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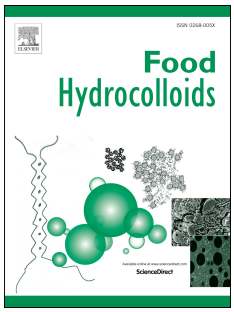

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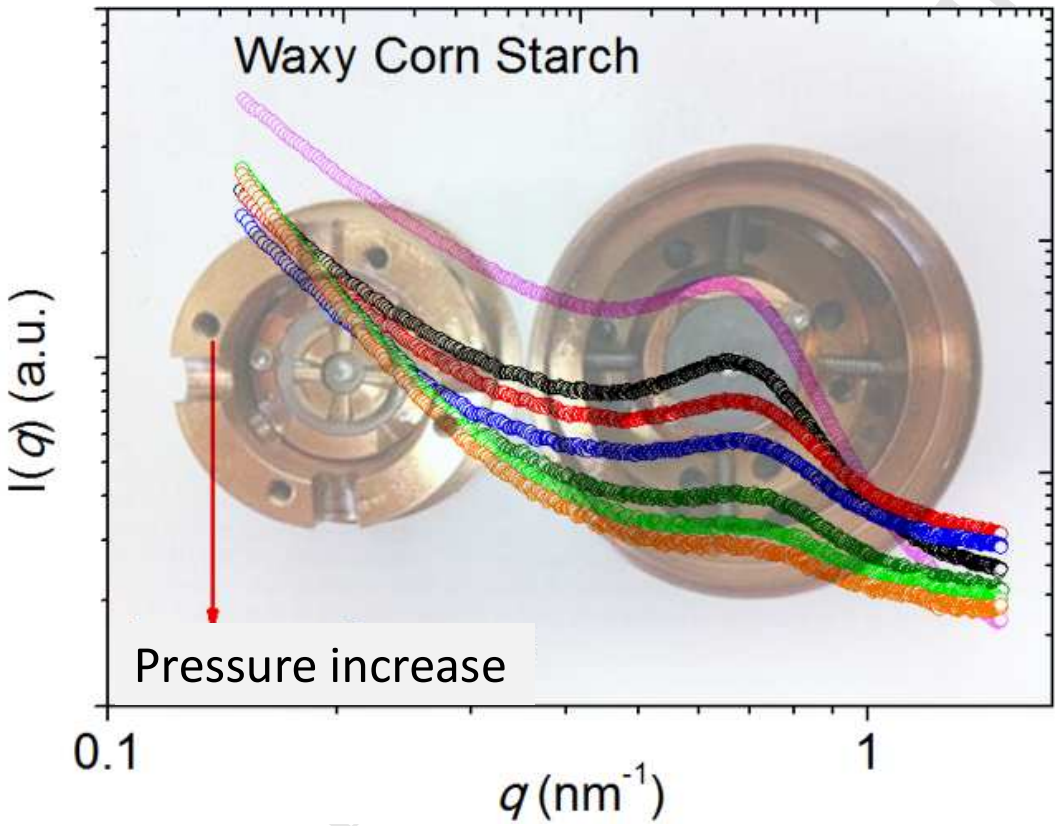
Graphical abstract

In situ synchrotron SAXS patterns of waxy corn starch under different pressure conditions. The background is a picture of the diamond anvil cell (DAC) used in this study.



In situ study starch gelatinization under ultra-high hydrostatic pressure using synchrotron SAXS

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Abstract

The gelatinization of waxy (very low amylose) corn and potato starches by high hydrostatic pressure (HHP) (up to ~1 GPa) was investigated *in situ* using synchrotron small-angle X-ray scattering (SAXS) on samples held in a diamond anvil cell (DAC). The starch pastes, made by mixing starch and water in a 1:1 ratio (by weight), were pressurized and measured at room temperature. During HHP, both SAXS peak areas (corresponding to the lamellar phase) of waxy corn and potato starches decreased suggesting the starch gelatinization increases with increasing pressure. As pressure increased, lamellar peak broadened and the power law exponent increased in low q region. 1D linear correlation function was further employed to analyse SAXS data. For both waxy potato and waxy corn starches, the long period length and the average thickness of amorphous layers decreased when the pressure increased. While for both of waxy starches, the thickness of the crystalline layer first increased, then decreased when the pressure increased. The former is probably due to the out-phasing of starch molecules, and the latter is due to the water penetrating into the crystalline region during gelatinization and to pressure induced compression.

