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Microscopy in the Industrial Use of Starch

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Introduction

Microscopy of starch complements the standard study techniques when analyzing starch and starch products. Since starch is a natural product, it is subject to the variation seen in material of natural origin. Measuring this variation in raw materials and matching them to the various areas of application of starch or keeping them constant is part of the daily routine in the labs of Crespel & Deiters (C&D). At its headquarters in Ibbenbüren, Germany, C&D break down wheat into its original constituents and refine it to customized products used in many sectors of the food and nonfood industry.

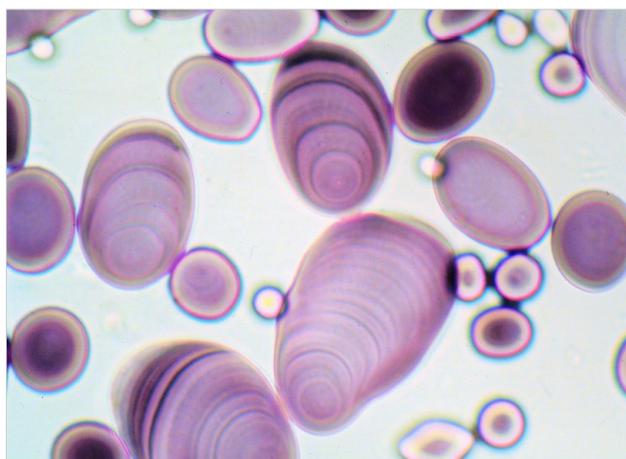


Figure 1 The layered structure of starch is easily visualized by microscopy: potato starch stained with Lugol's solution (iodine/potassium iodide). Courtesy of Prof. Dr. G. Wanner, LMU Biocenter, Munich, Germany

Good to know about starch formation and plant storage

As autotrophic organisms, plants produce all substances in their biomass themselves. The key is photosynthesis, a process started in special cell organelles, the chloroplasts. Photosynthesis usually takes place in the green leaves by day when lighting conditions are adequate. Initially, sugar (mostly α/β -D-glucose) is formed, which then is polymerized to assimilated starch. Since the sugar is now no longer in solution, it does not affect the osmotic equilibrium anymore. The light-independent reaction in photosynthesis takes place at night, when the plant uses amylase to metabolize assimilated starch granules into soluble disaccharides. The phloem (tubular guiding structures of the plant) transports this

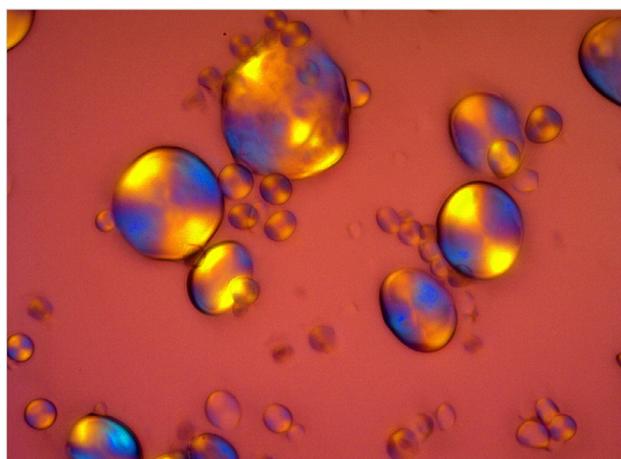


Figure 2 Wheat starch (40x) with bimodal distribution. Small granules have a size between 2 μm and 15 μm , large granules between 25 μm and 40 μm . Courtesy of L. Prange, Crespel & Deiters GmbH, Germany

sucrose into typical storage parts of the plant, such as pith parenchyma, sprout tubers, and root tubers, where the proplastids once again polymerize it to starch. The increasing starch level results in the formation of amyloplasts.¹ Starch formation takes place in layers around a center of starch formation easily recognizable, for example, with polarization microscopy. The layers themselves can be seen on brightfield microscopy with Lugol's iodine solution (iodine/potassium iodide).

