ESRF	**Experiment title:** In-situ study of the strain-induced structural evolution of amorphous starch films	**Experiment number**: 02-01-828
Beamline: BM02	**Date of experiment**: from: 29 september 2012 to: 02 october 2012	**Date of report**: 2013-03-05
Shifts: 9	**Local contact(s)**: Cyrille Rochas	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Leroy Lise, Unité Matériaux et Transformation (UMET), Lille, France
Dr. Stoclet Grégory, Unité Matériaux et Transformation (UMET), Lille, France
Pépin Julie, Unité Matériaux et Transformation (UMET), Lille, France
Defebvin Juliette, Unité Matériaux et Transformation (UMET), Lille, France
Dr. Séguela Roland, Matériaux, Ingénierie et Sciences (MATEIS) , Lyon, France

Report:

Starch actually knows an increasing interest as this biobased polymer is envisaged for the replacement of commodity polymer. Besides it is worth noticing that starch is highly moisture sensitive and that it consists in a blend of linear macromolecules, i.e. the amylose, and ramified ones, i.e. the amylopectine, and that the ratio amylose/amylopectine can strongly differs depending on the botanic origin of the starch.

Up to now, most of the studies dealing with starch were focused on the determination of its crystalline structure depending on its origin and few were devoted to the study of amorphous starch. Nevertheless recent studies reported that amorphous starch exhibits a shape memory behavior. To explain the latter it was proposed that locally ordered domains, induced upon stretching, act as network points, but there are presently no evidences of this assumption. Moreover a review of the litterature show that mechanical behavior and structural evolution upon stretching of amorphous starch is a poorly addressed topic and to our knowledge, nothing is know about:

i) the occurrence of a strain-induced crystallization process, phenomenon largely reported in the case of common polymers.
ii) the influence of the amylose/amylopectine ratio on the strain induced structural evolution of starch.

To assess these points we followed, by means of WAXS and SAXS, the structural evolution upon streching of amorphous starch having different amylose contents. Besides, a particular interest has been paid to put in evidence the formation of ordered domain upon

stretching. Particularly it has been supposed that these ordered domains can correspond to either nano crystals or to mesomorphic domains as reported by our research group in the case of other biopolymers.

In this study four starches with different compositions were studied: potato and wheat starch (25-30% amylose), amylomaize starch (60% amylose) and waxy maize (less than 1% amylose). Worth noticing is that all the elaborated materials were initially in their amorphous state. As starch is extremely sensitive to water, all the samples were coated with vaccum grease before experiments in order to keep the water content constant into the material.
Samples were drawn at an initial constant speed of $0.01s^{-1}$ on a wide draw temperature range varying from 25°C to 110°C.

1) <u>In-situ study of the gelatinization process.</u>

The first part of the experiments was focused on the study of the gelatinization process. Indeed a review of the litterature underline some uncertainties remain regarding the structural evolution during this process and also about the origin of the two endotherms observed.
Starch gelatinisation was studied by SAXS and WAXS in order to follow the structural evolution during heating. Samples of natives starches with differents water contents were heated at 3°C/min. These experiements have been compared with DSC analyses (figure 1) carried out in our laboratory in order to explain the different transitions obseved.

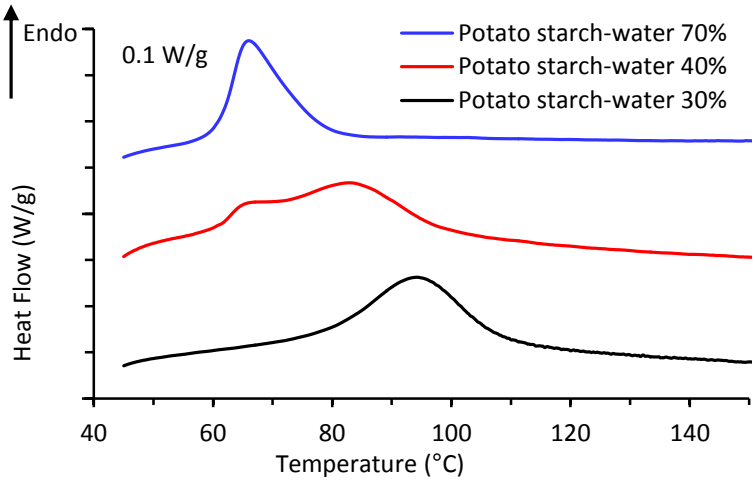


Figure 1 : thermal analysis of native potato starch with different water contents (10°C/min).

