. The extremely dispersed OSA around 0.1 μm could not be detected because of the effect of the big droplets. The results proved that there were unsolvable OSA droplets in the starch slurry during the reaction. When the size of unsolvable OSA droplets decreased to 0.1 μm, it can tend to migrate inside the granule through surface pores and channels. Therefore, the dissolved or some extremely dispersed OSA could penetrate the entire maize starch granule via the channels. However, either OSA droplets or dissolved OSA only could react with the granular surface of potato starch; thus, the granular interior of maize starch has higher fluorescence intensity than that of potato starch (Figure 1). In other words, more OS groups were distributed to the inner region of the OS maize starch when compared to the OS potato starch.

OS Substituent Distribution in Starch Granules Determined by Surface Gelatinization. To further study the OS substituent distribution in starch granules, OS maize starch and OS potato starch were treated with 4 mol/L $CaCl_2$ solution and a similar degree of gelatinization was obtained.

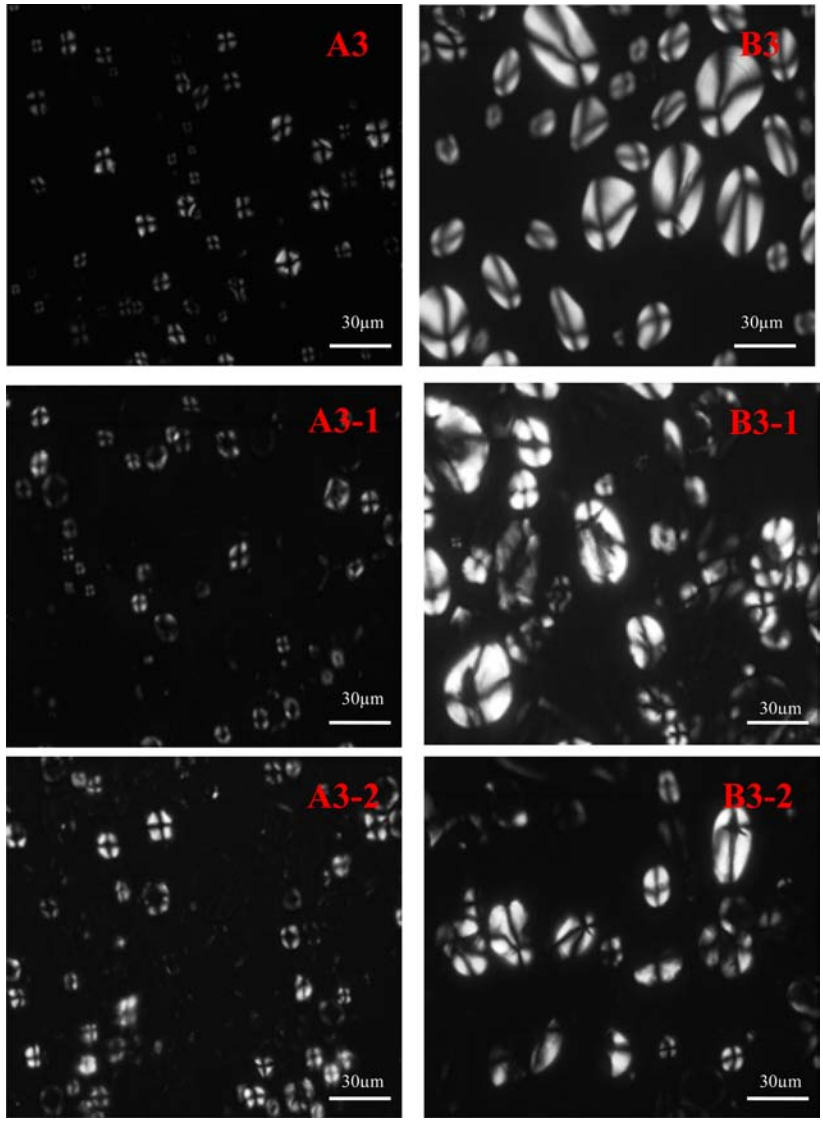

Figure 3. Polarized light micrographs of OS starch: (A3) OS maize starch, DS = 0.0336; (B3) OS potato starch, DS = 0.0341; and remaining granules of (A3-1 and A3-2) OS maize starch and (B3-1 and B3-2) OS potato starch after 20 and 50% degree of gelatinization, respectively.

