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Alkaline treatment
6 days

Alkaline treatment
12 days
A further study on supramolecular structure changes of waxy maize starch subjected to alkaline treatment by extended-q small-angle neutron scattering

Binjia Zhang\textsuperscript{a}, Elliot P. Gilbert\textsuperscript{b,c}, Dongling Qiao\textsuperscript{d,*}, Fengwei Xie\textsuperscript{e,f,†}, David K. Wang\textsuperscript{g}, Siming Zhao\textsuperscript{a}, Fatang Jiang\textsuperscript{d}

\textsuperscript{a} Group for Cereals and Oils Processing, Key Laboratory of Environment Correlative Dietology (Ministry of Education), College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

\textsuperscript{b} Australian Centre for Neutron Scattering, Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, NSW 2232, Australia

\textsuperscript{c} The Australian Institute for Bioengineering and Nanotechnology, and Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Qld 4072, Australia

\textsuperscript{d} Glyn O. Philips Hydrocolloid Research Centre at HBUT, Hubei University of Technology, Wuhan 430068, China

\textsuperscript{e} International Institute for Nanocomposites Manufacturing (IINM), WMG, University of Warwick, Coventry CV4 7AL, United Kingdom

\textsuperscript{f} School of Chemical Engineering, The University of Queensland, Brisbane, Qld 4072, Australia

\textsuperscript{g} School of Chemical and Biomolecular Engineering, The University of Sydney, Darlington, NSW 2006, Australia

\textsuperscript{*} Corresponding Author. Email: qdttk1@163.com (D. Qiao)

\textsuperscript{†} Corresponding Author. Emails: d.xie.2@warwick.ac.uk, f.xie@uq.edu.au (F. Xie)
Abstract: Recently, we reported the effect of mild alkaline treatment on the structure and enzymatic hydrolysis of waxy maize starch. Here, we have used extended-$q$ small-angle neutron scattering (SANS) to examine the extent to which this treatment causes structural changes on even greater length scales — to yield a complete picture from the nanometre to micron — including on the lamellar distribution and larger-scale structural features of the same starch. For this A-type polymorph starch containing mostly amylopectin, we found the changes caused by NaOH solution treatment (0.1% or 0.5% (w/v)) were confined mainly to double helices and crystallites, whereas the lamellar and supramolecular structures were conserved. Although the overall lamellar ordering was reduced in the 0.5% (w/v) alkaline treatment for 6 days, further treatment until 12 days could restore the overall lamellar ordering. Despite changes in starch double helical order and crystallites, there were minimal changes at larger length scales (e.g., blocklets and growth rings). This combination of methods reveals the extent of structural changes occurring as a result of mild alkaline treatment of waxy maize starch; it also provides insight into the rational design of granular starch products with varied functionality via hydrolytic control.

Keywords: starch; double helices, lamellae; large-scale structure; extended-$q$ small-angle neutron scattering; SANS


1 Introduction

Starch, as a storage polysaccharide in green plants such as maize, potato, wheat, and rice (Xie, Zhang, & Wang, 2017), serves as a food ingredient providing energy to humans (Juansang, Puttanlek, Rungsardthong, Puncha-armon, & Uttapap, 2012). Starch is also relevant for the design of functional foods (Fuentes-Zaragoza et al., 2011), bioactive carriers (Pu, Chen, Li, Xie, Yu, & Li, 2011) and biomaterials (Situ, Li, Liu, & Chen, 2015). There are two starch polymers biosynthesised during plant growth, namely amylose and amylopectin (Liu, Halley, & Gilbert, 2010; Jiang, Gao, Li, & Zhang, 2011). These two polymers can be organized on different scales in the starch granule to construct a multiscale supramolecular system; this system involves the whole granule, growth rings, blocklets, semicrystalline lamellae, crystallites, and double/single helices (Buleon, Colonna, Planchot, & Ball, 1998; Luengwilai & Beckles, 2009; Perez & Bertoft, 2010; Blazek & Gilbert, 2011; Doutch & Gilbert, 2013; Flanagan, Gidley, & Warren, 2015; Zhang et al., 2015). The structures with scales larger than that of lamellae can be loosely termed as ‘large-scale’ structures, including the growth rings and blocklets (Doutch et al., 2013).

The multiscale structural features of starch such as crystallinity, the degree of lamellar ordering, and the bulk density of the granule surface (associated with pores in cereal starches) are crucial in governing starch properties and functions (e.g., thermal behaviours and digestibility) (Lopez-Rubio, Flanagan, Shrestha, Gidley, & Gilbert, 2008b; Liu, Xie, Yu, Chen, & Li, 2009; Blazek & Gilbert, 2010; Xie, Halley, & Avérous, 2012). For instance, a multiscale supramolecular structure with tightly assembled starch chains can weaken the enzyme diffusion and hydrolysis, thus making the digestion rate of untreated starch several times lower than that of cooked starch (Bertoft & Manelius, 1992;
Noda et al., 2008; Blazek et al., 2010). Therefore, revealing the starch multiscale structure using advanced analytical techniques can help in understanding such structure–function relationships.