More precisely the goal here was to understand the origin of the two endotherms observed in the case of starch having intermediate water contents. Indeed while the low temperature endotherm that occurs at constant temperature for high water contents is ascribed to a gelatinization process the other one, at higher temperature is observed for low water contents and its position strongly depends on this parameter. Thus the observation of these two endothermic peaks for the intermediate water contents may originates from the fact that there are some inhomogeneities into the material, i.e. there is the coexistence of domains rich in water with domains where the water content is lower. Consequently SAXS should be the appropriate tool to assess this assumption.

Thermal analyses show a plastification of starch by the water molecules : when the water content increases, melting temperature decreases. At medium water content (40%), two endothermic peaks are observed : a melting one at 80-85°C, and a gelatinization one at 60°C. At higher water content, only the gelatinisation peak, always at 60°C, appears.

WAXS patterns recorded before, during and after the endotherms (see figure 2) were analyzed in order to determine the kinetics of the structural evolution of the crystalline structure.

45°C 50°C 65°C

Figure 2 : WAXS patterns of gelatinization of native potato starch with a 40%wt water content.

The patterns show that during heating, cristalline structure desappear around 60°C.This temperature be equivalent to DSC result, that is to say that during gelatinization of starches there are destruction of cristalline phase and starches become in amorphous state.
Contrary to the DSC analysis that indicates that melting occurs in two steps (two endotherms), the evolution of the crystalline peaks computed from the WAXS patterns show a one step process with a continuous decrease of the intensity of the diffraction peaks thus a constant decrease of the crystallinity into the material with the increase of the temperature.
Regarding SAXS results, no conclusions can be drawn to date regarding the evidence of inhomogeneities into the material. Indeed this requires a quantitative and carrefull analyses that is still in progress.

2) Study of stretching and structural evolution induced

The second part of this study has been devotes to studying the mechanical behavior and the concomittent structural evolution of different amorphous starchs drawn at different temperatures. Mecanical behaviors of starch samples drawn at Td = 80°C are presented in figure 3. Worth noticing is that all the starch samples exhibit the same glass transition temperature (Tg) around 40°C as determined by preliminarly DSC experiments.

It appears that the mechanical behvior is strongly influenced by the composition of starch. Indeed, while poatato and wheat starches have the same compositions exhbit almost the same mechanical properties, waxy maize (with 100% amylopectine) show a higher elongation at break while amylomaize (with 60% amylose) is more brittle.

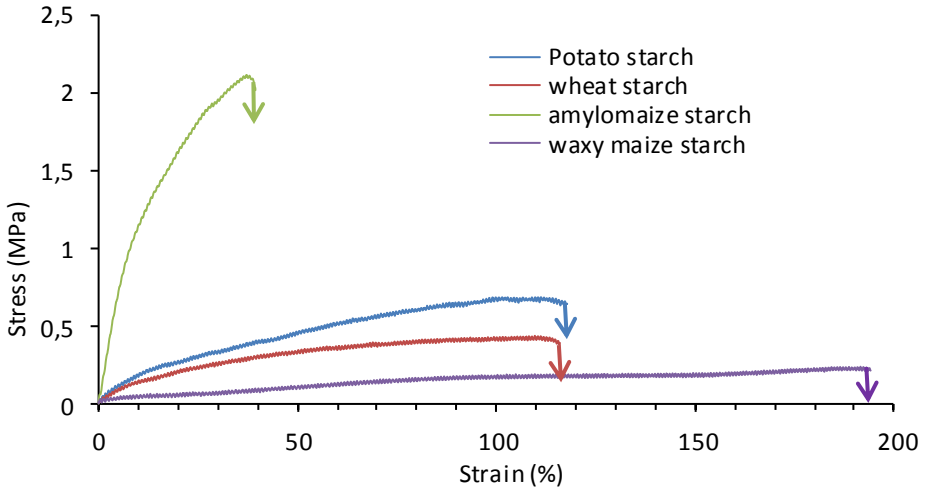
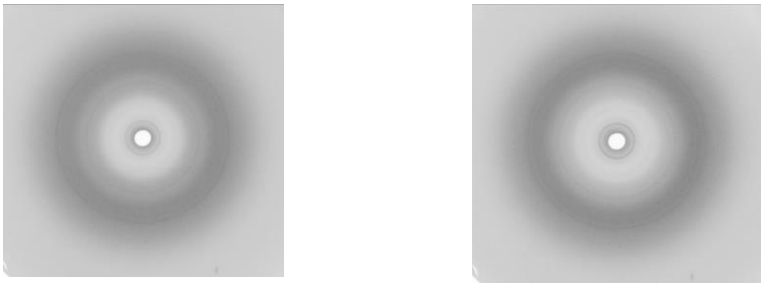