What do plants use starch for?

Plants use starch as a source of energy (enzyme-catalyzed α -D-glucose breakdown) and as a structural substance (linking of β -D-glucose units to cellulose) for the cell walls of the plant.²

Light and electron microscopy can easily identify the various types of starch. In electron microscopy it has proven of value to study fasting plants with broken-down assimilated starch because leaves rich in starch are hard to section into ultrathin pieces and cause staining problems.

Before use, starch must be exposed

There are numerous uses for starch both as a source of food and industrial raw material. Precondition for such use is the removal of the storage layer of the starch.

To illustrate this, two typical human-induced breakdown techniques for this layer in starch digestion are presented below:

- A) Oatmeal production by mechanical destruction of the endosperm and exposure of the starch in the oats with rollers.
- B) Controlled enzymatic metabolism of the amyloplast endosperm when brewing beer, the so-called "malting" (biological/chemical). Mashing is preceded by malting. When the grains are made to germinate they produce a large amount of amylase. The subsequent mashing rapidly breaks down the starch after gelatinization at 60–75 °C. The resulting product is primarily maltose because the enzymes are mostly β -amylases.

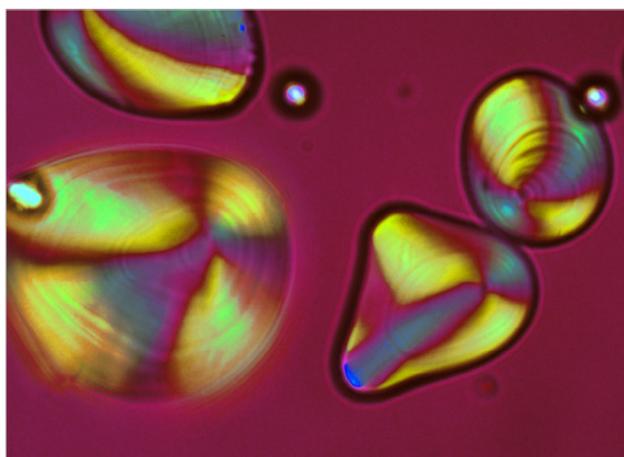


Figure 3 The size of potato starch granules (40 \times) is between 15 μm and 100 μm . Upon closer inspection the granules display their layering. Courtesy of L. Prange, Crespel & Deiters GmbH, Germany

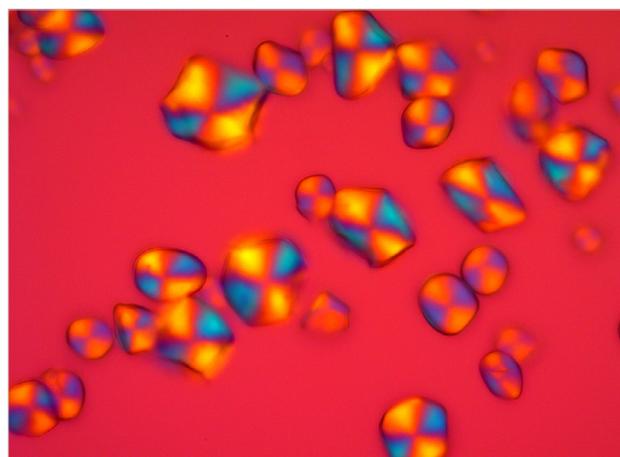


Figure 4 Corn starch (40 \times). Here, the diameters range from 10 μm to 25 μm with mostly irregularly shaped pentagons. Such polygonal shapes arise when the plastids are overflowing with starch granules (e.g., as in rice starch). The interfaces of the initial starch granules are in contact with each other during growth and therefore form a linear interface. Courtesy of L. Prange, Crespel & Deiters GmbH, Germany

¹ If forced by environmental conditions, plastids can undergo transformation. The transformation of amyloplasts into chloroplasts is exemplified, for example, by potato tubers, which will turn green when struck by light.