Small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) are among the most powerful techniques for characterizing the lamellar and large-scale structures of starch (Chanvrier et al., 2007; Lopez-Rubio, Htoon, & Gilbert, 2007; Lopez-Rubio, Flanagan, Gilbert, & Gidley, 2008a; Lopez-Rubio et al., 2008b; Blazek et al., 2010; Blazek et al., 2011; Witt, Doutch, Gilbert, & Gilbert, 2012; Doutch et al., 2013; Bai, Cai, Doutch, Gilbert, & Shi, 2014; Shrestha et al., 2015; Yang et al., 2019a). With these two scattering techniques, it is straightforward to extract information on the supramolecular structural features of starch such as the thickness of semicrystalline lamellae, crystalline and amorphous block dimensions (Cardoso & Westfahl, 2010; Zhang et al., 2014), and the size of blocklets (Doutch et al., 2013).

Alkaline treatment is widely used for the isolation of starches with high yields and purity from agro-products (Han & Hamaker, 2002; Correia & Beirão-da-Costa, 2012) and for the production of a wide variety of starch-based foods such as tortillas, yellow alkaline noodles, and dumplings (Campus-Baypoli, Rosas-Burgos, Torres-Chavez, Ramírez-Wong, & Serna-Saldivar, 1999; Lai, Karim, Norziah, & Seow, 2002). Alkalis, such as sodium hydroxide and sodium carbonate, can impart foods with typical aroma and flavour as well as a firm and elastic texture (Karim, Nadiha, Chen, Phuah, Chui, & Fazilah, 2008). It has been shown that alkaline treatment can alter the molecular structure (amylose content), hierarchical structures (granule morphology, lamellae, crystallites, and short-range order), and properties (e.g., swelling, pasting, rheological, thermal, and digestion behaviours) of various starches (e.g., corn, rice, potato, sago, and pea) (Lai et al., 2002; Cardoso, Putaux, Samios, & da Silveira, 2007; Méndez-Montealvo, Trejo-Espino, Paredes-López, &
Bello-Pérez, 2007; Karim et al., 2008; Thys, Westfahl, Norea, Marczak, Silveira, & Cardoso, 2008; Nor Nadiha, Fazilah, Bhat, & Karim, 2010; Wang & Copeland, 2012; Cai et al., 2014; Jiang et al., 2014; Wang, Luo, Zhang, Zhang, He, & Wang, 2014; Qiao et al., 2016). However, how alkaline conditions (especially mild) change the lamellar distribution and large-scale characteristics is not well defined. Moreover, as the susceptibility of starch to alkaline treatment greatly depends on its native granule architecture and molecular structure (Nor Nadiha et al., 2010; Jiang et al., 2014), it is worth studying how alkaline treatment could have varied effects on a variety of cultivars. Our previous study (Qiao et al., 2017) has shown that mild alkaline treatment (0.1% and 0.5% NaOH, w/v) can effectively alter the digestion behaviour of waxy maize starch. However, how alkaline treatment alters the supramolecular structure of an A-type polymorphic starch (the mechanism) is still not fully understood. To answer this question, an investigation of changes occurring on multiple and relevant length scales is essential. To this end, we have used an extended-\(q\) SANS to interrogate the lamellar distribution and large-scale features of starch spanning scales up to the micron and their evolution under alkaline treatment. Note that SAXS and SANS are complementary methods. SAXS is sensitive to electron density differences, whereas SANS is sensitive to differences in neutron scattering length density (SLD) (a nuclear property); the latter can be manipulated through solvent contrast variation and selective deuteration, for example. In the current case, neutron scattering has been employed to enable access to a wide \(q\) range to examine the hierarchical structures exhibited by starch; the latter is readily available on the QUOKKA SANS instrument (Gilbert, Schulz, & Noakes, 2006; Wood et al., 2018). Neutron focussing, enabling access to scattering vectors down to about 0.00045 Å\(^{-1}\), has also been used to simultaneously characterize the lamellar and large-scale structure of alkali-treated starch from the nanoscale to the micron-scale. A modified method was established to
fit the SANS data comprising a Gaussian-plus-Lorentzian peak function for the lamellar peak scattering, and a unified model (a Guinier function plus a power-law function) for larger length scales. With this method, the semicrystalline lamellae have been found to be distributed in a narrow range; moreover, the large-scale information related to blocklets and growth rings can be revealed.

2 Materials and methods

2.1 Materials

The starch used in this work was waxy maize starch commercially available from Penford Australia Pty Ltd. (Lane Cove, NSW, Australia). The amylose:amylopectin ratio of this starch was ca. 3:97, as determined using the iodine colourimetric method (Tan, Flanagan, Halley, Whittaker, & Gidley, 2007). The moisture content (ca. 13.6%) was measured using an MA35 moisture analyser (Sartorius Stedim Biotech GmbH, Germany). All chemicals (including sodium hydroxide, sodium azide, and ethanol) were of analytical grade and were provided by Tianjin Kemeou Chemical Reagent Co., Ltd. (Tianjin, China).

