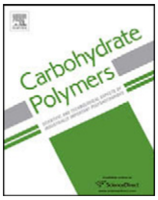
Carbohydrate Polymers 84 (2011) 1276–1281

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol



Effects of octenylsuccinylation on the structure and properties of high-amylose maize starch

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ARTICLE INFO

Article history:
Received 5 December 2010
Received in revised form 8 January 2011
Accepted 13 January 2011
Available online 20 January 2011

Keywords:
High-amylose maize starch
OS-starch
Digestibility
CLSM

ABSTRACT

Structural changes from the octenylsuccinylation of high-amylose maize starch were investigated by FT-IR and differential scanning calorimetry (DSC), with observations made on the distribution of octenyl succinate (OS) groups using confocal laser scanning microscopy (CLSM). Results of the octenylsuccinylation on *in vitro* digestion property (uncooked and cooked) were also determined. DSC data revealed a broad gelatinization endothermic peak ranged from 72.5 °C to 96.4 °C for the native starch, and a downward trend for products of increasing degree of substitution (DS). The OS groups appeared to be distributed throughout the OS-starch granules, especially on the surface. Octenylsuccinylation decreased the slowly digestible starch content of low DS OS-starch after cooking, while increased the resistant starch (RS) level in uncooked OS-starch. Even though uncooked OS-starch (DS ≈ 0.04) has 86.3% RS content, its RS content was reduced to 7.5% after cooking.

1. Introduction

Octenyl succinate (OS) starches are widely used as emulsifiers in food, pharmaceutical, and cosmetic industry. OS-starch is usually prepared by an alkaline catalyzed reaction of alkenyl succinic anhydride with granular starch in an aqueous suspension. The most attractive characteristic of OS-starch is its emulsification property, resulting from the addition of bi-functional groups that are both hydrophilic and hydrophobic.

For nutritional purposes, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). RDS, which is digested rapidly in the small intestine, contributes to a high postprandial glycemic index. SDS is digested slowly but completely, and sustains plasma glucose levels over time (Lehmann & Robin, 2007). RS is a fraction of starch that escapes digestion in the small intestine and enters the colon for fermentation (Englyst et al., 1992). Recent studies have showed that OS-normal maize, -waxy maize, -potato, and -tapioca starches could be used as functional dietary fibers because of their resistance to digestive enzymes, which would increase the content of SDS and RS (Han & BeMiller, 2007; He, Liu, & Zhang, 2008; Viswanathan, 1999). It has been hypothesized that the key to producing a functional modified starch with appreciable SDS and RS content is restriction of digestion resulting from introduction of bulky derivative groups (Han & BeMiller, 2007).

The location of OS groups within the OS-starch granule may also affect the digestibility. Normally, the reaction between octenyl succinic anhydride (OSA) and starch is retarded because of poor penetration of the big oily droplets of OSA into starch granules in an aqueous suspension, and the site of reaction is limited to the granule surface. As a consequence, the OS groups are not evenly distributed throughout the starch granule. Shogren, Viswanathan, Felker, and Gross (2000) suggested that OS groups are located mainly on the surface of the waxy maize starch granule, as observed by OsO_4-staining and backscattered electron imaging. Huang et al. (2010) proposed that the surface of OS normal maize starch is enriched with OS groups by a factor of 2–4 over that of the whole granule.

Little work has been reported on the structure and properties of OS high-amylose maize starch. The objective of this study was to develop a better understanding of the distribution of OS groups in high-amylose maize starch granules using confocal laser scanning microscopy (CLSM). We further investigated the physiochemical properties and digestibility of the OS-starch with different degrees of substitution (DS) by Fourier transform infrared (FT-IR) spectroscopy, differential scanning calorimetry (DSC), and *in vitro* starch digestibility methods.

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doi:10.1016/j.carbpol.2011.01.020

2. Materials and methods

2.1. Materials

High-amylose maize starch (Hylon V) was a gift of National Starch and Chemical Co. (Bridgewater, NJ, USA). 2-Octen-1-ylsuccinic anhydride was from Aroma Chemical Co. (Hangzhou, China). Pancreatin from porcine pancreas (Cat. No. P7545) and amyloglucosidase (Cat. No. A7095, activity 300 unit/mL) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The pancreatin used had eight times the activity specified in United States Pharmacopeia (USP), that is, at least 200 USP units of amylase (and protease) and at least 16 USP units of lipase per mg of product. Other chemicals used in the study were all analytical grade.