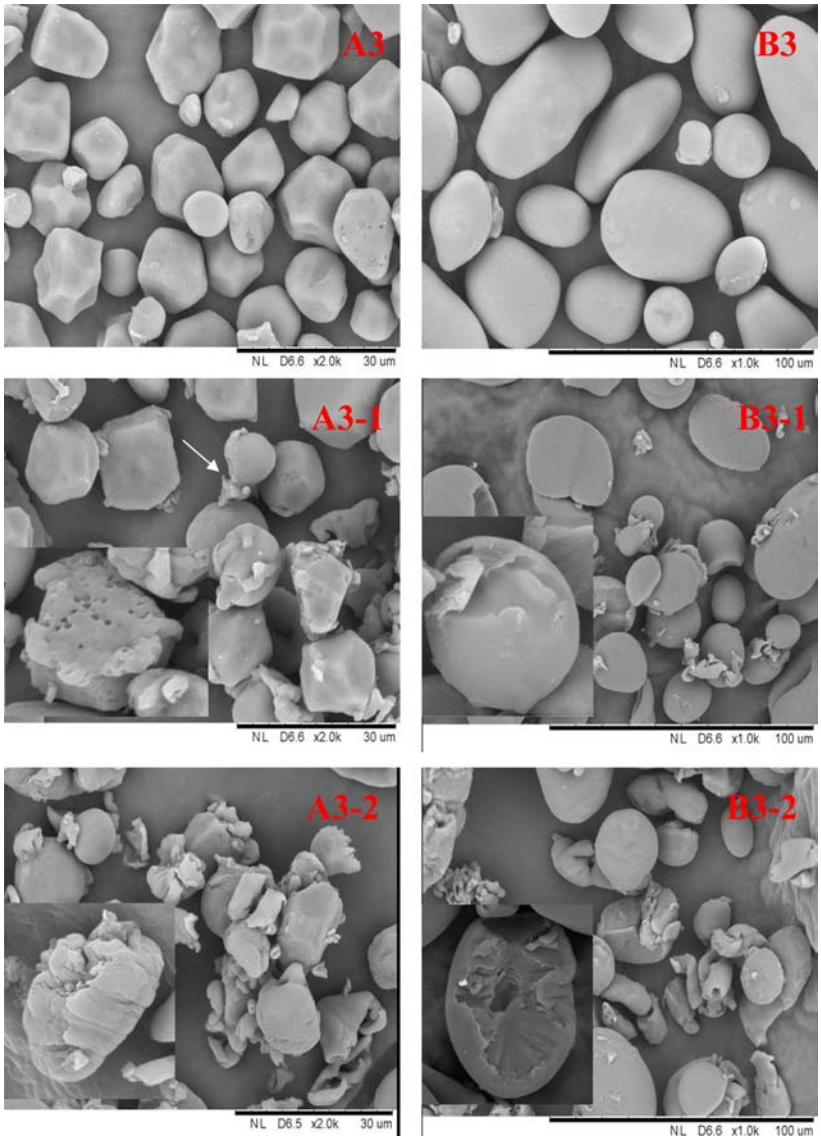

Figure 4. Scanning electron micrographs of OS starch: (A3) OS maize starch, DS = 0.0336; (B3) OS potato starch, DS = 0.0341; and remaining granules of (A3-1 and A3-2) OS maize starch and (B3-1 and B3-2) OS potato starch after 20 and 50% degree of gelatinization, respectively.