2.2 Alkaline treatment

Although a concentrated alkali solution can quickly and effectively disrupt the starch structure, it may also result in the degradation of starch molecules (Han & Lim, 2004) due to the β-elimination of reducing semi-acetal groups. In this work, NaOH aqueous solutions of 0.1% (w/v) and 0.5% (w/v) concentrations were employed for the treatment of the starch for different times (6 and 12 days).
Such mild alkali solutions were selected as they are typical for the processing of starch and starch-based foods where the modest alteration of the starch multiscale structure without degrading or dissolving starch molecules is desirable (Nordiha et al., 2010; Praznik, Buksa, Ziobro, Gambuś, & Nowotna, 2012; Wang et al., 2012; Cai et al., 2014; Jiang et al., 2014). Specifically, NaOH aqueous solutions of 0.1% (w/v) or 0.5% (w/v) concentration were prepared with 0.1% (w/v) sodium azide as a chemical preservative. About 10 g of the starch was added to 150 mL of the alkali solutions. The starch slurries were then placed at 35 °C for different treatment days (6 or 12), accompanied by intermittent shaking to effectively re-suspend the starch. Afterwards, each alkali-treated starch sample was washed using deionized water and then 95% ethanol (Wang et al., 2012; Jiang et al., 2014), followed by centrifugation for at least 3 times until a neutral starch slurry was acquired. The starch sediments were dried in an oven at 35 °C for 48 h and then were placed into zip-lock bags for further use.

In the following, codes such as “S-0.1-6” will be used, in which “S” denotes the waxy maize starch, “0.1” indicates the alkali concentration being 0.1% (w/v), and “6” means the days of treatment. In addition, a code of only “S” in the figures and tables indicates the native starch without any treatment.

2.3 Extended-\(q\) small-angle neutron scattering (SANS)

SANS measurements, extended to an ultra-small angle \(q = \text{ca.} \ 0.00045 \ \text{Å}^{-1}\), were performed on the 40-m QUOKKA instrument at the OPAL reactor (Sydney, Australia) (Gilbert et al., 2006; Wood et al., 2018). The sample aperture diameters were of 50 and 7.5 mm, respectively. Three instrument
configurations, with sample-to-detector distances of 1.3, 8.0 and 20.0 m at a wavelength ($\lambda$) of 5.0 Å and a fourth with wavelength 8.1 Å, and 10% wavelength resolution, were used to provide a continuous $q$ range of ca. 0.00045 to 0.70 Å$^{-1}$, where $q$ is the scattering vector defined as $q = \frac{4\pi\sin \theta}{\lambda}$ ($2\theta$ is the scattering angle ($^\circ$)). The instrumental resolution, $\Delta q$, at the lamellar peak (0.06 to 0.07 Å$^{-1}$) is 0.003 Å$^{-1}$. Note that $q_{\text{min}}$ is almost one order of magnitude lower than that used in SAXS ($0.0020 < q < 0.20$ Å$^{-1}$) previously (Qiao et al., 2017) enabling access to larger size range. The starch slurries in heavy water with a starch concentration of 10 wt.% were loaded into demountable cells with quartz windows which, in turn, were placed in a multi-position sample tumbler to prevent sample sedimentation; the scattering of pure heavy water within a quartz cell of 1 mm thickness was used as a background.

### 2.4 Reduction of SANS data

The resultant SANS data sets were reduced, normalised, and radially averaged using a package of macros in Igor software modified to accept HDF5 data files from QUOKKA (Blazek et al., 2010). The scattering curves are plotted as a function of absolute (SANS) intensity, $I$ (cm$^{-1}$), versus $q$ (Å$^{-1}$).

### 2.5 Fitting of SANS data

The extended-$q$ SANS data covering $q$ values of ca. 0.00045 to 0.20 Å$^{-1}$ were fitted in Origin 8 software (OriginLab. Inc., USA). Curve fitting was conducted iteratively; for each iteration, the fitting coefficients were refined to minimise chi-squared via a nonlinear, least-squares refinement procedure. As detailed in Section 3.3, the scattering data were fitted with a unified model including...
two power-law regimes (Doutch et al., 2013; Zhang et al., 2015) plus a Gaussian-plus-Lorentzian peak function to describe the lamellar peak range (ca. 0.015 to 0.20 Å\(^{-1}\)). Data are expressed as means ± standard deviations (SD).

3 Results and discussion

3.1 Short- and long-range structural changes of alkali-treated starch (previous study)

In our previous paper (Qiao et al., 2017), we reported the structure and in-vitro digestion behaviour of waxy maize starch under the same alkaline treatment conditions. Alkali concentration has been shown to largely influence the short-range structures (double helix and crystallite order) of waxy maize starch (Table 1) (Qiao et al., 2017). Using differential scanning calorimetry (DSC), we found the 0.1% (w/v) alkaline treatment could decrease the onset temperature (\(T_o\)), increase the conclusion temperature (\(T_c\)), and slightly reduce the enthalpy (\(\Delta H\)) of gelatinisation. X-ray diffraction (XRD) data indicated a slightly reduced degree of crystallinity (\(X_c\)). Similar changes in DSC results were observed for other types of starch (Méndez-Montealvo et al., 2007; Nor Nadiha et al., 2010; Wang et al., 2012; Cai et al., 2014; Wang et al., 2014). For waxy maize starch with A-type crystallites, the only endothermic peak represents an overlap of the transitions from smectic to isotropic phase and the helix–coil transition associated with the unwinding of amyllopectin double helices (starch molecular order) (Waigh, Gidley, Komanshek, & Donald, 2000a). It was proposed that the 0.1% (w/v) alkaline treatment could weaken the hydrogen-bonding interactions between double helices, disrupt some double helices, and increase the degree of perfection of some double helices.
helices. With a stronger alkaline (0.5% NaOH, w/v) treatment, the starch displayed increased $T_o$ and $T_c$ and decreased $\Delta H$ and $X_c$. In this regard, the 0.5% (w/v) NaOH solution could effectively disrupt some starch double helices especially those with flaws or a lower degree of perfection and increase the level of perfection for some existing starch double helices.