2.2. Preparation of OS-starch

High-amylose maize starch (25.0 g, dry starch basis, dsb) was suspended in distilled water (35%, w/w) with agitation. The pH of the suspension was adjusted to 8.0 with a pH meter by adding 3% NaOH solution. A weighed quantity of OSA (1.5, 3.0, 6.0, and 10.0% of the dry starch base, respectively) was added slowly within 2 h while maintaining the pH at 8.0. The reaction was allowed to proceed for a total of 3 h at 35 °C. After the reaction, the pH was adjusted to 6.5. The mixture was centrifuged and washed two times with distilled water and two times with ethanol. The OS-starch was oven-dried at 40 °C for 24 h, and then passed through a 100 mesh nylon sieve (Ruan, Chen, Fu, Xu, & He, 2009).

2.3. Determination of the degree of substitution

OS-starch sample (5 g, dsb) was accurately weighed and suspended by stirring for 30 min in 25 mL of HCl-isopropyl alcohol solution (2.5 M). 100 mL of aqueous isopropyl alcohol solution (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 any longer (using 0.1 M $AgNO_3$ solution). The starch was re-dispersed 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 (Ruan et al., 2009). The DS was calculated using the following equation:

$$\mathrm{DS} = \frac{0.162 \times (A \times M)/W}{1 - \left[0.210 \times (A \times M)/W\right]}$$

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.

The reaction efficiency (RE) was calculated as follows:

$$\mathrm{RE} = \frac{\text{Actual DS}}{\text{Theoretical DS}} \times 100\%$$

The theoretical DS was calculated by assuming that all the added OSA reacted with starch to form the ester derivative.

2.4. Fourier transform infrared spectroscopy

The FT-IR spectra were obtained from samples in KBr pellets using a Vector 33 FT-IR spectrophotometer (Bruker, Ettlingen, Germany). A spectral resolution of 4 cm^{-1} was employed and 64 scans were acquired for each spectrum.

2.5. Confocal laser scanning microscopy

Native starch and OS-starch samples were dye-stained for CLSM as follow. Specimens (0.5 g) were suspended in 30 mL water. The pH of the suspensions was adjusted to 8.0, and 1% Methylene Blue (MB^+) solution was added into each sample. The mixture was incubated in a shaking water bath at room temperature for 3 h and the granules were washed with methanol to remove the excess dye.

A TCS SP5 CLSM equipped 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 (Jayaraj, Umadevi and Ramakrishnan, 2001). During the image acquisition, each line was scanned four times and averaged to reduce noise.

2.6. Differential scanning calorimetry

A Diamond-1 differential scanning calorimeter (Perkin-Elmer, Norwalk, CT, USA) with an intra cooler was used to examine the thermal properties of starch samples. Starch samples (10–20 mg) were mixed with de-ionized water (moisture level 70%) and hermetically sealed in high-pressure stainless steel pans (PE No. BO182901) with a gold-plated copper seal (PE No. 042-191758). After equilibrating for 24 h at room temperature, samples were scanned at a heating rate of 5 °C/min from 30 to 150 °C. The enthalpy change (ΔH), onset (T_o), peak (T_p), and conclusion (T_c) temperature of starch gelatinization were calculated by using a Pyris software (Perkin-Elmer, Norwalk, CT, USA).

2.7. In vitro starch digestibility

2.7.1. Uncooked starch

In vitro digestibility was analyzed by a slightly modified Englyst procedure (Englyst, Englyst, Hudson, Cole, & Cummings, 1999). Starch samples (600 mg, dsb), sodium acetate buffer (20 mL, 0.1 M, pH 5.2) and five glass beads (5 mm diameter) were added into 50 mL polypropylene centrifuge tubes. The tubes were capped and mixed by vortexing for 5 min. The suspension was equilibrated horizontally in a shaking water bath (160 strokes per min) at 37 °C for 30 min. Pancreatin (3×10^3 USP) and amyloglucosidase (40 unit) in 5 mL sodium acetate buffer were added to each tube. Aliquots (1.00 mL) were taken at 20 and 120 min intervals and mixed with 10 mL 80% ethanol, and the glucose content in the mixture was measured by the 3,5-dinitrosalicylic acid method.

The different carbohydrate fractions were calculated according to the timeline of digestion: RDS, glucose released within the first 20 min; SDS, glucose released between 20 and 120 min; RS, glucose not released within 120 min, calculated by difference. The percentage of the different fractions was calculated by applying a factor of 0.9 to convert values for monosaccharide to starch. The samples were analyzed in duplicate.