The polarized light micrographs of OS maize starch (DS = 0.0336), OS potato starch (DS = 0.0341), and their remaining granules after 20 and 50% chemical surface gelatinization are shown in Figure 3. The remaining granules still displayed Maltese cross, suggesting that the granular structure of the remaining starch was preserved.[17] Chemical surface gelatinization seems to take place on all granules as demonstrated by the presence of both large and small granules even after 20 and 50% surface removal. Therefore, the results from this study could represent the whole granule population of maize starch and potato starch. The polarized light microscopic observation of normal starch showed strong birefringence patterns with a distinct and integrated Maltese cross centered at the hilum, whereas birefringence patterns were less distinct on some remaining granules and the Maltese cross of some remaining granules were deformed.

The morphology of the remaining granules obtained after treatment with $CaCl_2$ solution was also investigated by SEM (Figure 4). These remaining granules were left with a rough surface, indicating that the granule was peeled from the surface. Furthermore, erosion occurred to both large and small granules, which confirmed the uniformity of chemical gelatinization (panel A3-1 of Figure 4). The granules were more evenly eroded with the higher degrees of chemical gelatinization. The salt attack has taken place not only on the protruding edges of maize starch of the granules but all over the granules. Meanwhile, maize starch granules exhibit pores that could be sites for salt attack (panel A3-1 of Figure 4). However, the salt attack has taken place asymmetrically on the one side of the potato starch. The results are consistent with the previous studies by Koch and Jane[17] and Huang et al.[18] It was suggested that there were holes on the surface of potato-remaining granules, therefore possibly rendering this region more prone to salt attack. The results indicate that structural diversity exists between maize and potato starch granules.

The remaining granules with the higher degrees of chemical gelatinization showed lower degrees of substitution for all starch samples (Table 1). These data indicate that the OS substituent distribution depends upon the starch structure and level of substitution. The total OS groups of gelatinized peripheral starch granules could be calculated as eq 2

Table 1. Degree of Substitution on Native Starches and Remaining Granules after Different Degrees of Surface Removal[a]

sample	degree of chemical gelatinization (w/w, %)	degree of substitution
A1	0	0.0072 ± 0.0005
	21	0.0050 ± 0.0003
	51	0.0042 ± 0.0007
B1	0	0.0073 ± 0.0006
	23	0.0049 ± 0.0011
	49	0.0040 ± 0.0008
A2	0	0.0185 ± 0.0005
	20	0.0130 ± 0.0021
	52	0.0106 ± 0.0030
B2	0	0.0188 ± 0.0008
	20	0.0124 ± 0.0009
	49	0.0097 ± 0.0014
A3	0	0.0336 ± 0.0019
	20	0.0267 ± 0.0016
	54	0.0197 ± 0.0010
B3	0	0.0341 ± 0.0006
	23	0.0243 ± 0.0021
	51	0.0156 ± 0.0008

[a]OS starches with different DS were prepared by different amounts of OSA used (1, 3, and 7% of the dsb, respectively) in the reaction. (A1) OS maize starch, DS = 0.0072; (B1) OS potato starch, DS = 0.0073; (A2) OS maize starch, DS = 0.0185; (B2) OS potato starch, DS = 0.0188; (A3) OS maize starch, DS = 0.0336; and (B3) OS potato starch, DS = 0.0341.

$$T = \frac{DS_0 - DS_1(1 - D)}{DS_0} \times 100\% \quad (2)$$

where T is the total OS groups of gelatinized peripheral starch, DS_0 is the DS of original OS starch granules, DS_1 is the DS of remaining granules, and D is the degree of chemical gelatinization.

On the basis of the DS value, degrees of gelatinization, and eq 2, it was clear that 36−45% of the total OS groups were present in 20−21% of the maize granule at the periphery and 45−48% of the total OS groups were present in 20−23% of the potato granule at the periphery. The results showed that the DS of the OS maize starch degraded less than the DS of OS potato starch under a similar degree of gelatinization. The higher the DS of original OS starch, the bigger the differences were displayed (Table 1).