Table 1 Crystalline and thermal parameters of native and alkali-treated starch samples.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>S</th>
<th>S-0.1-6</th>
<th>S-0.1-12</th>
<th>S-0.5-6</th>
<th>S-0.5-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_c$ (%)</td>
<td>49.21±0.68$^a$</td>
<td>47.31±0.75$^b$</td>
<td>44.70±1.02$^c$</td>
<td>39.62±1.10$^e$</td>
<td>42.52±0.96$^d$</td>
</tr>
<tr>
<td>$T_o$ (°C)</td>
<td>72.31±0.20$^b$</td>
<td>68.96±0.35$^c$</td>
<td>67.95±0.19$^d$</td>
<td>77.35±0.23$^a$</td>
<td>77.17±0.37$^a$</td>
</tr>
<tr>
<td>$T_p$ (°C)</td>
<td>78.57±0.15$^c$</td>
<td>75.98±0.22$^d$</td>
<td>75.38±0.16$^e$</td>
<td>81.95±0.26$^a$</td>
<td>81.27±0.18$^b$</td>
</tr>
<tr>
<td>$T_c$ (°C)</td>
<td>86.14±0.32$^d$</td>
<td>89.11±0.24$^e$</td>
<td>89.88±0.38$^b$</td>
<td>90.06±0.43$^a$</td>
<td>89.73±0.23$^{ab}$</td>
</tr>
<tr>
<td>$\Delta T$ (°C)</td>
<td>13.83±0.12$^c$</td>
<td>20.15±0.11$^b$</td>
<td>21.93±0.19$^a$</td>
<td>12.71±0.20$^d$</td>
<td>12.56±0.14$^c$</td>
</tr>
<tr>
<td>$\Delta H$ (J/g)</td>
<td>15.42±0.16$^a$</td>
<td>15.12±0.21$^b$</td>
<td>14.28±0.38$^c$</td>
<td>10.55±0.26$^e$</td>
<td>11.52±0.22$^d$</td>
</tr>
</tbody>
</table>

$^a$ Parameter obtained by XRD: $X_c$, relative crystallinity. Thermal transition parameters measured by DSC: $T_o$, onset temperature; $T_p$, peak temperature; $T_c$, conclusion temperature; $\Delta T (T_c - T_o)$, transition temperature range; $\Delta H$, transition enthalpy. Values are means of three determinations ($n=3$) values. Different letters within a row mean a significant difference ($P < 0.05$). All the values are represented in average ± standard deviation.
3.2 Features of extended-\(q\) SANS data

Fig. 1A presents the extended-\(q\) SANS patterns on a logarithmic scale of the starch subjected to the 0.1% (w/v) or 0.5% (w/v) alkaline treatment for different days (6 or 12 days). All starch samples had a typical scattering peak at about 0.065 Å\(^{-1}\), ascribed to the semicrystalline lamellar structure within the starch granule (Zhang et al., 2017a). This peak may be somewhat weakened by the stronger alkaline solution resulting from the alkali-induced disruption of semicrystalline lamellae. As revealed by the Lorentz-corrected \((I^*q^2 \text{ vs. } q)\) SANS profiles included in Fig. 1B, there is an inflection region between the two regimes. A randomly-oriented, dilute system of lamellae would give rise to a \(q^{-2}\) dependence (Doutch et al., 2013). Here, the power-law dependence for the starch lamellar peak regime extends to \(ca. 0.015\) Å\(^{-1}\) (the inflection region). Below this regime, the \(q\) dependence of starch neutron scattering changes to \(q^{-3}\) or \(q^{-4}\), which is consistent with interfacial scattering; such interfacial scattering can be observed more clearly on a Porod \((I^*q^4 \text{ vs. } q)\) SANS plot (Fig. 1C).
Fig. 1 Double-logarithm (A), Lorentz-corrected ($I^* q^2$ vs. $q$) (B and D), and Porod ($I^* q^4$ vs. $q$) (C) extended-$q$ SANS patterns for waxy maize starch subjected to 0.1% (w/v) NaOH or 0.5% (w/v) NaOH solution treatment for 6 or 12 days. In A-C, data have been shifted vertically for clarity; in D, data are directly overlaid. In the latter, differences in SANS at $q$ values below the lamellar peak can be clearly observed.
### 3.3 Data fitting

The fitting curves for the extended-\(q\) SANS data are shown in Fig. 2. For the power-law regime, a unified model Eq. (1) (Beaucage, 2004; Zhang et al., 2017b) was used to fit the relevant scattering data.

\[
I(q) = G \exp \left( -\frac{R_g^2 q^2}{3} \right) + C \left( \frac{\text{erf} \left( \frac{qR_g}{\sqrt{6}} \right)}{q} \right)^{3\delta} 
\]

In this equation, \(G\) is the prefactor of the Guinier function corresponding to a radius \(R_g\); \(C\) and \(\delta\) are the prefactor and the exponent of the power-law function, respectively.