2.7.2. Cooked starch

Starch samples (600 mg, dsb) and water (10 mL) were added into 50 mL polypropylene centrifuge tubes. The tubes were capped and mixed by vortexing for 5 min, and then placed in a boiling water bath for 30 min and vortexed in each 5 min to avoid agglomeration. Sodium acetate buffer (10 mL, 0.1 M, pH 5.2) and five glass beads were added to each tube. The dispersion was equilibrated horizontally in a shaking water bath at 37 °C for 30 min. The same procedure of digestibility was followed.

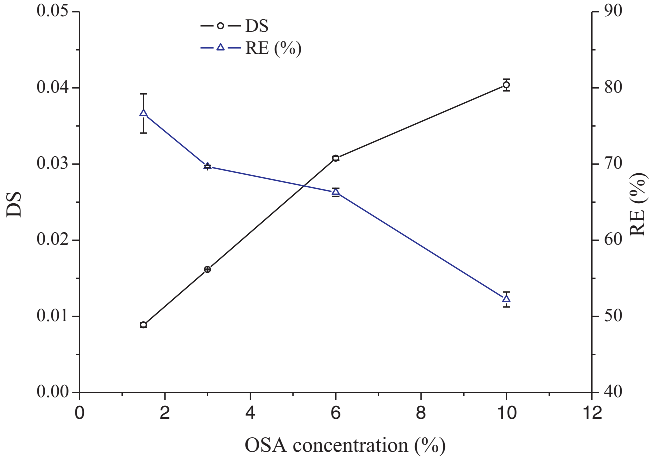

Fig. 1. Effect of OSA concentration on DS and RE. Reaction temperature was 35 °C; pH 8.0; starch concentration, 35% (pooled data from three experiments are presented as means ± standard deviation of the mean ($n = 3$)).

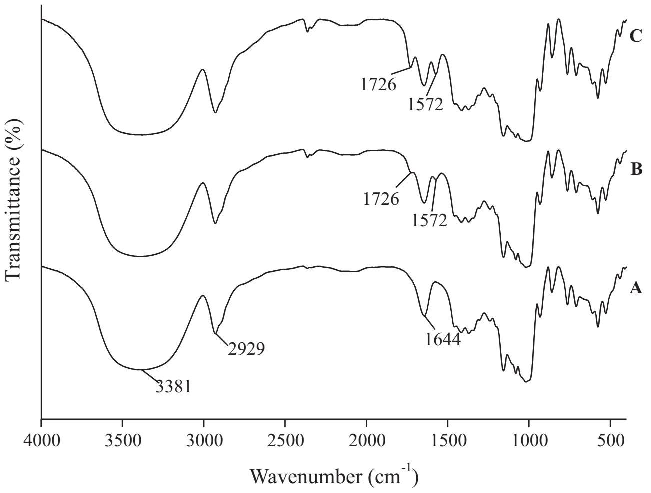

Fig. 2. FT-IR spectrum of native and OS-starches (DS: A, 0; B, 0.016; C, 0.040).

2.8. Statistical analysis

Analyses of variance and the mean separations were performed by Duncan's least significant test ($p < 0.05$) by SPSS 13.0 statistical software for Windows (SPSS, Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Effects of varying OSA concentration on esterification

Fig. 1 shows the results of variation in OSA concentration while holding the starch concentration at 35%, pH at 8.0, and reaction time of 3 h. With the increasing of OSA added, DS increased from 0.009 to 0.040. The increasing rate of DS, however, was not linear, and RE decreased remarkably from 76.6 to 52.2%, which was due to insufficient mixing between the water-insoluble OSA and starch phases (Ruan et al., 2009). In addition, it could also be attributed to OSA and OS-starch hydrolysis during the prolonged time of reaction when a larger quantity of OSA was used (Song, He, Ruan, & Chen, 2006). The results were consistent with previous report (Ruan et al., 2009).

3.2. FT-IR spectroscopy

The FT-IR spectra of native and OS-starches with different DS (0; 0.016; 0.040) are shown in Fig. 2. The characteristic peaks at 3381 cm^{-1} and 2929 cm^{-1} are the hydroxyl groups (O–H) and C–H stretching vibration of the glucose unit, respectively. The absorption at about 1644 cm^{-1} is due to residual bound water (Luo, Huang, Fu, Zhang, & Yu, 2009). Compared with the native starch curve (Fig. 2A, DS:0), two new absorption bands at 1726 cm^{-1} and 1572 cm^{-1} appeared in OS-starch curves (Fig. 2B, DS: 0.016; Fig. 2C, DS: 0.040). The band occurring at 1726 cm^{-1} is C=O stretching vibration of an ester group (Shogren, 2003). The peak at 1572 cm^{-1} was assigned to an asymmetric stretch of vibration of a carboxyl group (Liu et al., 2008). The intensity of absorption bands at 1726 cm^{-1} and 1572 cm^{-1} increased with an increasing DS in starch (Fig. 2C). The results confirmed the OS-starch formation at different DS.