Figure 3: Stress-strain diagrams of different starches.

Thus it seems that the higher the amylopectine content, the higher the strain at break. This result is quite intriguing as the amylopectine is the ramified molecule. Indeed one would think that the higher strain at break would have been obtained for the materials having the higher amylose content, the latter being the linear molecule.

Regarding the structural analysis, WAXS patterns recorded during wheat starch drawing don't show a significant structural evolution. Particularly, as can be seen on figure 4, no strain-induced crystallization is evidenced on the patterns even large draw ratios are achieved. Indeed a diffuse halo characteristic of an amorphous material is observed on both the stretched and unstretched samples.



unstretched Stretched at $\varepsilon = 62\%$

Figure 4 : WAXS patterns of wheat starch during streching at 75°C.

The same behavior has been observed for all the starches stretched at differents temperatures (75, 90, 110°C). Below 75°C samples exhibit a brittle behavior

Comparison of the integrated intensity profiles obtained for an undrawn and a drawn potatoe starch sample, reported in figure 5, do not evidence the presence of a crystalline phase. Nevertheless one can note that a small diffraction peak around $2\theta = 2°$ appears. This may be relevant of the formation of a mesomorphic phase into the material that can be ascribed to the nano-ordered domains previously proposed to explain the shape memory behavior of starch.

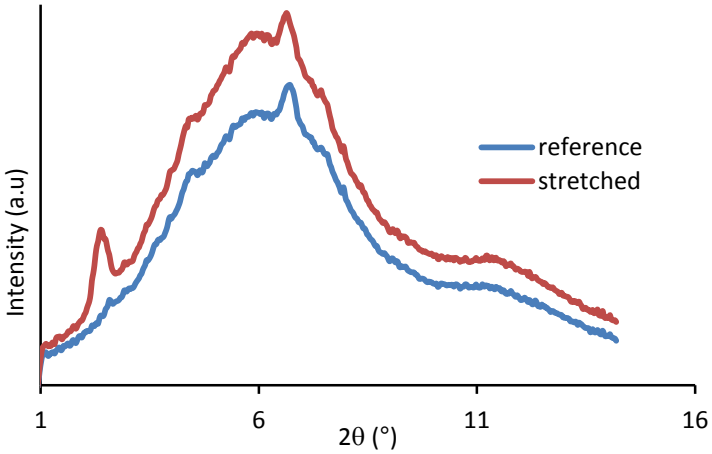
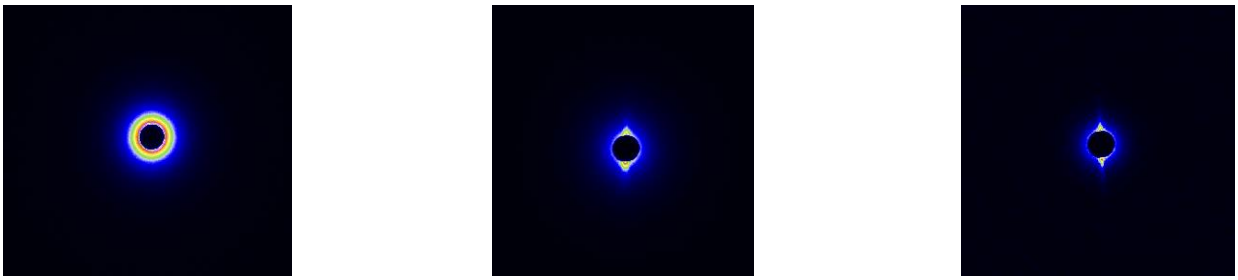


Figure 5 : X-ray diffractograms of potato starch before and after stretching (120%).