For the lamellar peak regime of 0.015–0.20 Å\(^{-1}\), an additional Gaussian-plus-Lorentzian function (Eq. (2)) were used to fit the peak scattering \(I_{\text{peak}}(q)\).

\[
I_{\text{peak}}(q) = B + f A \sqrt{\frac{4}{\pi}} \exp \left( -\frac{2 \ln 4 (q - q_{\text{peak}})^2}{W^2} \right) + (1 - f) \frac{2A}{\pi} \frac{2W}{4(q - q_{\text{peak}})^2 + W^2}
\]

In this equation, the first term \(B\) is the scattering background; the second and third terms are the Gaussian and Lorentzian functions, respectively, describing the lamellar peak centred on about 0.065 Å\(^{-1}\); \(f\) is the prefactor for the peak. In the Gaussian and Lorentzian functions, \(A\) is the peak area, \(W\) (Å\(^{-1}\)) is the peak full width at half maximum (FWHM) in reciprocal space, and \(q_{\text{peak}}\) (Å\(^{-1}\)) is the peak centre position. Fig. 2 shows satisfactory fitting results for the scattering data.
Fig. 2 Extended-$q$ SANS patterns and their fitted curves of waxy maize starch subjected to 0.1% (w/v) or 0.5% (w/v) NaOH treatment for 6 or 12 days.
3.4 Lamellar thickness distribution

Fig. 3 illustrates the SANS data (including the scattering from the background, power-law, and lamellae) in the lamellar peak regime (0.015 to 0.20 Å\(^{-1}\)) and the fitted lamellar peaks (the Gaussian-plus-Lorentzian scattering in Eq. (2)) for the untreated and alkali-treated starches. The starches displayed a semicrystalline lamellar peak in the range of \(ca. 0.03\) to \(0.10\) Å\(^{-1}\), corresponding to a lamellar distribution with mean thickness \((d_{ave})\) of \(ca. 9.70–10.00\) nm (see Fig. 4).
Fig. 3 SANS patterns in the lamellar scattering range, and their fitted lamellar peaks, for waxy maize starch subjected to 0.1% (w/v) NaOH or 0.5% (w/v) NaOH solution treatment for 6 or 12 days.
Table 2 shows the fitted peak positions ($q_{\text{peak}}$) and the FWHMs in reciprocal space ($W$) of lamellar peak for different starch samples. The peak position was used to calculate the average thickness ($d_{\text{ave}}$) of semicrystalline lamellae according to the Woolf-Bragg equation, $d_{\text{ave}} = \frac{2\pi}{q_{\text{peak}}}$. Based on Eq. (3), the associated reciprocal of the space value ($W$ in Table 2), $W_{\text{real}}$, is positively related to the thickness distribution width, or equivalently, the lamellar polydispersity, of semicrystalline lamellae based on Eq. (3) (Cardoso et al., 2010; Witt et al., 2012).

$$W_{\text{real}} = \frac{2\pi W}{q_{\text{peak}}^2} \quad (3)$$

The $d_{\text{ave}}$ and $W_{\text{real}}$ values for different starch samples are illustrated in Fig. 4. For the untreated, native starch, $d_{\text{ave}}$ was about 9.70 nm and $W_{\text{real}}$ about 3.90 nm. Both the 0.1% (w/v) and 0.5% (w/v) alkaline treatments slightly increased $d_{\text{ave}}$ to similar extents while the 0.1% (w/v) alkaline solution caused a greater increase in $W_{\text{real}}$. On the other hand, a longer treatment time did not appear to change $d_{\text{ave}}$ or $W_{\text{real}}$ further.
Table 2 Fitted parameters of the lamellar peak SANS data for waxy maize starch before and after 0.1% (w/v) or 0.5% (w/v) NaOH solution treatment for 6 or 12 days.

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>S-0.1-6</th>
<th>S-0.1-12</th>
<th>S-0.5-6</th>
<th>S-0.5-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$ ($\text{Å}^{-1} \text{cm}^{-1}$)</td>
<td>0.0027±0.0001</td>
<td>0.0027±0.0001</td>
<td>0.0029±0.0001</td>
<td>0.0018±0.0001</td>
<td>0.0023±0.0001</td>
</tr>
<tr>
<td>$W$ ($\text{Å}^{-1}$)</td>
<td>0.0264±0.0006</td>
<td>0.0281±0.0009</td>
<td>0.0276±0.0007</td>
<td>0.0269±0.0008</td>
<td>0.0266±0.0008</td>
</tr>
<tr>
<td>$q_{\text{peak}}$ ($\text{Å}^{-1}$)</td>
<td>0.0645±0.0001</td>
<td>0.0628±0.0002</td>
<td>0.0627±0.0002</td>
<td>0.0629±0.0002</td>
<td>0.0627±0.0002</td>
</tr>
<tr>
<td>$B$ ($\text{cm}^{-1}$)</td>
<td>0.021±0.002</td>
<td>0.022±0.002</td>
<td>0.025±0.002</td>
<td>0.023±0.001</td>
<td>0.022±0.001</td>
</tr>
<tr>
<td>$f$</td>
<td>0.59±0.10</td>
<td>0.71±0.13</td>
<td>0.67±0.10</td>
<td>0.62±0.11</td>
<td>0.67±0.12</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>1.98</td>
<td>1.98</td>
<td>1.98</td>
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<td>1.98</td>
</tr>
</tbody>
</table>