Keywords:

Waxy corn and potato starches; High pressure; Synchrotron small-angle X-ray scattering; Diamond anvil cell

1. Introduction

Starch is one of the most common biomacromolecules present in nature and consists of two major types of α-glucans at the molecular level: the linear amylose and the branched amylopectin. The former is mostly linear with few branches and a molecular weight of 10^5-10^6 Da, and the latter is extensively branched with about 5-6% branches scattered along the backbones with a molecular weight in the range of 10^7-10^9 Da (Buléon, Colonna, Planchot & Ball, 1998; Pérez & Bertoft, 2010; Zobel, 1988). The starch granules display a hierarchical structure periodicity and are organised into concentric rings radiating out from the central hilum to the surface of the granule. The number and size of the rings depend on the botanical origin of the starch, and it is generally believed to display an onion like organization with alternating 120-400 nm thick amorphous and semi-crystalline growth rings (Chen, Yu, Simon, Liu, Dean & Chen, 2011; Vermeylen, Derycke, Delcour, Goderis, Reynaers & Koch, 2006). It is believed that the amorphous rings consist of amylose and amylopectin in a disordered conformation, whereas the semi-crystalline rings are formed by a lamellar structure of alternating crystalline and amorphous regions with regular repeat distance of 9-10 nm as revealed by small angle x-ray scattering (SAXS) (Cameron & Donald, 1992).

Gelatinization is one of the most important processes in the industrial application of starch. Besides heating starch in water, high hydrostatic pressure (HHP) can also be employed to gelatinize starch (Katopo, Song & Jane, 2002; Oh, Pinder, Hemar, Anema & Wong, 2008). It is suggested that during HHP, the transition of starch crystalline structures could occur (e.g. from A-type to B-type) (Katopo, Song & Jane, 2002). When enough high pressure is exerted, the starch granule can be fully gelatinized and loose its crystalline structures (Yang, Gu & Hemar, 2013). Besides the crystalline structure changes induced by HHP, the other supramolecular structures (e.g. lamellae characteristics, fractal structures, etc.) are expected to be affected by HHP. Unfortunately until very recently, studies on the *in situ* effects of HHP on starch systems are scarce (Gebhardt, Hanfland, Mezouar & Riekel, 2007; Yang, Gu & Hemar, 2013). Gebhardt et al. (2007) employed synchrotron SAXS/WAXS to study *in situ* potato starch gelatinization under HHP, and showed that the onset of gelatinization starts with the hydration of the semicrystalline lamellae and lateral breakdown of the crystalline domain. The SAXS analysis in their study did not investigate the changes in the amorphous and

crystalline layers. Yang et al. (2013) utilised synchrotron X-ray powder diffraction (WAXS) to investigate *in situ* waxy and high amylose corn starch gelatinization under HHP focusing on their crystalline structure changes (e.g. crystalline type and *d*-spacing) and demonstrated that starch retrogradation starts immediately after pressure removal.

In this study, synchrotron SAXS is used to probe *in situ* the effect of HHP on the structure of waxy corn and potato starches. Synchrotron SAXS has the advantages over lab-bench SAXS, due to its higher intensity and collimation, enabling data to be obtained in real-time, and waxy starches were selected as they show clearly a peak corresponding to the lamellar phase. To the best of our knowledge, this study is the first to report *in situ* synchrotron SAXS measurements on waxy starch dispersions in water under high pressure using a diamond anvil cell (DAC) in order to probe the changes in waxy starch amorphous and crystalline layers under HHP.

2. Materials and Methods

2.1 Materials and sample preparation

Waxy maize (amylose content 1.37±0.09 w/w%) and potato (amylose content 1.69±0.64 w/w%) starches were donated by Avebe Food (Auckland, New Zealand). Starch powder (0.2 g) was mixed with 1 ml Milli-Q water at room temperature and vortexed for 3 min. The starch suspensions were centrifuged at 10,000 rpm for 5 min and the supernatants were removed. The water content in the starch paste was ~ 50% (w/w).