However, in order to confirm the latter point, complementary structural characterization experiments are currently in progress. Besides FTIR and NMR experiments may allow to highlight the formation of this mesomorphic phase.

An other result bring out by this study is that even if strain don't induce the formation of a crystalline structure, SAXS study show that there's a structural evolution at a mesoscopic scale. An example depicted in figure 6 shows the structural evolution of potato starch during stretching. As can be seen an equatorial scattering signal is observed for draw ratios above 30%. The analysis of the scattering curves let's think that this scattering corresponds to the occurrence of a fibrillation phenomenon. Nevertheless complementary studies carried out by means of SEM and TEM are actually in progress to confirme this hypothesys.



No stretching Stretching at 30% Stretching at 42%

Figure 6 : SAXS patterns of potato starch during stretching at 110°C.

3) <u>Others materials studied : plastified starch</u>

In this work, amorphous potato starch samples plasticized with lactic acid have also been studied. Besides this type of material has never been studied before and one of its main feature is that it exhibits interesting toughness properties. Thus the goal here was to analyze the influence of the plasticizer on both the intial structure of the material and on the structural evolution upon drawing.

Integrated intensity profiles obtained for plasticized and unplasticized unstretched potato starch samples are depicted on figure 7. As can be seen there are no significant

differences between them, particularly no cristalline peaks appear on the diffractogram for plasticized starch that still exhibit an amorphous character.

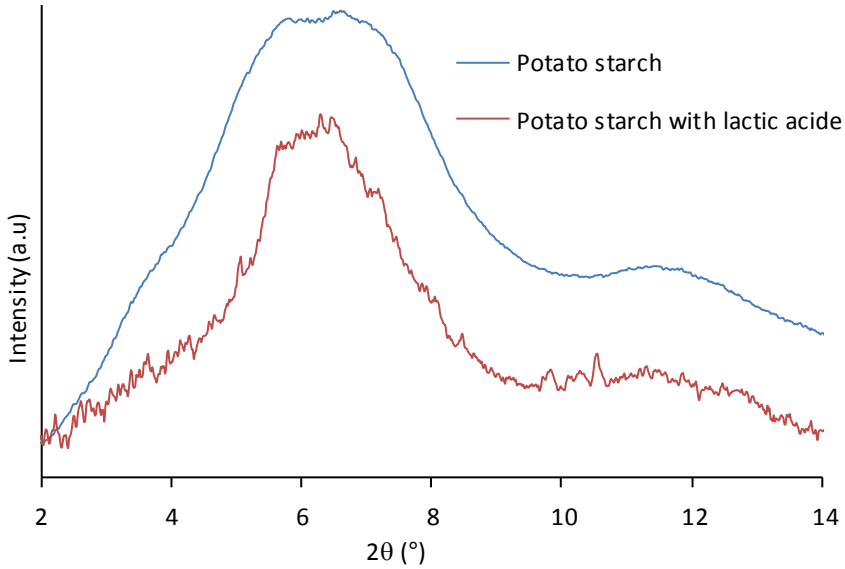


Figure 7 : X-rays diffractograms of amorphous potato starch and potato starch with 3% lactic acide.

Regarding mechanical behavior it appears that the plasticizer doesn't significantly improve stretchability of starch.

To conclude, this study has allowed to show that :

- Even if two endotherms are observed during heating of native starch with intermediate water content, the melting process, monitored by WAXS, has a monotonic kinetic.
- The mechanical behavior of starch is strongly influenced by the amylose/amylopectine ratio as well as the water content.
- No strain-induced crystallization occurs whatever the amylose/amylopectine content but it seems that mesomorphic domains are formed.
- A fibrillation process occurs upon stretching and regarding WAXS results the strain-induced fibrills are mainly amorphous.

Finally the outlook of this work are :

- An in depth analysis of the SAXS patterns in order to determine if there are heterogeneites into hydrated native starch.
- To confirm the formation of both mesomorphic domains and fibrills upon stretching by complementary structural analyses methods.

<u>Communications of the results</u> :

- An oral presentation will be given at the 3rd EPNOE International Polysaccharide Conference, Nice, october 2013.
- A publication is in preparation.