$q$ values used for fitting based on Eq. (2): 0.015 to 0.20 Å$^{-1}$. The resultant parameters: $A$, area of the lamellar peak; $W$, full width at half maximum (FWHM) in reciprocal space; $q_{\text{peak}}$, position of the lamellar peak; $B$, scattering background; $f$, prefactor for the lamellar peak function. All the values are represented in average ± standard deviation.
Fig. 4 Average semicrystalline lamellar thickness ($d_{\text{ave}}$) (A) and width of distribution ($W_{\text{real}}$) (B) for waxy maize starch subjected to 0.1% (w/v) NaOH or 0.5% (w/v) NaOH solution treatment for 6 or 12 days.

Table 2 also records the fitted lamellar peak area ($A$), which can be positively correlated with the degree of lamellar ordering (Pikus, 2005; Wang, Wang, Li, Chen, & Zhang, 2017). The 0.1% (w/v) alkaline treatment did not change $A$ even after 12 days. In contrast, $A$ was reduced by 0.5% (w/v) NaOH solution, and this reduction was even greater for 6 days than for 12 days. Our previous SAXS data (Qiao et al., 2017) indicates that the thickness of crystalline lamellae ($d_c$) slightly increased and that of amorphous lamellae ($d_a$) marginally decreased by alkaline treatment. In an earlier study (Qiao et al., 2016), 0.5% (w/v) alkaline treatment of regular maize starch, which is also an A-type polymorphic starch, resulted in a similar increase in $d_c$ while the decrease in $d_a$ was less apparent. In contrast, high-amylose maize starch Gelose 50 (B-type) exhibited increases in both $d_c$ and $d_a$ (Qiao et al., 2016). For other starches subjected to alkaline treatment, a reduction in amylose content has generally been reported (Karim et al., 2008; Nor Nadiha et al., 2010; Wang et al., 2012; Cai et al.,...
In particular, Cai et al. (2014) reported that for a high-amylose rice starch (C-type), 0.4% NaOH treatment resulted in leaching of amylose from starch granules, which was accompanied by changes in granule morphology and electron density between crystalline and amorphous lamellae. In our case here, we observed only slight changes in $d_c$ and $d_a$, which may due to the very low amylose content in waxy maize starch. Besides, the amorphous lamellae are normally thought to include the branching points of the amylopectin macromolecules. The decreases in $d_a$ due to mild alkali treatment may arise from either a physical process, for example, the rearrangement of branching points, or a chemical process due to cleavage/hydrolysis of these branching points or, indeed, a combination thereof. On the other hand, the changes to crystalline lamellae were accompanied by the reduced crystallinity and degree of molecular ordering (Table 1). When the 0.1% (w/v) alkaline solution was used, any changes to the molecular order and crystallites within crystalline lamellae did not cause significant variation to the overall lamellar ordering (as reflected by an unchanged $A$). Nevertheless, a prominent decrease in $A$ was observed for S-0.5-6, indicating the stronger alkaline solution could disrupt the overall lamellar ordering to some extent. This disruption of the lamellar ordering was accompanied by (or should result from) the misalignment of starch double helices (a crystalline smectic–nematic transition), the disruption of less-stable double helices, and the rearrangement of double helices with high degrees of perfection (as reflected by increased $T_o$ and $T_c$, see Table 1). Interestingly, S-0.5-6 presents lower $A$ than S-0.5-12, which may indicate that the disruption of the overall lamellae by strong alkaline treatment was temporary and a longer time of such a treatment could restore the lamellar ordering to some extent although more detailed studies are required to confirm this.
Irrespective of the alkali concentration and treatment time, the four treated samples exhibited higher $d_{\text{ave}}$ and $W_{\text{real}}$ values with the increase in $d_c$ being greater than the decrease in $d_a$ (Qiao et al., 2017) (Fig. 4). We propose that the strong alkaline treatment can more effectively decrease the thickness of amorphous lamellae and increase crystalline lamellae. As a result, there were lower $W_{\text{real}}$ values of S-0.5-6 and S-0.5-12 than those of S-0.1-6 and S-0.1-12 (Fig. 4).

