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Short communication

In situ study of maize starch gelatinization under ultra-high hydrostatic pressure using X-ray diffraction

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ABSTRACT

The gelatinization of waxy (very low amylose) and high-amylose maize starches by ultra-high hydrostatic pressure (up to 6 GPa) was investigated *in situ* using synchrotron X-ray powder diffraction on samples held in a diamond anvil cell (DAC). The starch pastes, made by mixing starch and water in a 1:1 ratio, were pressurized and measured at room temperature. X-ray diffraction pattern showed that at 2.7 GPa waxy starch, which displayed A-type XRD pattern at atmospheric pressure, exhibited a faint B-type-like pattern. The B-type crystalline structures of high-amylose starch were not affected even when 1.5 GPa pressure was applied. However, both waxy and high-amylose maize starches can be fully gelatinized at 5.9 GPa and 5.1 GPa, respectively. In the case of waxy maize starch, upon release of pressure (to atmospheric pressure) crystalline structure appeared as a result of amylopectin aggregation.

1. Introduction

Next to cellulose, starch is the second most abundant biopolymer present in nature, and can be found in many different botanical sources such as wheat, peas, and potato. Starch is mostly composed of a mixture of glucose polymers named amylose and amylopectin. Amylose is mostly a linear polymer of molecular weight ranging between 5×10^5 and 1×10^6; whereas amylopectin is a much branched polymer of molecular weight of several millions (Wang, Bogracheva, & Hedley, 1998). There are three different types of sub-chains within the amylopectin molecular, namely A, B and C. The A chains are the shortest (DP 6–15) and are linked by a single α 1-6 linkage, which identifies the A chains as 'outer' chains. B chains are longer (DP 15–50) and support both A chains and other B chains. There is only one C chain per amylopectin molecule and it is identified as having the only non-reducing end (Donald, 2004). The native starch granule is present in a semi crystalline form with degrees of crystallinity ranging from 15% to 45% depending on the starch (Cairns, Bogracheva, Ring, Hedley, & Morris, 1997), and it has been shown that it is the amylopectin that forms the crystalline region within the granule (Donald, 2004).

Due to its semi crystalline structure, X-ray powder diffraction (XRD) has been employed extensively to characterize the physico-chemical properties of starch. It is generally recognized that there are mainly three XRD patterns for starches namely A, B and C (Sarko & Wu, 1978). A-type patterns are generally characteristic of cereals, such as waxy maize, rice, and wheat, with amylopectin of relatively shorter chain length, while a B-type pattern is shown by tubers (*e.g.* potato) and high amylose maize (>40%) starches with amylopectin of longer chain length. The C-type pattern is mainly exhibited by legumes (Cairns et al., 1997; Karim, Norziah, & Seow, 2000). Both A and B types of starch have essentially the same helical structure within their polymorphic forms (Imberty, Buléon, Tran, & Pérez, 1991). However, the packing of these double helices within A-type structure is relatively compact with low water content, while the B-type has a loosely packing of these double helices containing a hydrated helical core (Pei-Ling, Xiao-Song, & Qun, 2010). C-type is now believed to represent a combination of A-type and B-type polymorphic structures (Tester, Karkalas, & Qi, 2004).

High hydrostatic pressure (HPP) has been employed to gelatinize or physically modify starch dispersions (Błaszczak, Fornal, Valverde, & Garrido, 2005; Błaszczak, Valverde, & Fornal, 2005; Błaszczak et al., 2007; Buckow, Heinz, & Knorr, 2007; Douzals, Cornet, Gervais, & Coquille, 1998; Gebhardt, Hanfland, Mezouar, & Riekel, 2007; Hibi, Matsumoto, & Hagiwara, 1993; Muhr & Blanshard, 1982). However, to the best of our knowledge, most of HHP treatments were performed up to 600–800 MPa, due to the limit in the maximum pressure achievable on most commercial HHP equipments (Liu, Selomulyo, & Zhou, 2008). Further, most studies on the effect of HHP on starch samples (especially for starch–water-suspensions) are carried out post pressure treatment, and usually undergo several sample preparation steps

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Table 1
Chemical composition (w/w, %) of the maize starches used.

	Waxy starch	High-amylose starch
Water	11.26 ± 0.61	12.29 ± 0.69
Amylose	1.37 ± 0.09	89.78 ± 0.96
Lipid	0.24 ± 0.01	0.14 ± 0.01
Ash	0.05 ± 0.01	0.10 ± 0.01
Protein	1.76 ± 0.33	2.66 ± 0.41

including filtration and drying prior to XRD measurements (Hibi et al., 1993; Katopo, Song, & Jane, 2002). To overcome these problems, in this study starch gelatinization at very high pressures is monitored *in situ* using synchrotron XRD in combination with a diamond anvil cell (DAC) which is known to generate pressures of up to 30 GPa (Jayaraman, 1983). Synchrotron XRD has advantages over the lab-bench XRD due to its higher intensity and short acquisition time, enabling XRD pattern to be obtained in real-time condition and is not restricted to starch samples in powder form (Cai, Bai, & Shi, 2012). To investigate the effect of amylose content a waxy starch with very low amylose content and a high-amylose starch were studied. The reversible effects occurring upon release of pressure were also considered.

