

Starch Gelatinization as Detected by Proton Magnetic Resonance

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ABSTRACT

It has been shown that proton magnetic resonance (PMR) data can be used to follow starch gelatinization. Data are presented for corn, wheat, and potato starches. Before onset of gelatinization, the PMR line-width of water increased with increasing temperature. This increase was temperature reversible, and is assumed to indicate reversible water adsorption. As the temperature was increased, it was observed: For high water concentrations, there was a rapid increase in water line-width, assumed to indicate rapid hydration; for low water concentrations the line-width decreased rapidly, assumed to indicate an increase in mobility of the water already adsorbed on the starch owing to breakdown of starch structure. During this initial stage, all soluble starch went into solution as measured by PMR. After this initial stage, as the temperature was increased further, there was a less-rapid decrease in water line-width, assumed to indicate further increase in mobility of water and starch.

Various methods have been used to measure starch gelatinization (1). Some are based on the granular properties of the starch or its suspension. These include viscosity determinations, microscopic examination of swelling, and light transmittance. Transmission of polarized light is widely used to determine gelatinization temperatures, but changes in polarized light are limited to a narrow temperature range.

With the introduction of commercially available proton magnetic resonance (PMR) equipment, a new means of observing starch gelatinization has been introduced. The changes in the PMR spectrum originate on a molecular level, and can be followed over a wide temperature range.

Collison and McDonald (2) were the first to note the difference in the PMR water line-width between ungelatinized and gelatinized starch suspensions. They report data obtained at 35°C. only. PMR peak areas are not reported. It will be shown that water line-width and starch solubility data can be obtained from PMR spectra over a wide temperature range, and that these data can complement starch gelatinization information obtained by conventional means.

MATERIALS AND METHODS

The starches used were: commercial corn starch at pH 6.0, an unmodified wheat starch supplied as "Starbake" by the Hercules Powder Company, and a potato starch supplied commercially as Crown Potato Starch by Penick & Ford, Ltd. Specifications show 17% moisture and a pH (1:10) of 8.3.

The Varian A-60 Analytical NMR spectrometer was employed, which operates at a frequency of 60 MHz. The water line-width was determined at one-half signal height. The total area under the curve was measured with the Varian V-6040 electronic integrator. The starch samples were blended on an "as-is" basis with distilled water. One gram of the starch-water slurry was heated in the A-60 sample tube using the variable temperature probe heater of the spectrometer. The heating

and cooling were done in steps of either 10°C. per 5 min. or 5°C. per 5 min.

The temperature dial was calibrated by measuring the chemical shift of methanol and propylene glycol and using the calibration data of Van Geet (3).

Calculation of Water-Solubles

In PMR spectra of 50% starch-in-water, the PMR peaks of starch could not be resolved completely from that of water. Therefore, only the total area of all the peaks could be measured. A plot of total area vs. temperature was found to first decrease, then increase between about 50° and 80°C., and then decrease again. The first decrease in area was caused by increasing temperature. The following increase was assumed to be caused by starch solubilization, while the final decrease was again attributed to increasing temperature.

The validity of this assumption was checked at low concentrations of starch in deuterium oxide. There, after starch solubilization, the peaks from the anomeric proton could be resolved from that of water and the other starch protons appeared as a shoulder on the water peak.

Therefore, an increase of the ratio of total PMR peak area to the absolute temperature indicated an increase in starch solubilization. The ratio of area to temperature was normalized by measuring the maximum ratio. This normalized value was assumed to give the percentage of "soluble" starch in solution at a particular temperature.

RESULTS AND DISCUSSION

Figure 1 shows plots of signal line-width vs. temperature for two concentrations of potato starch. The plot illustrates how the average water mobility can influence the results which are used for interpretation of the determination.

At the lower temperatures, the line-width of water for the 20% starch sample is close to the width for pure water, which under these instrumental conditions measured about 0.5 Hz. The 40% starch sample has more water molecules bonded to the solid starch. This lowers the average water mobility and is measured as a wider line-width (2).