3.5 Large-scale features

To interrogate the large-scale structures such as growth rings and blocklets, Table 3 lists the fitted parameters for the two power-law regimes extended to an ultra-small angle (0.00045 Å$^{-1}$). By fitting the data at low $q$ values (below 0.015 Å$^{-1}$), power law scattering, with an exponent consistent with surface fractal behaviour ($\delta_1 > 3$), was observed for the starch without or with alkaline treatment, with a Guinier scattering from a structure with a radius of gyration ($R_{g1}$). $R_{g1}$ ranges from 326 to 343 nm, corresponding to a diameter of about 1 µm by assuming spherical objects (Beaucage, Kammler, & Pratsinis, 2004), which could be related to larger blocklets or growth rings with finite roughness (Gallant, Bouchet, & Baldwin, 1997). Larger ‘blocklets’ have been proposed in the hard semicrystalline rings of the starch granule with smaller blocklets in the softer amorphous rings (Gallant et al., 1997; Pérez, Baldwin, & Gallant, 2009; Pérez & Bertoft, 2010). The length of a blocklet has also been purported to correspond to the length of a single amylopectin molecule (Szymońska & Krok, 2003). In Table 3, no statistically apparent changes in $R_{g1}$ by alkaline treatment could be found. The 0.1% (w/v) alkaline treatment did not change $\delta_1$ and the 0.5% (w/v) alkaline
solution only reduced δ₁ slightly (especially for S-0.5-6). Structures at these larger length scales were hardly altered by the alkaline conditions used in this work, irrespective of strength or time.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>S-0.1-6</th>
<th>S-0.1-12</th>
<th>S-0.5-6</th>
<th>S-0.5-12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G₁ (10⁵)</strong></td>
<td>1.59±0.35</td>
<td>1.79±0.41</td>
<td>1.84±0.43</td>
<td>0.98±0.22</td>
<td>1.55±0.36</td>
</tr>
<tr>
<td><strong>R₁ (nm)</strong></td>
<td>342.5±13.7</td>
<td>342.0±13.6</td>
<td>339.2±13.8</td>
<td>326.2±13.9</td>
<td>342.8±15.7</td>
</tr>
<tr>
<td><strong>C₁ (10⁻⁷)</strong></td>
<td>1.46±0.33</td>
<td>1.01±0.23</td>
<td>1.04±0.24</td>
<td>5.92±1.54</td>
<td>3.32±0.71</td>
</tr>
<tr>
<td><strong>δ₁</strong></td>
<td>3.40±0.04</td>
<td>3.46±0.04</td>
<td>3.46±0.04</td>
<td>3.15±0.04</td>
<td>3.30±0.03</td>
</tr>
<tr>
<td><strong>G₂</strong></td>
<td>0.93±0.18</td>
<td>0.91±0.24</td>
<td>0.93±0.17</td>
<td>1.51±0.16</td>
<td>1.57±0.20</td>
</tr>
<tr>
<td><strong>R₂ (nm)</strong></td>
<td>12.8±1.37</td>
<td>13.1±1.9</td>
<td>12.5±1.4</td>
<td>12.0±0.8</td>
<td>11.9±1.0</td>
</tr>
<tr>
<td><strong>C₂ (10⁻⁴)</strong></td>
<td>3.59±1.90</td>
<td>3.79±2.37</td>
<td>3.06±1.58</td>
<td>1.15±0.44</td>
<td>1.69±0.71</td>
</tr>
<tr>
<td><strong>δ₂</strong></td>
<td>1.68±0.14</td>
<td>1.65±0.17</td>
<td>1.74±0.14</td>
<td>2.16±0.11</td>
<td>2.09±0.12</td>
</tr>
</tbody>
</table>

*a 1st level covers ca. 0.00045 to 0.015 Å⁻¹; 2nd level covers 0.015 to 0.20 Å⁻¹. Parameters obtained by fitting SANS data with Eq. (1): **G₁** and **G₂**, prefactors of the Guinier scattering for the first- and second-level **q**-ranges, respectively; **R₁** and **R₂**, radii of gyration for the first- and second-level **q**-ranges, respectively; **C₁** and **C₂**, prefactors of the power law for the first- and second-level **q**-ranges, respectively; δ₁ and δ₂, power-law exponents for the first- and second-level **q**-ranges, respectively. All the values are represented in average ± standard deviation.
In the $q$ range above 0.015 Å$^{-1}$, the untreated starch showed a power-law exponent ($\delta_2$) ranging from 1.60 to 2.20, that may be related to mass fractal structures, and a gyration radius $R_{g2}$ of 12–13 nm. Assuming monodisperse spherical objects of uniform scattering density, $R_{g2}$ corresponds to a diameter of about 40 nm, which is within the size range of small starch blocklets as shown by other methods (Doutch et al., 2013). Blocklet size is reported to vary in location within the granule and with botanical source (Tang, Mitsunaga, & Kawamura, 2006; Pérez et al., 2010). Thus, these two parameters could be associated with the blocklets and/or regions of organisation within the branching structure of growth rings. Here, the 0.1% (w/v) alkaline treatment did not lead to apparent changes in $\delta_2$ whereas there was an increase in $\delta_2$ with the use of 0.5% (w/v) NaOH solution. These differences on length-scales greater than lamellae are apparent in Figure 1D. However, no apparent variation in $R_{g2}$ after either the 0.1% (w/v) or 0.5% (w/v) alkaline treatment could be observed, suggesting little change to the blocklets and/or organisation regions (with $R_{g2}$ of 12–13 nm) within the branching structure of growth rings. Our previous SAXS data showed that the 0.1% (w/v) alkaline treatment had no apparent effect on blocklets and mass fractals (with $R_{g2}$ of ca. 195 Å), whereas the 0.5% (w/v) alkaline treatment lead to an increase in $R_{g2}$, which may be linked to the changes to larger-scale structural features as discussed above.