2.2 Methods

A diamond anvil cell (DAC) (easyLab) with 1 mm anvil culet size was used. The drilled hole (500 µm diameter and 150 µm thickness) of stainless steel gasket was used to host the starch sample and ruby balls of *ca.* 20 µm diameters were loaded with the sample to measure the pressure. The pressure was generated by tightening the four cap screws of the DAC step by step. The pressure was measured from the shift of the ruby fluorescence using Ocean optics system (FL, USA).

In situ synchrotron small angle X ray scattering (SAXS) experiments were conducted on the beamline BL16B1 at the Shanghai Synchrotron Radiation Facility (SSRF, China). A monochromatic beam of 0.1240 nm wavelength was used, and the sample-to-detector distance was set to 2150 mm. Scattering was detected in the q ranges of 0.15-1.5 nm^{-1} in which q= $(4\pi\sin\theta)/\lambda$ (where 2θ is the scattering angle and λ is the wavelength). FIT2D software (http://www.esrf.fr/computing/scientific/FIT2D/) was used to convert the one-dimensional (1D) data from the 2D scattering pattern. All the data were background subtracted and normalized.

2.3 Analysis of SAXS data

SAXS scattering curve were fitted to a power-law function plus a Gaussian peak (Blazek & Gilbert, 2010):

$$I(q) = B + Pq^{-\alpha} + \frac{A\sqrt{\ln 2}}{W\sqrt{\pi/4}}\exp\left(-\frac{4\,ln\,2\;(q-q_0)^2}{W^2}\right) \qquad (1)$$

where B is the background; the second term is the power-law function where P is the power law pre-factor and α is the power-law exponent; the third term is a Gaussian function where A is the Gaussian peak area, W (nm^{-1}) the full width at half maximum of the peak, and q_0 (nm^{-1}) is the peak centre position.

The SAXS data are also analysed by the 1D linear correlation function *L(r)* which is derived from Fourier transition of the scattering curves (Fan, et al., 2014; Zhang, et al., 2015).

$$r(z) = \frac{\int_0^\infty I\,(q)q^2\cos(qz)dq}{\int_0^\infty I\,(q)q^2dq} \qquad (2)$$

In equation (2), r (nm) is the distance in real space. Determinations of the lamellar parameters of starch samples are conducted as follows: long period (d), i.e. the lamellar repeat distance is the value of z at the second maximum of r (z); the average thickness of the amorphous lamellae is expressed as d_a, which can be acquired by the solution of the linear region and the flat r (z) minimum. Thus, d_c, the average thickness of crystalline lamellae can be calculated

by d_c=d- d_a. The correlation function analysis was conducted using S programme package (Li, 2013).

3. Results and Discussion

In situ SAXS patterns of waxy corn and potato starches, under HHP, are reported in Figure.1A and B, respectively. The curves are all characterized by intense scattering at low scattering vector (q). A typical SAXS peak could be observed at a q of 0.6 to 0.7 nm^{-1}, indicating a 9-10 nm semi-crystalline structure, according to Bragg's law $d = 2\pi/q$ (Blazek & Gilbert, 2011). HHP treatment resulted in the reduction in the peak intensity after normalization. To analyse the SAXS curves Equation. (1) is used and the goodness of the fits can be seen in the supporting Figures. S1 and S2. The resulting parameters are reported in Figure. 2A-F. For both starches, the peak area, which indicates the degree of lamellae ordering (Pikus, 2005), decreases with increase in pressure due to gelatinisation. Conversely, the peak width which depends on the regularity of the lamellar arrangement within starch granule (Blazek & Gilbert, 2010), increases with the increase in pressure. It could be due to the fact that at the limit of lamellae compression, excess compression may be accommodated by lamellar 'buckling'. This leads to an increase of the distribution of lamellar sizes thus broadening the SAXS peak. A similar SAXS peak broadening has been suggested to occur due to compression induced by water freezing (Perry & Donald, 2000). In the low-q region the curves comply with a simple power law equation ($I\ (q) \sim q^{-\alpha}$) (Martin & Hurd, 1987); where the exponent α gives insight into the surface/mass fractal structure of the starch granules (Suzuki, Chiba & Yarno, 1997). Moreover, the mass fractal dimension ($0<\alpha<3$) is used to indicate the compactness, whereas the surface fractal dimension ($3<\alpha<4$) is regarded as an indicator of the degree of smoothness of the scattering objects (Zhang, et al., 2014). In the case of the fractal dimension of the two starches under HHP, the mass fractal dimension ($1<\alpha<3$) increased suggesting that the starch structures become more compact due to compression during pressurization. Note that for waxy corn starch, when the pressure is increased to $\geq$ 670 MPa, the value of α is higher than 3; suggesting the appearance of surface fractal structure. It is suggested that the scattering objects of surface fractals are more compact than those of mass fractals (Zhang, et al., 2014).