As the temperature is increased from 20° to 50°C., more water will be taken up

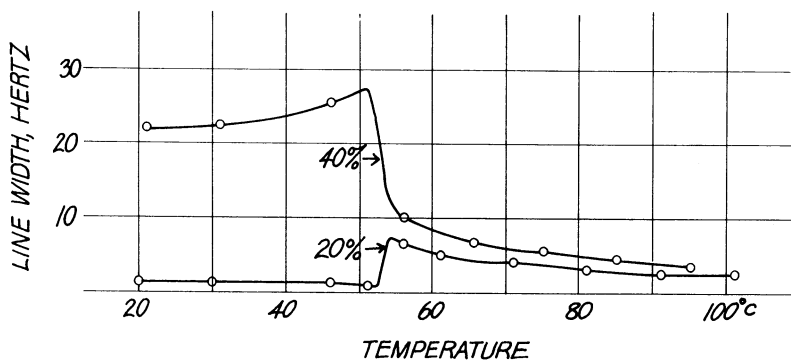


Fig. 1. Plot of PMR water line-width vs. temperature for potato starch in water at two concentrations.

by starch (1). With more water molecules being bonded to solid starch between 20° and 50°C., the average water mobility will decrease, and is measured as increasing line-width for the 40% sample. In the 20% sample, the average is still dominated by free water and therefore no increase in line-width can be measured with increasing temperature below gelatinization.

At the onset of gelatinization (52°C. for potato starch in Fig. 1), the line-width of the 40% sample drops suddenly, indicating a sudden increase in water mobility. Increased water mobility is caused by the increase in mobility of the macromolecules, as pointed out by Collison and McDonald (2).

Although more water molecules become associated with starch above 52°C., the mobility of partially gelatinized starch increases over that of ungelatinized starch and the mobility of the water associated with the 40% sample also increases, resulting in a narrower line. The 20% sample shows a sudden increase in line-width at that temperature. This decrease in average water mobility is caused by the sudden increase in hydration upon gelatinization which decreases the water mobility over that of free water. The structure of the macromolecule might even extend beyond the water molecules immediately bonded to starch, as proposed by the above two authors (2).

The average water mobility will thus depend on the ratio of water molecules in close association with starch to the total water. It will also depend on the mobility of the starch molecules with which the water molecules are associated.

Above 55°C. the water mobility increased, as shown by the slowly decreasing line-width. The increase in water mobility was probably due to decreasing microviscosity of the macromolecules with increasing temperature, similar to a decrease of macroviscosity (and microviscosity) of pure water with increasing temperature.

In conclusion, then, two observations can be made. The start of gelatinization can be distinctively observed by measuring the PMR line-width. At a higher starch concentration, information about water mobility before gelatinization can be obtained because of the relatively larger number of absorbed water molecules.

Kerr (1) distinguishes between three phases of breakdown of the starch structures during gelatinization. In the first phase, "water is slowly and reversibly taken up." If the decrease in water mobility as shown by an increasing line-width below 52°C. in Fig. 1 for the 40% sample is Kerr's reversible phase, then the increase in line-width should be reversible.

To illustrate the reversibility, 60% wheat starch was used. Sixty percent rather than 40% was used since it gave a larger change in line-width between ambient temperature and the temperature of maximum line-width. The results are given in Table I.

The increasing line-width with increasing temperature in Table I indicates water mobility due to increasing hydration of ungelatinized starch. Lowering the temperature to its original value decreases the water line-width again to approximately its original value. The reversibility of the increase in line-width at

TABLE I. PMR LINE WIDTH OF WATER IN 60% UNMODIFIED WHEAT STARCH DURING HEATING AND COOLING

Temperature, °C.	34	40	50	60	50	34
Width, Hz	60	64	71	80	72	62

TABLE II. TEMPERATURES OF BEGINNING GELATINIZATION AS INDICATED BY PMR LINE-WIDTH MAXIMUM FOR SAMPLES OF 50% STARCH HEATED AT TWO RATES AND FROM OTHER SOURCES

Starch	PMR 10°C./5 min.	PMR 5°C./5 min.	By Schoch and Maywald (4)	By Seidemann (5)
Corn	63	58	62	64-67
Wheat	61	56	52	54-58
Potato	54	53	56	57-60

temperatures below the line-width maximum is predicted if this is the region where water is reversibly taken up (1, p. 165).

To compare PMR with other methods of determining gelatinization, three different starches were tested in duplicate. Routinely the PMR samples in this laboratory are heated at a rate of 10°C. per 5 min. To determine whether the samples were at equilibrium, a slower rate of 5°C. per 5 min. was also used. The slower rate should also increase precision by providing more points in a plot of line-width vs. temperature. The results are given in Table II, which shows that at the faster heating rate, the samples were not at equilibrium at the time of measurement. By comparing the gelatinization temperatures in Table II with values obtained by loss of polarization (4) and by swelling (5), it is seen that beginning of decreasing water line-width falls into the general range of beginning gelatinization. Differences between the methods are probably caused by differences in definition of gelatinization.