2. Materials and methods

2.1. Materials and sample preparation

Waxy (Novation2300) and high-amylose (Gelose80) maize starch powders were donated by National Starch Food Innovation (Auckland, New Zealand). Their chemical compositions are reported in Table 1. Starch powder (0.2 g) was mixed with 1 ml Milli-Q water at room temperature and vortexes (Labnet VX100, Australia) for 1 min. The starch suspensions were centrifuged at 10,000 rpm for 5 min on a Mini Spin eppendorf and the supernatants were removed. The water content in the starch paste was approximately 50% (w/w).

2.2. Methods

The diamond anvil cell (DAC) used in this study was developed at the Australian Synchrotron (Melbourne - Australia). It uses a 0.5 mm diamond culet (easyLab, UK), a 0.3 mm thick 100 mm diameter rhenium gasket (easyLab, UK) with a 0.2 mm hole which was drilled by Boehler micro driller (easyLab, UK). The drilled hole is used to host the starch sample and ruby balls 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 Optiprexx Ruby Lux system (easyLab, UK).

In situ synchrotron X-ray diffraction (XRD) experiments were performed at the Australian Synchrotron PD (Powder Diffraction) beam line, on the sample held in the DAC. All measurements were performed at room temperature. The beam with photon energy 18 keV from the bending magnet is monochromated to a wavelength $\lambda = 0.6881$ Å by a double crystal monochromator (DCM). A vertical collimating mirror was used to reject higher harmonics. Two JJ slits are put about 0.5 m and 1.5 m upstream from sample. A nominal beam size of 0.15 mm × 0.15 mm is used to ensure that the beam goes through the hole within DAC gasket. The diffraction patterns were recorded for 10 min with 20 s per cycle and multi read 30 times for each sample using a Mar165 CCD detector (165 mm diameter). The sample-to-detector distance was determined to be 250 mm. The XRD of a NIST 640C-Silicone Line Position is used to calibrate and correct the detector distortions. Australian Synchrotron developed software and FIT2D analysis program were employed to analyze the data.

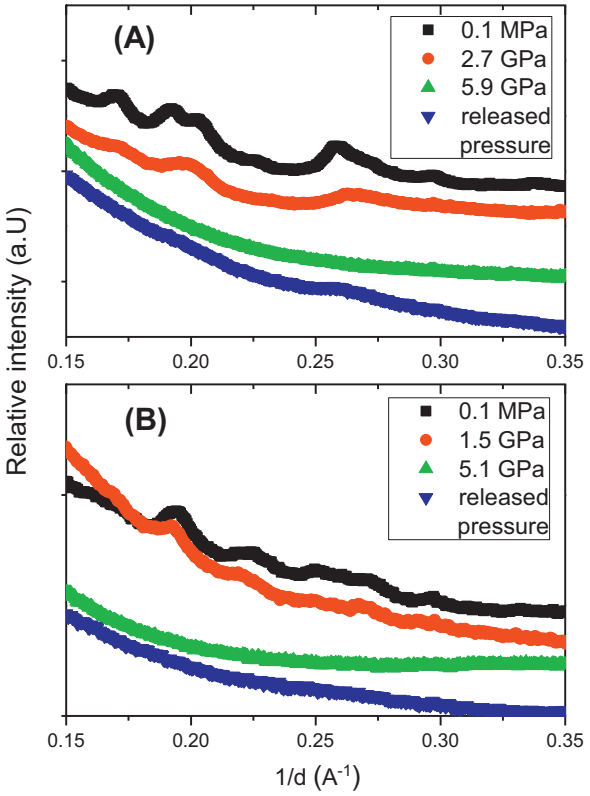

Fig. 1. *In situ* synchrotron X-ray diffraction patterns of waxy (A) and high-amylose (B) maize starches under different pressure conditions.

3. Results and discussion

X-ray diffraction patterns of waxy and high-amylose maize starches are reported in Fig. 1. The measured raw X-ray intensities are shifted in the direction of *y*-axis to allow comparison between the different HHP conditions. Due to the high amount of water present in the samples, the XRD spectrums show very broad and non intensive diffraction peaks. It could be seen that at atmospheric pressure the waxy starch exhibit a typical A-type XRD pattern, showing four strong peaks at 5.95, 5.21, 4.96 and 3.86 Å. When a pressure of 2.7 GPa is applied a faint B-type like XRD pattern is obtained; this is indicated by the transition from a double peak (at 5.21 and 4.96 Å) to a single peak at 5.05 Å. Nevertheless, the single peak around a 3.86 Å which is a characteristic of the A-type XRD pattern was not affected. These results are in agreement with those reported in previous studies on the effect of HHP on waxy maize starches (Hibi et al., 1993; Katopo et al., 2002). The transition from A-type to faint B-type, for waxy maize starch was previously explained in terms of the B-type structure being more favored by HHP because of the higher number of associated water molecules stabilizing the helix structure through van der Waals forces (Buckow, 2006). The XRD pattern recorded from waxy maize starch at 2.7 GPa slightly shifted toward higher values of $1/d$ compared to a classical B-type XRD pattern, and this is likely due to lattice compression as suggested by (Gebhardt et al., 2007). When a higher pressure is applied (5.9 GPa), the XRD spectrum does not show any peak. This indicates that the waxy starch dispersion is fully gelatinized. In the case of high-amylose maize starch, at atmospheric pressure the typical B-type XRD pattern, with peaks at 5.88, 5.21, 4.48, and 4.00 Å is observed (Fig. 1B). The observed peak at