3.3. Distribution of OS groups revealed by CLSM

CLSM was used to study the internal structure of starch granules to avoid the use of complicated or invasive sample preparation methods, such as sectioning and chemical etch (Chen et al., 2009; van de Velde, van Riel, & Tromp, 2002). Previous researchers have used CLSM to visualize the internal structures of starch granules, such as channels, growth rings, equatorial grooves, distribution of amylose, protein and phosphate (Chen et al., 2009; Glaring, Koch, & Blennow, 2006; Huber & BeMiller, 2000; Jiang et al., 2010; van de Velde, van Riel, & Tromp, 2002).

In this study, the distribution of OS groups in the starch granules was investigated using CLSM after being stained MB$^+$ dye. MB$^+$ is one of the fluorescence dyes that highlight anionic substances. It has previously been used for specific labeling of anionic head groups of sodium dodecyl sulphate (Jayaraj, Umadevi, & Ramakrishnan, 2001). Fig. 3 shows the CLSM optical sections of OS-starch granules of different DS obtained at the same photomultiplier (PMT) after being stained with MB$^+$. The native starch granules (DS: 0; Fig. 3A) did not show fluorescence while the OS-starch granules (DS: 0.016; 0.031; 0.040; Fig. 3B, C and D respectively) had fairly uniform bright fluorescence. This showed that MB$^+$ specifically stained the $-COO^-$ of OS groups, and the distribution of OS groups in starch granules could be clearly revealed. It was revealed from Fig. 3 that the higher DS (0.031; 0.040) of OS-starch granules showed stronger fluorescence intensity, indicating that there were more negatively charged groups available to stain with MB$^+$. In addition, OS groups appeared to be distributed throughout the OS-starch granules, especially on the surface.

OSA has low solubility in water and as a result, a mixture of dissolved reagent and small drops of reagent in dispersion exists during the reaction. OSA droplets could react with the granular surface or, depending on drop size, penetrate granule channels and arrive at the inner cavity (Segura-Campos, Chel-Guerrero, & Betancur-Ancona, 2008; Shogren et al., 2000). Only dissolved or extremely dispersed OSA could penetrate the entire granule. It would seem likely that the granular surface has higher fluorescence than the interior because the granular surface can react with OSA droplets as well as with dissolved OSA.

The distribution of OS groups in starch granules has been studied by backscattered electron microscopy and X-ray photoelectron spectroscopy previously (Huang et al., 2010; Shogren et al., 2000), and it has been noted that: (a) the OS groups were probably distributed in the interior, amorphous domains of amylopectin molecules as well as on the outside of the granule; (b) the concentration of OS groups on the surface of the OS-starch granules was about 2–4 times than that of the bulk. To the best of our knowledge, this is the first time the distribution of OS groups in the starch granules has been directly observed under CLSM. The con-