As a result of succinylation, the hydroxyl groups in the starch were substituted by carbonyl groups of OSA. To detect whether the esterification successfully formed, the FTIR spectra of OS starches with different DS were measured (Figure 5). Previous reports showed that the spectra of OS starch showed two new absorption bands at 1724 and 1573 cm^{-1}.[29,30] The peak at 1724 cm^{-1} originated from C═O stretching vibration of an ester group.[30] The band occurring at 1573 cm^{-1} corresponded to the asymmetric stretch of vibration of carboxylate $RCOO^-$. The intensity of absorption peaks at 1726 and 1572 cm^{-1} increased with an increasing DS of starch.

The FTIR spectra of the samples: OS maize starch (A3) and OS potato starch (B3) with the closest DS (0.0335 and 0.0341, respectively) showed the same remarkable peaks at 1573 and 1724 cm^{-1}. However, the remaining granules of OS maize starch (A3-2) showed more conspicuous peaks compared to the counterpart of OS potato starch (B3-2), suggesting that the

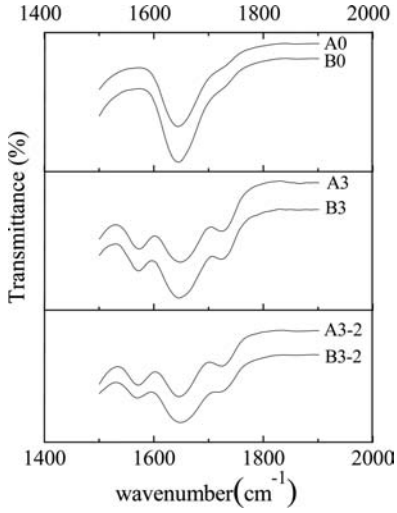

Figure 5. FTIR spectra of OS starch: (A0) maize starch; (B0) potato starch; (A3) OS maize starch, DS = 0.0336; (B3) OS potato starch, DS = 0.0341; and remaining granules of (A3-2) OS maize starch and (B3-2) OS potato starch after 50% degree of gelatinization.

OS maize starch has retained more OS groups than the OS potato starch after a similar degree of gelatinization.

Amylose is modified to a greater extent than amylopectin has been reported for acetylated starch, methylated starch, and hydroxypropylated starch.[31−34] Amylose is more concentrated at the periphery than at the core of the granules, for both maize starch and potato starch. In this study, we found that a greater proportion of the OS groups were present at the periphery than at the core of the granules, which is consistent with previous reports.[8,9]

Huber and BeMiller[16] observed the reaction sites within phosphorylated potato starch and hydroxypropyl waxy maize starch at the granular level by SEM compositional backscattered electron imaging. In waxy maize starch, the flow of reagent into the granule matrix occurred from channels and cavities. In potato starch granules, which do not possess channels, reagent diffuses inward through exterior granule surfaces. The investigation on the distribution of substituent at the granular level for OS maize starch and OS potato starch showed that the distribution of OS groups was uneven within one granule. However, in comparison to potato starch, maize starch displayed a much more homogeneous OSA reaction pattern. Through analysis of structural differences between maize starch and potato starch, it is deduced that not only dissolved OSA but also OSA droplets diffuse into internal maize granules primarily through channels and cavities. For potato starch, OSA droplets started reaction probably at the periphery first and proceeded to the core of the granules, because OSA reacted rapidly before it could permeate homogenously throughout the starch granules. With the special molecular architectures (pinholes and channels) of maize, it is easier to change the location of OS groups than with potato starch by changing reaction conditions or starch pretreatments.

This paper acted as a precursor and inspired further research that will determine how the distribution of OS groups might affect physical properties, such as emulsification activity, and what new physical properties are obtained at higher DS.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

CLSM, confocal laser scanning microscopy; DS, degree of substitution; $d_{3,2}$, volume−surface average diameter; FTIR, Fourier transform infrared spectroscopy; MB^+, methylene blue; OS, octenylsuccinic; OSA, octenylsuccinic anhydride; SEM, scanning electron microscopy

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