² Nature has another blueprint for carbohydrates: If the C_2 atom of the cross-linked monomers (sugar units) has an N-acetyl group instead of the hydroxyl group, this results in chitin, which forms part of the hard outer integument especially of beetles and crustaceans.

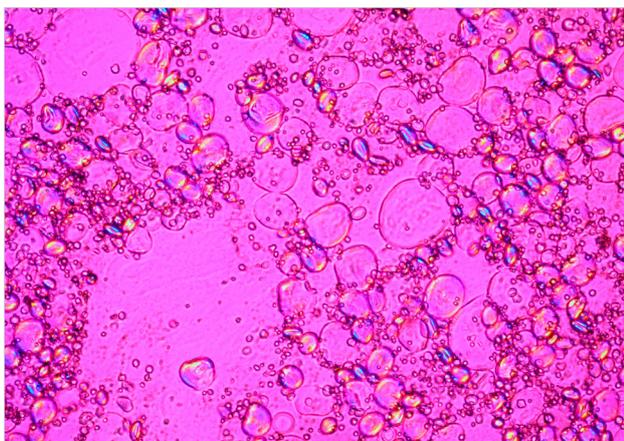


Figure 5 Imbibed wheat starch (10×). Starch granules in different stages of imbibition, the polarization cross is not visible anymore. Courtesy of L. Prange, Crespel & Deiters GmbH, Germany

Maltose can then be metabolized as substrate by the yeast. The protective double membrane of the amyloplast must be removed before the subsequent breakdown of the starch by amylases can take place, resulting in the final product. Examples of such tears and damages are illustrated in the appendix. They represent the microscopically visible starting point for utilizing starch in the brewing process.

Routine study of starch

In the presence of starch granules light microscopy is used to differentiate between the types of starch (e.g., wheat starch, corn starch, and potato starch). The granules differ in their size and shape depending on the type of starch.

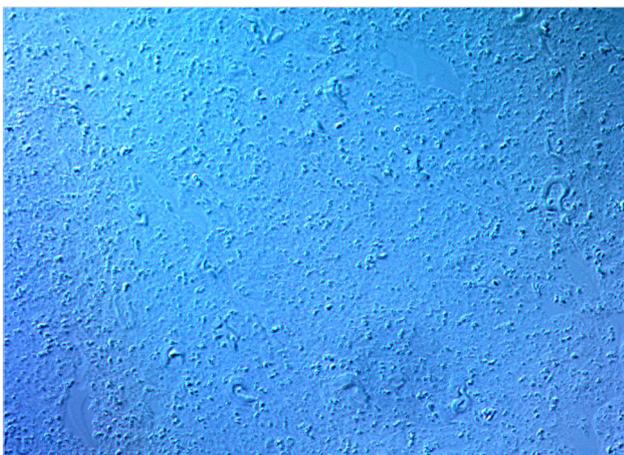


Figure 6 Suspension gelatinized in the "Viscograph E" (10×). Differential Interference Contrast (DIC) visualizes the dissolved structure of the starch. Courtesy of L. Prange, Crespel & Deiters GmbH, Germany

One important parameter in starch use is gelatinization where the starch granules produce a paste in the presence of water and heat. Tears, contamination, and destruction of the starch granules, easily demonstrated on light microscopy, may at times have a negative impact on the gelatinization characteristics and therefore the gelatinization temperature. These are easily recognized with light microscopy. The so-called "Viscograph E" device by Brabender can monitor this gelatinization.

Starch microscopy relies on an upright light microscope, such as ZEISS Axio Lab.A1 or ZEISS Axio Scope.A1 with DIC option.

Routine practice relies on 10×, 20× and 40× objectives as well a polarization unit with lambda plate. The camera employed (e.g. ZEISS AxioCam ERc 5s) and the software should have a sufficient dynamic range and a white balance with color channels to visualize the polarization-induced play of colors in a true-to-life manner. Routine DIC applications require adequate camera performance.