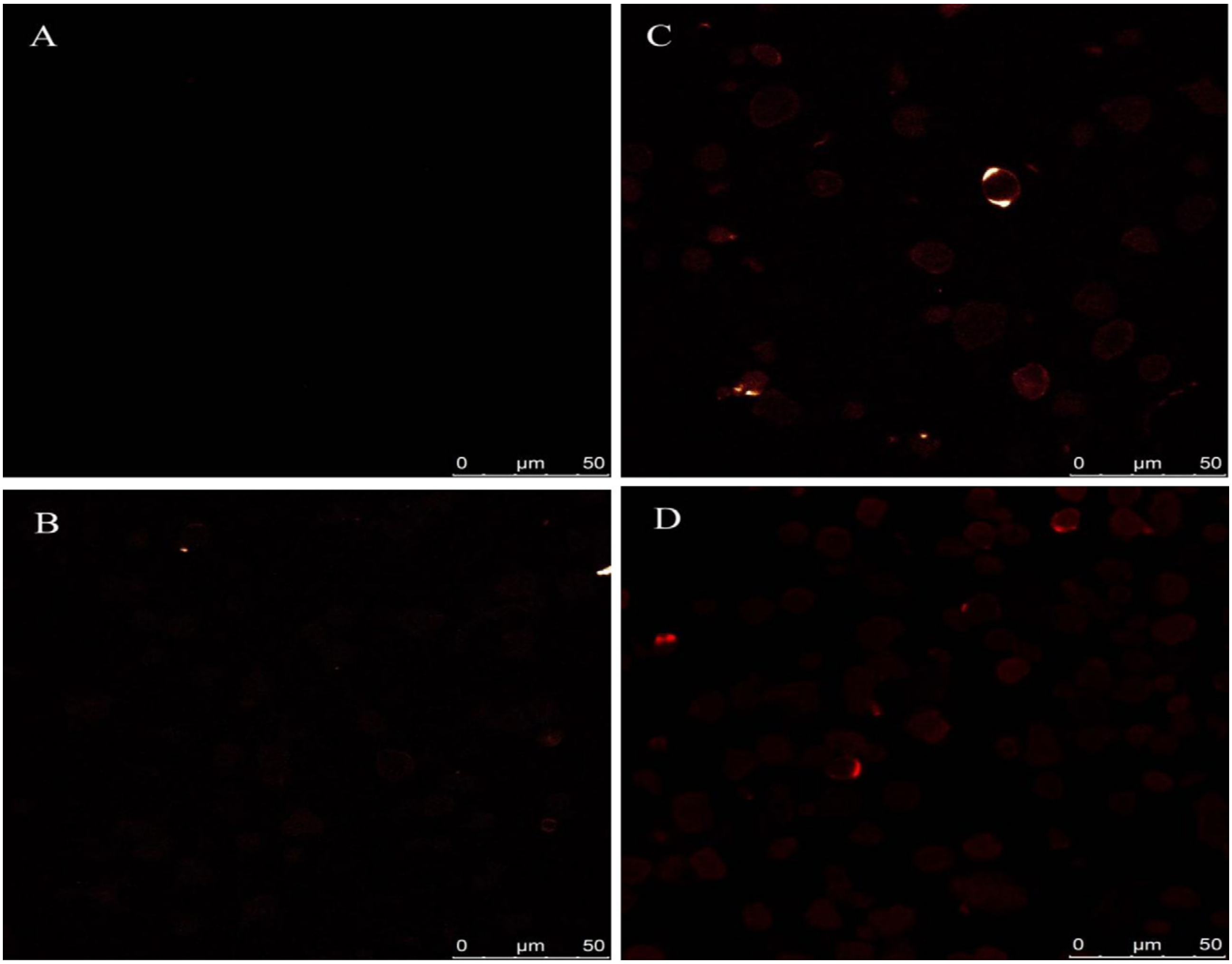

Fig. 3. The CLSM optical sections of native and OS-starches (DS: A, 0; B, 0.016; C, 0.031; D, 0.040).

focal microscopy clearly showed promise to further explore the distribution of chemical groups in the starch granules.

3.4. Thermal properties

The gelatinization parameters (T_o, T_p, T_c and ΔH) of native and OS-starches with DS 0.009 to 0.040 are presented in Table 1. T_o, T_p and T_c have been shown to be influenced by amylose content, amylopectin chain length distribution and lipid complexed amylose chains (Chung, Hoover, & Liu, 2009). Gelatinization of the native starch started at 72.5 °C (T_o), peaked at 77.8 °C (T_p), and completed at 96.4 °C (T_c) with an enthalpy change of 8.0 J/g. These results were consistent with the previous report (Jane et al., 1999). T_o, T_p, T_c, and ΔH values decreased ($p < 0.05$, Table 1) with increasing DS of OS-starches. The lower gelatinization temperature and enthalpy change could be attributed to the disruption of the crystalline structure of the native starch, resulting from the increase in the DS of the OS group. The anion (carboxyl group) repulsion effect also helped OS-starch to swell at lower temperature (Bhosale & Singhal, 2007; Jane et al., 1999). Additionally, the OSA effect on the gelatinization temperature of starch was dependent on starch base and DS (Thirathumthavorn & Charoenrein, 2006). Similar trend in thermal properties of OS-starches has been reported by earlier researchers (Bhandari & Singhal, 2002; Bhosale & Singhal, 2007; Thirathumthavorn & Charoenrein, 2006).