Further analysis of the SAXS measurements were performed using the linear correlation functions (Equation (2)) (Figure. 1C-D). The most relevant morphological parameters

obtained through correlation function analysis are d (long period distance), d_a (average thickness of amorphous layers), and d_c (crystalline and amorphous layer thickness) (Figure .2D-F). For both waxy corn and potato starch, d decreases with the pressure increase. This is due to lamellae compression under high pressure (Gebhardt, Hanfland, Mezouar, & Riekel, 2007). Compared to waxy corn starch, the decrease of d with pressure (up to 750 MPa) is less for potato starch compared to corn starch. It is suggested that having longer amylopectin chains, B-type starch (waxy potato) is much less compressible compared to A-type starch (waxy corn), since lamellae phases made of long amylopectin chains require an overall layer bending to accommodate the internal stresses generated by the compression (Daniels & Donald, 2003; Lan, Li, Xie & Wang, 2015). As pressure is increased, the amorphous layer starts to decrease for both starches probably due to simple compression (Gebhardt, Hanfland, Mezouar & Riekel, 2007) or/and due to the leaching out of starch molecule from the amorphous layer (Zhang et al., 2015). Similar trend is also observed in thermal gelatinization of starch (Jenkins & Donald, 1998; Zhang et al., 2015). Further, the amorphous regions of starch granules may be expected to act as 'shock absorbers' upon the application of compressive forces (Morgan, Furneaux & Larsen, 1995; Perry & Donald, 2000). This is also consistent with the idea that amorphous regions within lamellar systems 'protect' the crystalline regions by preferential compression upon impact. The crystalline layer thickness d_c of both waxy corn and potato are falling in the range of 5-7 nm, as suggested by Putaux, Molina-Boisseau, Momaur & Dufresne, 2003. Contrary to the amorphous layers, the thickness layers thickness d_c initially increased slightly followed by a decrease in very high pressure region for both waxy corn and waxy potato starches. Compared to the amorphous layers, the crystalline layers have higher degree of order and rigidity thus are expected to be more resistant to compression (Perry & Donald, 2000). During gelatinization, the water penetrates into crystalline region, which results in an increase of the d_c and eventually the formation of a disordered phase through disruption of the helical packing (Gebhardt, Hanfland, Mezouar & Riekel, 2007). The decrease of d_c at very high pressure could attribute to the crystalline layer compression.

Overall, in our view an important observation from these experiments is the fact that all the parameters considered through the analysis of both the SAXS data and their corresponding correlation functions are affected by pressure. While most of the published literature gives a threshold pressure for starch gelatinisation, depending on the starch type and amylose content (Buckow, Jankowiak, Knorr, & Versteeg, 2009), this study indicates that the starch granule

fine structure is affected as soon as pressure is applied. In all cases, this study demonstrates the potential of using synchrotron SAXS in combination with a DAC to monitor *in situ* starch gelatinization under HHP.

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Highlights

- *In situ* SAXS is performed on starch dispersions under pressures of up to ~ 1 GPa.
- The starch granule fine structure is affected as soon as a pressure is applied.
- Waxy corn and potato starches showed similar structure changes due to pressure.