It should be noted that the absence of structural changes within the extended-$q$ SANS scattering data here only reflects changes on length scales up to about 1 µm. Ultra-small-angle neutron scattering (USANS) can readily provide data about structural changes on even larger scales, corresponding to the alteration to granule morphology caused by alkaline treatment as observed by scanning electron microscopy (SEM) (Wang et al., 2012; Cai et al., 2014; Wang et al., 2014; Qiao et al., 2017). As an example, Yang et al. (Yang et al., 2019b) recently used USANS to study pore size
and distribution during amyloglucosidase hydrolysis of corn starch which could be directly correlated with SEM; they noted that USANS provides information on internal pores as well as surface pores. The difference in the large-scale parameters, extracted from unified model fits to the SAXS and SANS data, may arise from scattering contrast sensitivities due to differential solvent accessibility or the different fitted $q$ ranges, with the extended-$q$ SANS accessing lower $q$.

3.6 Further discussion

Early studies (Wootton & Ho, 1989; Ragheb, Abdel-Thalouth, & Tawfik, 1995) have shown that NaOH solutions of high concentration could cause starch gelatinization. The question is to what extent relatively low concentrations (0.1% and 0.5%, w/v) of NaOH could vary the starch structures without degrading starch molecules. Despite the apparent changes in starch molecular order (double helices) and crystallinity (Qiao et al., 2017), the variations by alkaline treatment at the large-scale structural features (growth rings and blocklets) measured by SAXS and extended-$q$ SANS were minor except for the lower overall lamellar ordering (reflected by $A$) exhibited by S-0.5-6. We, therefore, propose the changes caused by NaOH solutions at low concentration start from the smaller-scale structural features (double helices and crystallites), whereas the fundamental arrangement of lamellae, growth rings, and blocklets are largely conserved, at least under the treatment conditions and times studied here. Alkaline treatment may cause slight changes to starch double helices resulting in the slightly increased thickness ($d_{\text{ave}}$) of semicrystalline lamellae, while the lamellar ordering was mostly not varied. Only S-0.5-6 displayed a reduced degree of the lamellar ordering. Regarding this, the 0.5% (w/v) alkaline treatment may initially lead to a crystalline
smectic–nematic transition in some parts of crystalline lamellae (similar to the case of starch gelatinization with limited water (Waigh et al., 2000a; Waigh, Kato, Donald, Gidley, Clarke, & Riekel, 2000b)). However, a longer time of alkaline treatment could bring the double helices back to a smectic state so a restored A value for S-0.5-12 was observed. The latter nematic-smectic transition should be contributed by the small-scale changes such as the enhanced order of starch double helices. These proposed changes are illustrated in Fig. 5, which shows the mild alkaline conditions primarily impact small-scale structural features.

Fig. 5. Schematic representation of the changes in starch lamellar structure and molecular order by alkaline treatment.
The patterns of change to waxy maize starch subjected to alkaline treatment is clearly different from those suffered by other types of starch. An early study (Cardoso et al., 2007) has indicated that the granule organisation of rice starch could be significantly altered with NaOH solutions of concentrations higher than 0.24% (w/v). In a study of C-type starch granules (Thys et al., 2008), SAXS analysis indicated a gradual compression of the semicrystalline growth rings through the swelling of the amorphous growth rings, while wide-angle X-ray scattering indicated a partial degradation of B-type allomorph of starch when granules were alkali-treated. Cai et al. (2014) reported that the treatment of starch by 0.1% (w/v) or 0.4% (w/v) NaOH solution for up to 14 days decreased the difference in electron density between crystalline and amorphous lamellae of starch. Despite these, in these previous studies (Cai et al., 2014; Wang et al., 2014), the changes in amylose content and crystalline structure including long- and short-range ordered structure and crystallinity were not pronounced. As the susceptibility of starch to different physical and chemical treatments highly depends on its native granule architecture and molecular structure, it is always relevant to explore the mechanisms behind these starch structural changes caused by these different treatments. Our previous study (Qiao et al., 2017) has shown significant changes to the starch digestion behaviour caused by the same alkaline conditions. Taking the results from extended-\(q\) SANS into consideration, the varied digestion behaviour of waxy maize starch could be mainly linked to the changes in the small-scale structures (starch molecular order and crystallites) whereas the large-scale structural features (lamellae, growth rings, and blocklets) contributes little.
4. Conclusions

Using extended-\( q \) SANS, in concert with other complementary characterisation techniques, and spanning length-scales from the nanometer to micron, our work has revealed the extent of structural changes on multiple length scales of waxy maize starch subjected to mild alkaline treatment. For this A-type polymorphic starch containing mostly amylopectin, we found the action of either the 0.1\%(w/v) or 0.5\%(w/v) alkaline solution occurs at the smaller length-scale (i.e. starch double helices and crystallites), whereas the large-scale structural motifs such as lamellae, growth rings and blocklets were largely conserved. This approach and the information gained can provide insights into the rational design of granular starch products with varied functions as well as process optimisation.

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References


Highlights:

- Extended-$q$ SANS detects larger-scale structural changes of waxy maize starch
- Mild alkaline treatment mainly changes starch double helices and crystallites
- Lamellar and supramolecular structures were conserved during the alkaline treatment
- There were minimal changes at larger scales (e.g., blocklets and growth rings)
- The mechanism of mild alkaline treatment of waxy maize starch is revealed