Procedure: Place a mixture of glycerin and water, which suspends the starch specimen in a thin layer, on a slide and cover the latter with a cover slip (0.17). When working with the polarization filter, the double refraction will produce polarization crosses (fig. 5). The characteristic double refraction is evident in nonimbibed starch granules. Increasing imbibition results in the loss of double refraction all the way to complete gelatinization of the specimen, at



Figure 7 Cross-linked wheat starch subsequently gelatinized in the "Viscograph E" (10×). Despite the gelatinization cross-linking impairs grain structure breakdown, thereby leaving some residual structures intact. Courtesy of L. Prange, Crespel & Deiters GmbH, Germany

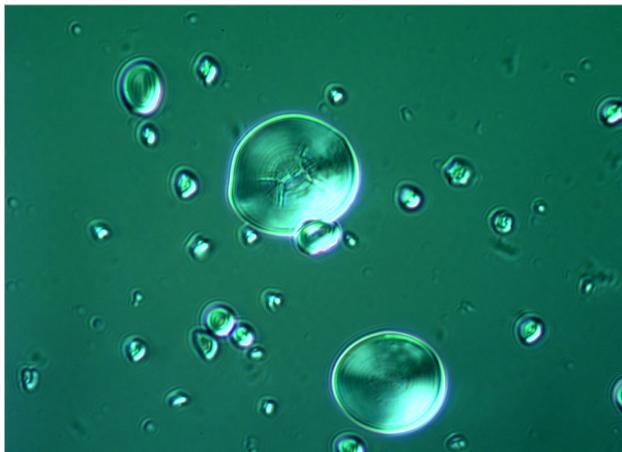


Figure 8 Acetylated starch with gallery (pitting, 40x). Courtesy of L. Prange, Crespel & Deiters GmbH, Germany



Figure 9 Starch oxidized with sodium hypochlorite (40x). Courtesy of L. Prange, Crespel & Deiters GmbH, Germany

which point it will have disappeared completely. Thus, microscopy is able to trace the steps and extent of imbibition.

After gelatinization of native starch

Even after gelatinization in the “Viscograph E,” the starch paste can be studied by microscopy with Differential Interference Contrast (DIC) (fig. 5). In complete gelatinization the granules undergo irreversible imbibition and swell to many times their original volume (fig. 6 and 7).

Galleries (pitting) in starch granules

A good example of galleries (pitting) are the granules of cross-linked acetylated starch. Chemical substitutions, and sometimes also enzymatic (long-term) attacks on the

granules, manifest themselves by galleries, evident as tubes/channels, to the center of the granule (fig. 8 and 9).

Tears and star-shaped formations on starch granules

Usually, star-shaped formations result from mechanical or oxidative processes. Figure 9 illustrates these formations in starch after oxidation (e.g., with sodium hypochlorite).

For the derivatization of starch (acetylation/hypochlorination) see reference 3 (Whistler). Figures 10 and 11 compare wheat starch with and without minor constituents from the manufacturing process.

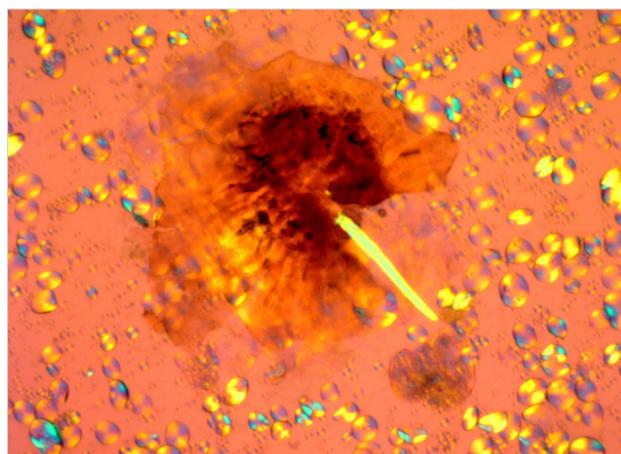
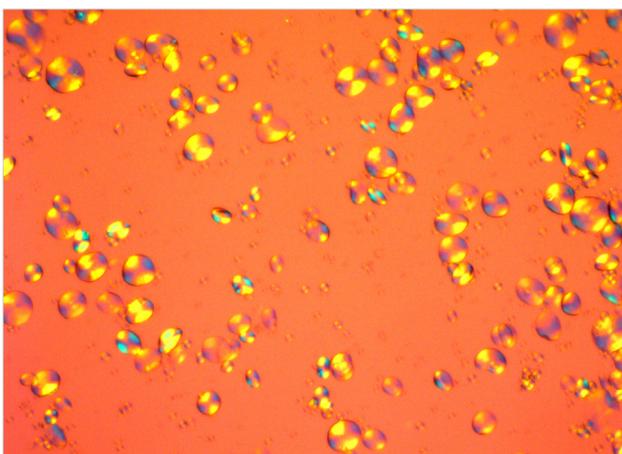