At the faster heating rate of 10°C. per 5 min., the samples in the PMR spectrometer should have reached the preset temperature. Therefore, it is an equilibrium of water mobility, influenced by hydration and gelatinization, and not a temperature equilibrium that is measured. PMR data in Table II show that potato starch takes less time to reach a beginning of water line-width narrowing than the other starches; i.e., it takes less time to affect an increase in starch mobility. This might be interpreted to indicate weaker internal bonding of potato starch (6).

It was noted that PMR measurements showed potato starch to solubilize at lower temperatures than the other two starches, which agrees with conventional solubility determinations. The PMR solubilization data in Fig. 2, however, differ in two aspects from the data reported in the literature (7). Only one stage of solubilization was evident. All the soluble starch was in solution at the end of the first stage of solubilization (7), as determined conventionally.

Two reasons may be considered for the one-stage solubilization as detected by PMR measurements in Fig. 2: either the water concentration was too low to make the second stage of solubilization possible, or "solution" as defined by classical methods might not be measured as such by PMR measurements.

To determine whether the water concentration was too low, 5% corn starch was heated in heavy water to make the PMR measurements more sensitive. PMR measurements showed that at least 90% of the "soluble starch" went into solution between 65° and 80°C., and only a small (less than 10%) increase was detected between 80° and 100°C.

Next, it should be determined what fraction of the total starch is the one

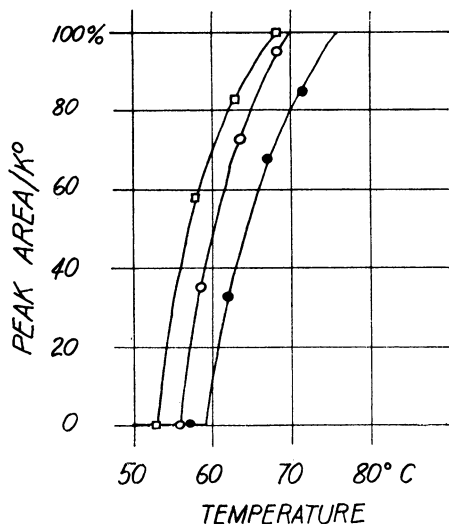


Fig. 2. Solubilization of 50% starch suspensions in water. Plot of total area under the PMR curve divided by the absolute temperature vs. temperature. Squares = potato starch, circles = wheat starch, and dots = corn starch.

measured by PMR as "soluble starch." To answer this the spectra of 5% starch in deuterium oxide were used.

Because of the relatively low viscosity of the 5% starch solution, three peaks could be resolved in the PMR spectrum. The peaks resulted from the anomeric proton, the protons in hydroxyl groups, and the protons from the other hydrogens of soluble starch. The starch in solution was then calculated from the ratio of anhydroglucose units measured to anhydroglucose units calculated for total solubility, both normalized on the basis of water (moisture) as explained in the following two paragraphs.

If all the starch were in solution, then based on the 8.5% moisture determination there should be $(0.915/162):(0.085/18) = 1.2$ anhydroglucose units of starch per H_2O molecule in the solution of 5% starch at 8.5% moisture in 95% deuterium oxide. Six and nine-tenths hydroxyls per starch anhydroglucose unit were measured as recorded in Table III. This value reduced to 5.8 hydroxyls when corrected for the proton impurities present in the deuterium oxide.

Since the three hydroxyls of the anhydroglucose of starch are susceptible to proton exchange (8), the value of 5.8 must be further corrected by subtracting 3. This leaves as the contribution of water 2.8 protons per anhydroglucose unit.

TABLE III. PMR RELATIVE AREA DETERMINATIONS AT 80°C. FOR 5% CORN STARCH IN D_2O AND OVEN MOISTURE DETERMINATIONS OF STARCH

Total Hydroxyls	Hydrogen Bonded to Carbon Less Anomeric Hydrogen	Hydroxyls Corrected for Water in Deuterium Oxide	Oven Moisture of Starch
6.9±0.1	6.0	5.8±0.1	8.5%

Therefore, the PMR measurements gives a molar ratio of 1:1.4 or 0.71 anhydroglucose units per water molecule. Since the apparatus measured only protons of starch molecules in solution, it was calculated ($0.71/1.2 = 0.6$) that 60% of the starch was in solution.

The value of 60% is higher than the value of about 30% obtained for corn starch at 80°C. by Leach and co-workers (7) at the end of the first stage of solubilization. This eliminates the possibility that the samples used for the PMR determination never went past the first stage of solubilization.

This, then, leaves us with only one explanation. Part of the starch must already be in solution inside the granule before it escapes out of the granule. That part of the starch in solution is measured in the PMR determination together with the soluble starch outside the granule.