Table 1
Gelatinization characteristics of native and OSA modified high-amylose maize starches.[a]

DS[b]	T_o[b] (°C)	T_p[b] (°C)	T_c[b] (°C)	$T_c - T_o$[c] (°C)	ΔH[d] (J/g)
0	72.5 ± 0.5^e	77.8 ± 0.1^e	96.4 ± 0.2^e	23.9	8.0 ± 0.1^e
0.009	68.3 ± 0.9^f	75.9 ± 0.1^f	90.0 ± 1.3^f	21.7	5.4 ± 0.5^f
0.016	66.7 ± 0.1^g	75.0 ± 0.1^g	88.2 ± 0.1^g	21.5	4.5 ± 0.2^g
0.031	63.8 ± 0.3^h	73.5 ± 0.2^h	87.6 ± 0.1^g	23.8	3.2 ± 0.1^h
0.040	59.9 ± 0.6^i	72.9 ± 0.1^i	84.7 ± 0.2^h	24.8	3.0 ± 0.1^h

[a] All data reported on dry basis and represent the mean of duplicates. Means within a column with different letters are significantly different ($p < 0.05$) by Duncan's least significant test.

[b] DS, degree of substitution. T_o, T_p and T_c indicate the temperatures of the onset, peak and conclusion of gelatinization, respectively.

[c] $T_c - T_o$ indicates the gelatinization temperature range.

[d] ΔH indicates the enthalpy change of gelatinization.

3.5. In vitro starch digestibility

The levels of RDS, SDS, and RS in native and OS-starch samples are presented in Table 2. Based on the Englyst method, percentages of RDS, SDS, and RS in uncooked high-amylose maize starch were 11.1%, 19.7%, and 69.2%, respectively. In contrast, uncooked normal maize starch contained higher RDS (23.4%) and SDS (56.9%) contents and lower RS content (19.7%) (Chung, Liu, & Hoover, 2009). The granule amylose content appears to be a factor involved in starch enzymatic resistance. This is evident since starches con-

Table 2
RDS, SDS and RS levels in uncooked and cooked OS-starches.[a]

Sample	Uncooked starch			Cooked starch		
(DS)	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
0	11.1 ± 0.7^{b}	19.7 ± 2.0^{b}	69.2 ± 2.7^{b}	87.3 ± 2.3^{c}	6.6 ± 1.2^{c}	6.2 ± 1.1^{b}
0.009	9.2 ± 0.4^{c}	17.7 ± 0.7^{bc}	73.1 ± 1.0^{bc}	91.7 ± 0.7^{b}	2.4 ± 0.4^{b}	5.9 ± 0.3^{b}
0.016	8.4 ± 0.2^{c}	17.0 ± 1.6^{bc}	74.5 ± 1.4^{c}	90.7 ± 0.5^{b}	3.0 ± 0.2^{b}	6.4 ± 0.3^{b}
0.031	8.2 ± 0.1^{c}	15.6 ± 1.8^{c}	76.2 ± 1.9^{c}	86.9 ± 1.3^{c}	6.4 ± 0.3^{c}	6.7 ± 0.9^{b}
0.040	4.4 ± 0.1^{d}	9.3 ± 0.4^{d}	86.3 ± 0.2^{d}	84.8 ± 0.6^{c}	7.7 ± 0.1^{c}	7.5 ± 0.4^{b}

[a] RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch. DS, degree of substitution. The data are averages of two measurements with standard deviation. Means within a column with different letters are significantly different ($p < 0.05$) by Duncan's least significant test.

taining high levels of amylose (e.g. amylomaize, wrinkled pea and the small B-granules of wheat) are more resistant to enzymatic attack than the corresponding starches of lower amylose content (e.g. normal and waxy maize, smooth pea and large A-wheat starch granules) (Gallant, Bouchet, Buleon, & Perez, 1992). The out layer of the high-amylose maize starch granules is more resistant to enzymatic and acid hydrolysis, because the starch molecules at the periphery are densely packed and have more amylose (Jiang et al., 2010). It is now known that significant enrichment of amylose exists towards the granule surface (Gallant, Bouchet, & Baldwin, 1997; Pan & Jane, 2000), which may be responsible for an increased resistance towards the granule surface. Recently, Zhang, Ao, & Hamaker (2006) suggested that B-type crystalline starches had no pores on the surface, and the side-by-side digestion mechanism produces a different hydrolysis pattern observed as “exo-pitting”, because the enzyme began digestion from the resistant surface.

Even though uncooked high-amylose maize starch had almost 70% RS content, its RS content decreased to 6.1% after cooking. The increase in RDS and decrease in SDS and RS after cooking was due to a disruption of the granular or supramolecular structures within the starch granule. Native high amylose starch had a broad gelatinization endothermic peak ranged from 72.5 to 96.4 °C, suggesting that partially granular structure would swell and deform during cooking. Greater swelling and solubility would result in greater digestibility.