4.48 Å (corresponding to 20° in conventional XRD pattern) indicates the presence of an amylose–fatty acid complex (V-pattern) (Katopo et al., 2002). At 1.5 GPa, an XRD spectrum similar to the B-type of the non pressurized high-amylose starch is observed. This confirms the resistance of high-amylose starches to HHP as previously reported in the literature (Błaszczak et al., 2007; Oh, Pinder, Hemar, Anema, & Wong, 2008). It has been suggested that under HHP, amylose–fatty acid complexes could be formed in starches with high amylose content and these complexes may restrict the swelling and gelatinization of the starch granules (Katopo et al., 2002). Note however that in these studies the HHP applied was always smaller than 1 GPa. Further, the water content in the starch dispersion is also an important parameter, and it was reported that the pressure threshold of starch gelatinization decreases with the increase in water content (Hibi et al., 1993); and (Stute, Klingler, Boguslawski, Eshtiaghi, & Knorr, 1996). It is worth noting that the B-type XRD pattern recorded from high amylose starch at 1.5 GPa is shifted toward lower $1/d$ when compared to XRD pattern recorded at ambient pressure. The B-type starch has relatively more open structure and larger water channels (Sarko & Wu, 1978). It is possible that more water molecules penetrate into the high-amylose starch granules due to high pressure thus increasing the d spacing, as previously suggested by Gebhardt et al. (2007). However at 5.1 GPa, no peak is observed in the XRD profile (Green line in Fig. 1B), and this would indicate that all crystalline structures, including those resulting from amylose–fatty acid complexes are disrupted. To the best of our knowledge this is the first time full gelatinization of high-amylose maize starch under HHP is reported.

To investigate reversible effects, XRD measurements were performed on the starch pastes after the high pressure in the DAC is brought back to atmospheric pressure. Measurements showed that for the waxy maize starch, broad peaks appeared between 3.33 Å and 4.45 Å (blue lines in Fig. 1A). This effect is instantaneous and is likely due to the rearrangement and association of the outmost short branches of amylopectin (DP = 15) through hydrogen bonding resulting in the formation of some crystalline structures during retrogradation (Hoover, 2001; Ring et al., 1987). Amylose crystallization occurs by the formation of double-helical associations of 40–70 glucose units through hydrogen bonding during retrogradation (Hoover, 2001; Jane & Robyt, 1984; Leloup, Colonna, Ring, Roberts, & Wells, 1992). However, no diffraction peaks characteristic from potential retrogradation is observed in the high-amylose starch XRD spectrums recorded after pressure release (blue lines in Fig. 1B). This could be attributed to the fact that the remaining amylose disordered amorphous regions are still connected to the aggregated regions thus the ordering and association of amylose is not regular enough to produce coherent Bragg peaks in XRD spectrums (Frost, Kaminski, Kirwan, Lascaris, & Shanks, 2009). Recrystallization of starch molecules, after heat treatment or pressure is well studied and is usually termed starch retrogradation. The results of the present study confirms the findings of Stute et al. (1996) who observed retrogradation peaks using Differential Scanning Calorimetery (DSC) on pressure-treated starch samples, and provide a direct demonstration to their suggestion that starch retrogradation occurs instantaneously after pressure treatment.

4. Conclusion

High pressure treatments of low amount of amylose maize starch (A-type) in the presence of water can be partly transformed first to a B-type crystallinity, while high amount of amylose content maize starch (B-type) can keep its original B-type crystallinity under similar HPP condition ($\leq$1.5 GPa). However, when exposed to very high pressure (>2.5 GPa), both waxy and high-amylose maize starches can be fully gelatinized shown through total loss of crystalline by XRD. When the pressure is released back to atmospheric pressure, waxy maize starches showed broad XRD peaks likely due to amylopectin reaggregation, an effect which is known to be at the origin of starch retrogradation. It is important to stress that this study was carried out at starch to water ratio of 1:1, and it is expected that the effect of HHP on starch crystallinity will depend on this water content. Overall, this study demonstrates the potential of using synchrotron XRD in combination with a DAC to monitor *in situ* starch gelatinization at pressure exceeding the GPa.

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