ESRF	**Experiment title:** Time-resolved monitoring of structural changes during in situ hydration of starch granules	**Experiment number:** A02-1-915
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Project outline and objectives

Starch, the major energy reserve of higher plants and the main source of calories in our diet, is widely used in food and non-food industries. Starch is a mixture of two homopolymers of glucose, namely linear amylose (~20 wt%) and branched amylopectin (~80 wt%). Although their shape and size (1 to 100 µm) depend on their botanical origin, all native starch granules exhibit a *multilayered organization* with different structural lengthscales: i) alternating semicrystalline and amorphous *growth rings* (200-600 nm) and ii) alternating crystalline and amorphous *lamellae* from the clustered distribution of amylopectin branches, with a repeat distance of 9-10 nm [Pérez 2010]. Native granules exhibit distinct WAXS signatures ("A" for cereals and "B" for tubers and amylose-rich starches) that differ by the packing of double helices formed by amylopectin branches (A is monoclinic, B is hexagonal) and the number of bound water molecules (8 per unit cell for A and 36 for B). Water thus plays a crucial structuring role, both in the lamellar order and degree of crystallinity of the granules. Studies carried out on a few hundred-milligram powder samples showed that once placed for several days in a chamber maintaining a 95% relative humidity (RH), starch spontaneously adsorbed up to 35 wt% water [Buléon 1982]. Conversely, the local hydration properties were studied by projecting microdrops of water on the surface of single granules and monitoring the intensity changes in synchrotron microdiffraction patterns [Lemke 2004]. While the impact of water content on the lamellar repeat was assessed from SAXS profiles of wheat starch granules [Cameron 1993], to our knowledge, the hydration dynamics of the crystalline fraction have not been studied yet. Our experiments aimed at shedding light on the hydration dynamics by monitoring the structural changes over the course of a few hours, as a function of the amylose/amylopectin ratio and allomorphic type of the granules, to rationalize the equilibration process.

Experimental method

Sample preparation. Powders of native starch granules, amylose spherulites and amylose-ibuprofen complexes were previously dried into primary vacuum during 24 h and the partial disappearance of the semicrystalline structure was assessed by laboratory WAXS. The semi-dry powders were rapidly poured into 3-mm (o.d.) glass tubes folded into a L-shape in a flame. The powders were maintained with of porous Teflon wool at both ends, close to the fold (*Fig. 1A*). The tubes were end-caped while being carried to the beamline.

Experimental set-up. The tubes were mounted on an automatic sample changer and the top of each tube was connected by silicon pipes to the hydration system that was lent by the PSCM platform (*Fig. 1B,C*). Nitrogen, dry or at maximum relative humidity was continuously blown from the top of the tubes through the

powders. Hydration / dehydration cycles were carried out over several hours (between 3 and 12). In an additional experiment, all specimens were soaked in liquid water to favor the lamellar ordering in the starch granules. WAXS and SAXS data were simultaneously collected over two *q*-ranges corresponding to the crystal structure (0.1 to 4 $Å^{-1}$) and molecular ultrastructure (0.1 to 1 nm^{-1}), using the WOS and D5 detectors, located at 13.4 and 161 cm of the specimens, respectively. Scattering patterns were collected from the tubes containing the starch powders during 20 s exposures, at a beam energy of 15.8 keV. For a given specimen, the time between two acquisition varied from 30 s to 20 min, depending on the experiment. The 2D scattering pattern of an empty glass tube was subtracted from those from the specimens and were rotationally averaged with a homemade program to yield scattering profiles. The profiles were calibrated using those collected from BeAg (SAXS) and LaB_6 (WAXS) standards.

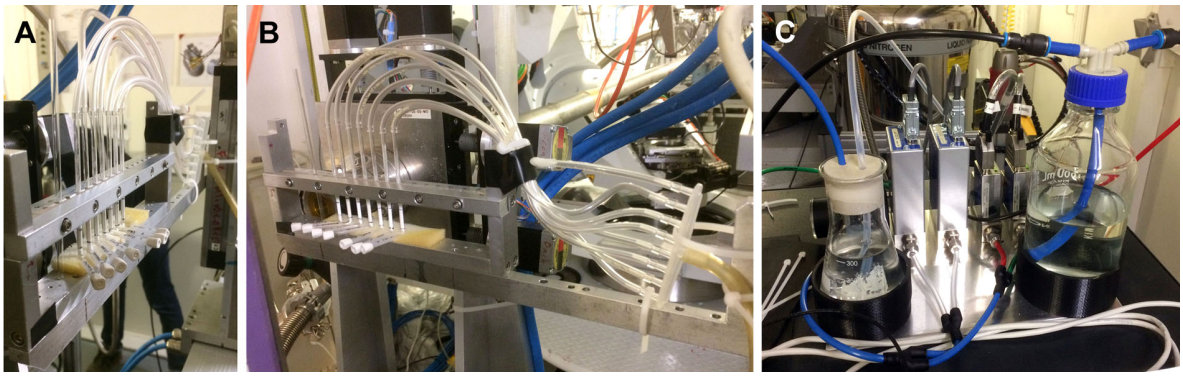

Fig. 1. *Experimental set-up: L-shaped glass tubes containing the various powders are placed inside the sample changer (A,B) and connected to the hydration system (C).*

Results

Native starch granules yield powder XRD patterns corresponding to one of two distinct allomorphs (mainly monoclinic A-type and hexagonal B-type) or a mixture of both. In both cases, the crystals contain double-helical chains stabilized by a number of water molecules. Preliminary results from the collected data show that changes in the relative intensities of the WAXS profiles upon hydration can be detected within the first hour of hydration, the crystallinity slowly developing afterwards (*Fig. 2A,C*). However, even after 12 h of hydration, the starch granules are still far from equilibrium in water uptake. The changes in the profiles of B-type starch granules are more easily visible, in particular due to the intensity variation of the 100 peak at about q = 0.40 $Å^{-1}$ that is known to be very sensitive to hydration (*Fig. 2A*).