In uncooked OS-starch granules, the presence of a small amount of substituent posed a great hindrance to the enzymes for degradation and this resistance is directly proportional to the DS of the sample. It should be noted that OS-starch with the higher DS (0.040) has the highest percentage of RS (86.3%). The RDS and SDS levels of the uncooked OS-starches ($p < 0.05$, Table 2) showed a decreasing tendency with the increase of DS. Octenylsuccinylation was known to reduce the enzyme-catalyzed degradation of starch and to slow the glycemic response (He et al., 2008; Viswanathan, 1999; Wolf et al., 2001). Our CLSM results (Fig. 3) showed that OS groups were located mostly on the surface of the granules. The OS groups coated on the surface of the granules and reduced activity of enzyme hydrolysis (Han & BeMiller, 2007; He et al., 2008; Viswanathan, 1999). As a result of prevention of digestion, uncooked OS-starch could also be a useful source of RS.

However, cooked OS-starches dramatically increased their *in vitro* hydrolysis by α-amylase and amyloglucosidase. The SDS level of the OS-starches showed an increasing tendency with the increase of DS, while the SDS level of the low DS starch ($DS \le 0.031$) was lower than that of native starch. This effect should be attributed to the fact that octenylsuccinylation decreased the gelatinization temperature and ΔH (Table 1), which caused the starch granule to be fully gelatinized and then hydrolyzed by amylase to a larger extent. As discussed earlier, the hydrogen bonds present between starches chains in starch granules may have been disrupted, thereby increasing the accessibility of the starch chains to hydrolyzing enzymes.

4. Conclusions

Structure and properties of OS-starches with different DS were investigated. FT-IR spectroscopy showed the characteristic vibration of the ester carbonyl groups in OS-starch at 1726 cm^{-1}. DSC results displayed a broad gelatinization endothermic peak ranged from 72.5 to 96.4 °C for native high amylose maize starch, and a downward trend for OS-starches with an increasing DS. The CLSM imaging showed that OS groups were probably located mainly on the surface of the granules. Starch susceptibility to enzyme digestion was substantially influenced by octenylsuccinylation. In uncooked OS-starch granules, the RS content increased while RDS and SDS contents decreased with an increasing of DS. Octenylsuccinylation decreased the SDS content of low DS OS-starch after cooking.

Acknowledgements

The authors gratefully thank Dr. Andrew Tang for his help in the preparation of the manuscript. Financial support received from the Fundamental Research Funds for Central Universities/SCUT (2009ZM0028, 2009ZM0058) and Department of Education of Guangdong Province (x2qs N9100250) is also acknowledged.

References

Bhandari, P. N., & Singhal, R. S. (2002). Effect of succinylation on the corn and amaranth starch pastes. *Carbohydrate Polymers*, *48*, 233–240.

Bhosale, R., & Singhal, R. (2007). Effect of octenylsuccinylation on physicochemical and functional properties of waxy maize and amaranth starches. *Carbohydrate Polymers*, *68*, 447–456.

Chen, P., Yu, L., Simon, G., Petinakis, E., Dean, K., & Chen, L. (2009). Morphologies and microstructures of cornstarches with different amylose-amylopectin ratios studied by confocal laser scanning microscope. *Journal of Cereal Science*, *50*, 241–247.

Chung, H. J., Hoover, R., & Liu, Q. (2009). The impact of single and dual hydrothermal modifications on the molecular structure and physicochemical properties of normal corn starch. *International Journal of Biological Macromolecules*, *44*, 203–210.

Chung, H. J., Liu, Q., & Hoover, R. (2009). Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. *Carbohydrate Polymers*, *75*, 436–447.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, *46*, S33–S50.

Englyst, K. N., Englyst, H. N., Hudson, G. J., Cole, T. J., & Cummings, J. H. (1999). Rapidly available glucose in foods: An in vitro measurement that reflects the glycemic response. *American Journal of Clinical Nutrition*, *69*, 448–454.

Gallant, D. J., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: Evidence of a new level of granule organization. *Carbohydrate Polymers*, *32*, 177–191.

Gallant, D. J., Bouchet, B., Buleon, A., & Perez, S. (1992). Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European Journal of Clinical Nutrition*, *46*, 3–16.

Glaring, M. A., Koch, C. B., & Blennow, A. (2006). Genotype-specific spatial distribution of starch molecules in the starch granule: A combined CLSM and SEM approach. *Biomacromolecules*, *7*, 2310–2320.