GENERAL DISCUSSION

From solubility data measured by conventional means and by PMR determinations, the following concept emerges. Most of the soluble starch solubilizes over a relatively narrow temperature range as determined by PMR area measurements. Only a part of the starch in solution, however, escapes the swollen granule and that part is measured in the first stage of solubilization.

As the temperature is raised, the structure of the insoluble starch granule breaks down, releasing the solution in the inside of the granule, and this is measured as the second stage of solubilization, The start of gelatinization can be easily detected by PMR via the water molecules absorbed on starch which show an increase in starch mobility as gelatinization begins. It is known, however, that starch gelatinizes over a definite temperature range.

In the PMR experiment, this range might be defined by the difference in mobility as measured by a difference in PMR line-width between the partially gelatinized system and the completely gelatinized paste. This difference can be determined from a plot of line-width vs. temperature recorded during heating and cooling the sample. Then

$$G = (F - (W_p - W_g))/F$$

where

G = fraction of starch gelatinized.

F = difference between maximum line-width of water and line-width of water in gelatinized starch at the temperature of maximum.

W_p = line-width of water peak in the partially gelatinized system at an arbitrary temperature.

W_g = line-width of water peak in the completely gelatinized system at a temperature corresponding to W_p.

The above definition of gelatinization also shows how the mobility, as determined by line-width, of the partially gelatinized system approaches that of the completely gelatinized system. The measure is normalized in order to compare different starch-water systems. With this equation, plots of percentage gelatinization vs. temperature can be obtained, as shown in Fig. 3.

Figure 3 shows that at first there is a rapid narrowing of line-width. This range

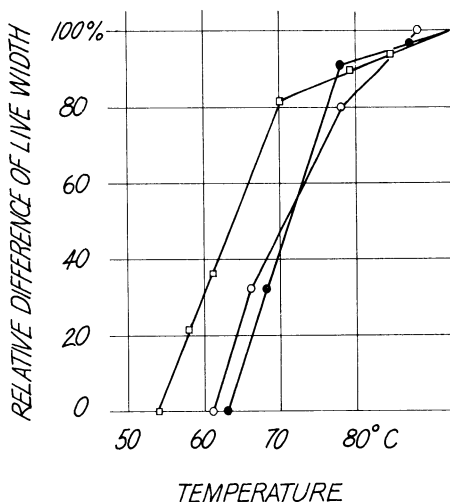


Fig. 3. Percentage gelatinization of 50% starch slurries by PMR. Plot of normalized difference between line-width of ungelatinized and of gelatinized starch — G (see General Discussion) vs. temperature. Squares = potato starch, circles = wheat starch, and dots = corn starch.

generally coincides with gelatinization as determined by polarized light and the first stage of solubilization as determined by conventional means. At higher temperatures there is a smaller and less-rapid increase in mobility, corresponding to the second stage of solubilization.

A comparison of PMR results on gelatinization with some other methods is made in Table IV. All the methods seem to indicate approximately the same starting temperature for gelatinization. The differences are probably due to differences in definition of the starting temperature.

At an intermediate temperature of 63° to 76°C., the starch granule has swollen to a point where its crystallinity has been destroyed. By this time, all of the soluble starch is in solution as determined by PMR.

Beyond this temperature, further breakdown of the granules will occur, shown conventionally by the increasing amounts of solubilized starch escaping the granule (second stage of solubilization) and by PMR as the still-increasing starch-chain mobility.

In conclusion, then, PMR measurements show a decrease in water mobility

TABLE IV. RANGE OF GELATINIZATION TEMPERATURE OF STARCH BY SWELLING, POLARIZED LIGHT, PMR, SOLUBILIZATION BY PMR, AND SOLUBILIZATION BY LEACHING OUT. ALL TEMPERATURES MEASURED IN °C.

Process	Wheat	Corn	Potato
Swelling (5)	56-65	66-74	58-68
Polarized light (4)	52-63	62-72	56-66
Mobility by PMR (Fig. 3)	61-90	63-92	54-92
Solubilization by PMR (Fig. 2)	56-70	60-76	53-68
Solubilization by leaching out (7)		65-95+	55-95+

during the stage of reversible water pick-up (1, p. 165). The decrease in average water mobility is probably caused by an increasing number of water molecules being absorbed in the solid starch. At the point of gelatinization, the mobility of absorbed water starts to increase because of the increasing mobility of the starch molecules with which the water is associated. At the same time, solubilization starts.

Most of the starch solubilizes over a relatively narrow temperature range, but not all of it escapes the still-intact granule. In this range, which corresponds to the first stage of solubilization, there is a large increase in mobility of starch. The rapid increase in mobility is followed by a slower increase. In this temperature range, the starch already in solution inside the granule escapes to the outside as the granule falls apart.

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