Figure 10/11 Comparison of wheat starch (10x) with and without minor constituents. The fiber is a probable residue of the beard of the grain. Courtesy of L. Prange, Crespel & Deiters GmbH, Germany

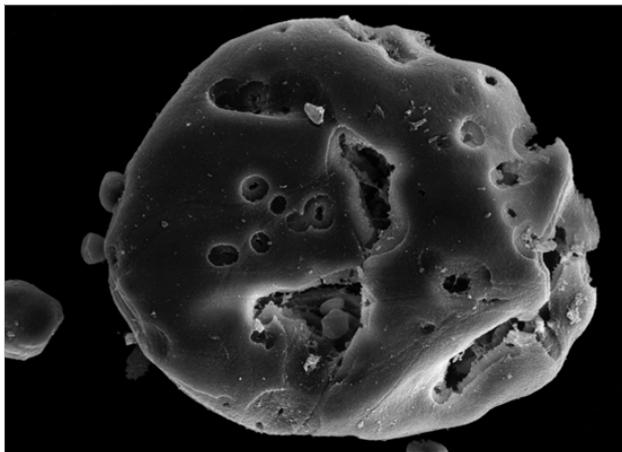


Figure 12 Galleries
Courtesy of Prof. Dr. G. Wanner, LMU Biocenter, Munich, Germany

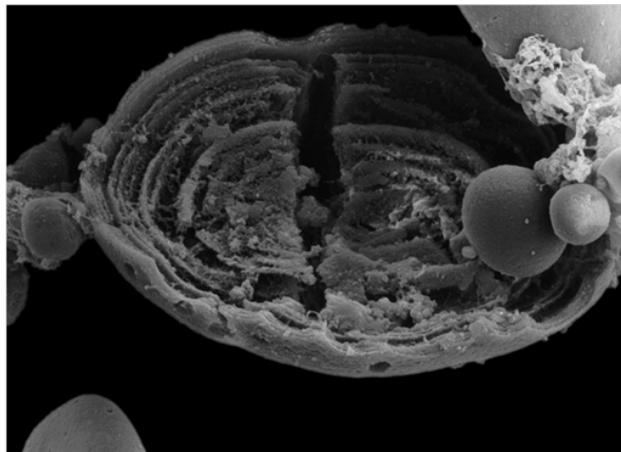


Figure 13 Starch layers
Courtesy of Prof. Dr. G. Wanner, LMU Biocenter, Munich, Germany

Appendix: SEM images of starch layers

Once the above-mentioned damage is present in the double membrane, light microscopy can demonstrate veritable amylase galleries. Figures 12 and 13 clearly illustrate the detailed breakdown also seen in the starch layering noted above. The SEM/FIB technique has demonstrated its usefulness for such images. However, such images may also be obtained with a ZEISS EVO SEM or ZEISS GeminiSEM FE-SEM.

According to Prof. Dr. Wanner, LMU Munich, the layering of starch is hard to visualize on TEM despite embedding, because the hydrophilic layers in the starch granule will produce faults in the specimen during dehydration and staining with uranyl acetate or lead acetate. However, the SEM images visualize the corroded starch granule quite well.

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