Han, J. A., & BeMiller, J. N. (2007). Preparation and physical characteristics of slowly digesting modified food starches. *Carbohydrate Polymers*, *67*, 366–374.

He, J. H., Liu, J., & Zhang, G. Y. (2008). Slowly digestible waxy maize starch prepared by octenyl succinic anhydride esterification and heat-moisture treatment: Glycemic response and mechanism. *Biomacromolecules*, *9*, 175–184.

Huang, Q., Fu, X., He, X. W., Luo, F. X., Yu, S. J., & Li, L. (2010). The effect of enzymatic pretreatments on subsequent octenyl succinic anhydride modifications of cornstarch. *Food Hydrocolloids*, *24*, 60–65.

Huber, K. C., & BeMiller, J. N. (2000). Channels of maize and sorghum starch granules. *Carbohydrate Polymers*, *41*, 269–276.

Jane, J.-L., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S., Radosavljevic, M., et al. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry*, *76*, 629–637.

Jayaraj, S. E., Umadevi, M., & Ramakrishnan, V. (2001). Environmental effect on the laser-excited fluorescence spectra of Methylene Blue and Methylene Green dyes. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *40*, 203–206.

Jiang, H. X., Horner, H. T., Pepper, T. M., Blanco, M., Campbell, M., & Jane, J. L. (2010). Formation of elongated starch granules in high-amylose maize. *Carbohydrate Polymers*, *80*, 533–538.

Lehmann, U., & Robin, F. (2007). Slowly digestible starch - its structure and health implications: A review. *Trends in Food Science and Technology*, *18*, 346–355.

Liu, Z. Q., Li, Y., Cui, F. J., Ping, L. F., Song, J. M., Ravee, Y., et al. (2008). Production of octenyl Succinic anhydride-modified waxy corn starch and its characterization. *Journal of Agricultural and Food Chemistry*, *56*, 11499–11506.

Luo, F. X., Huang, Q., Fu, X., Zhang, L. X., & Yu, S. J. (2009). Preparation and characterisation of crosslinked waxy potato starch. *Food Chemistry*, *115*, 563–568.

Pan, D. D., & Jane, J. L. (2000). Internal structure of normal maize starch granules revealed by chemical surface gelatinization. *Biomacromolecules*, *1*, 126–132.

Ruan, H., Chen, Q. H., Fu, M. L., Xu, Q., & He, G. Q. (2009). Preparation and properties of octenyl succinic anhydride modified potato starch. *Food Chemistry*, *114*, 81–86.

Segura-Campos, M., Chel-Guerrero, L., & Betancur-Ancona, D. (2008). Synthesis and partial characterization of octenylsuccinic starch from Phaseolus lunatus. *Food Hydrocolloids*, *22*, 1467–1474.

Shogren, R. L. (2003). Rapid preparation of starch esters by high temperature/pressure reaction. *Carbohydrate Polymers*, *52*, 319–326.

Shogren, R. L., Viswanathan, A., Felker, F., & Gross, R. A. (2000). Distribution of octenyl succinate groups in octenyl succinic anhydride modified waxy maize starch. *Starch/Stärke*, *52*, 196–204.

Song, X. Y., He, G. Q., Ruan, H., & Chen, Q. H. (2006). Preparation and properties of octenyl succinic anhydride modified early indica rice starch. *Starch/Stärke*, *58*, 109–117.

Thirathumthavorn, D., & Charoenrein, S. (2006). Thermal and pasting properties of native and acid-treated starches derivatized by 1-octenyl succinic anhydride. *Carbohydrate Polymers*, *66*, 258–265.

van de Velde, F., van Riel, J., & Tromp, R. H. (2002). Visualisation of starch granule morphologies using confocal scanning laser microscopy (CSLM). *Journal of the Science of Food and Agriculture*, *82*, 1528–1536.

Viswanathan, A. (1999). Effect of degree of substitution of octenyl succinate starch on enzymatic degradation. *Journal of Environmental Polymer Degradation*, *7*, 185–190.

Wolf, B. W., Wolever, T. M. S., Bolognesi, C., Zinker, B. A., Garleb, K. A., & Firkins, J. L. (2001). Glycemic response to a food starch esterified by 1-octenyl succinic anhydride in humans. *Journal of Agricultural and Food Chemistry*, *49*, 2674–2678.

Zhang, G. Y., Ao, Z. H., & Hamaker, B. R. (2006). Slow digestion property of native cereal starches. *Biomacromolecules*, *7*, 3252–3258.