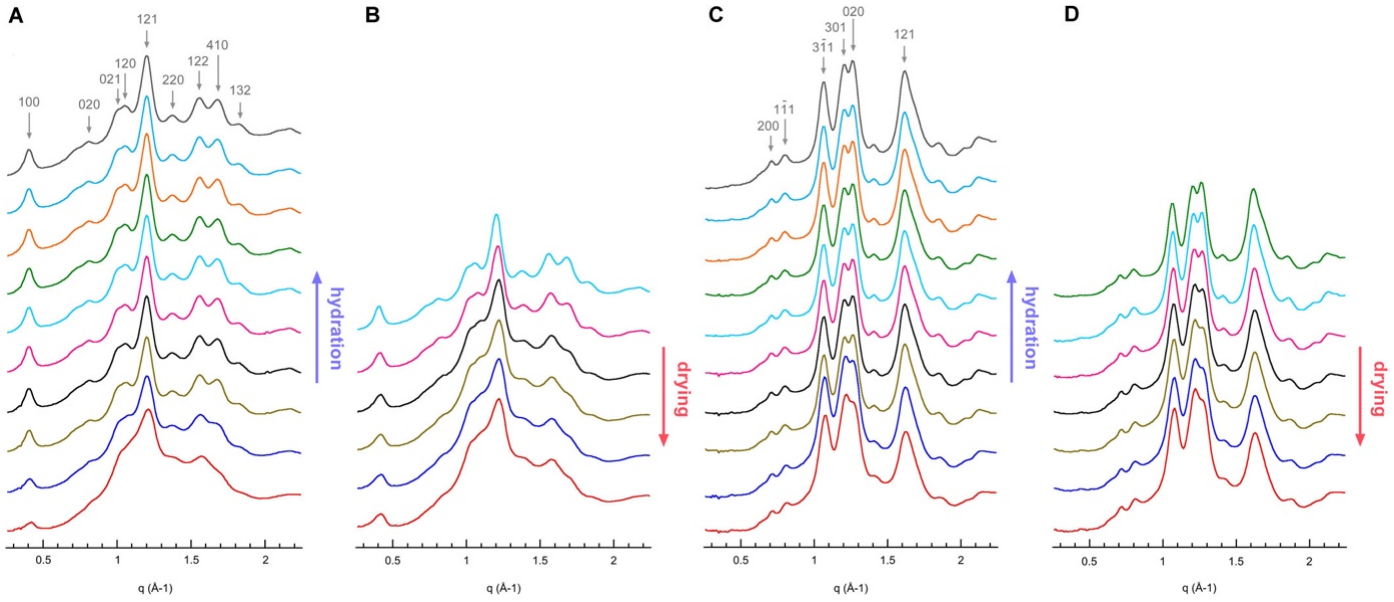

Fig. 2. *Series of WAXS profiles from starch granules from various cultivars upon hydration (A,C) and drying (B,D): A,B) standard potato (allomorph B); C,D) waxy maize (allomorph A). Hydration was carried out during 12 h and drying over 3 h.*

B-type is a more hydrated structure compared to A-type and the crystal contains "channels" that can rapidly incorporate water molecules. This peak corresponds to a lattice distance of 16 Å and is related to the spacing of the double helices. During water uptake, the resolution of diffraction peaks increases which means that the helix ordering in the unit cell improves. In addition, the position of the 100 peak slightly shift upon hydration (from q = 0.418 to 0.405 $Å^{-1}$ – *Fig. 2A*) suggesting that the unit cell expands upon hydration, and contracts back upon drying (q = 0.418 $Å^{-1}$ – *Fig. 2B*). Although the drying time was significantly shorter (3 h), the structural evolution was reversible for both A and B types. In our conditions, drying thus appeared to be faster than water uptake.

Conclusion

The literature reports that reaching maximum water uptake by a few hundred milligrams of starch granules requires several days of equilibration at a relative humidity of 95%. In our experiments, although we only used a few milligrams and blew a humid nitrogen atmosphere through the specimens, the hydration process, as judged by the evolution of crystallinity, remained rather slow. However, during experiments carried out over several hours (typically between 3 and 12 h), preliminary data treatment showed that it was possible to detect changes in peak position and relative peak intensity in WAXS patterns. Further processing of the WAXS and SAXS data should provide information on the dynamics of these early stages of hydration and subsequent drying, and on differences that may depend on the crystal type, granule size, amylose/amylopectin ratio, etc. We will evaluate the possible correlation of the ordering processes in starch granules at different lengthscales as well.

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