

Starch Retrogradation Study in Presence of Sucrose by Low-Resolution Nuclear Magnetic Resonance

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ABSTRACT

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We have studied the evolution of the solid nuclear magnetic resonance (NMR) signal of starch-sucrose-water gels with four starch and water ratios (1.3; 1.5; 1.6; and 2.0) and percent of sucrose content (0-42). We have measured the range of the increase of the signal ΔS coming from the solid part of the samples for the retrogradation. This solid signal was determined from a bi-gaussian fitting. As the evolution of ΔS has a decreasing linear profile according to the amount of sucrose added,

we made the assumption of a total inhibition of the effect of the water by the sucrose on starch for an amount of sucrose added corresponding to $\Delta S = 0$. Our results support the idea of an interaction of 4.6 water molecules per sucrose molecule, where sucrose finds itself totally in the liquid phase. The addition of sucrose reduces the amount of gelatinized starch, but the water-sucrose entity plays a plastifying part during the retrogradation.

Although the study of the influence of sugar on starch gelatinization has been dealt with by different techniques such as nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) (Carrillo et al 1989, Chinachotti et al 1991, Eliasson 1992), the part played by sugars, and more particularly by sucrose, is not always well determined. Some hypothesis have been made and results obtained from DSC experiments on the water-sucrose-starch system gelatinization are, of course, a very good starting point for a study of the retrogradation process of starch. It has long been known that an increase in the onset (T_o), peak maximum (T_m), and conclusion (T_c) temperatures of DSC endotherms is related to the added amount of sucrose (Eliasson 1992, Kim and Walker 1992). Several hypotheses have been expressed concerning the origin of these variations. 1) Sucrose interacts with starch in the amorphous region to form bridges between the chains, which delays the gelatinization temperature (Spies and Hoseney 1982). The G_1 , G_6 , F_1 , and F_3 carbon atoms of the sucrose molecule have the strongest interaction with starch during heating, just before the T_o (Hansen et al 1989). 2) Sucrose binds water and makes it less available for starch. Indeed, in limited water, the loss of crystalline order occurs at a higher temperature than it does in excess water because there is an insufficient water absorption to disrupt the crystalline order (Cameron and Donald 1993). However, this hypothesis is rejected by Eliasson (1992). 3) Sucrose causes a decrease in the mobility of water (Lim et al 1992) and in the water activity (a_w) of the water-sucrose solution (Spies and Hoseney 1982). 4) Sugars act as antiplasticizing agents (Slade and Levine 1988). 5) The addition of sucrose makes the gelatinization occur more easily (Eliasson 1992). Considering the great number of cereal products that contain sugar (and some of them in large proportion), there is an interest in understanding the mechanisms of interaction between the main ingredients. However, as it can be seen, available results concerning the interaction mechanisms by which sugar delays starch retrogradation and the interaction mechanisms within gels still remain unclear.

The advantages of ^{17}O over proton NMR techniques have been well discussed by Sobczynska et al (1990). In effect, the ^{17}O signal is not affected by chemical exchange between distinct states of water. ^2H and ^{17}O nuclei also avoid the problem of cross-relaxation between protons of the macromolecule and those of water. However, works performed with these techniques only concern the study of the mobility of water and exchangeable hydroxyls in a medium mobile enough for the transverse relaxation time of

oxygen to be obtained from the line width at half height of the peaks ($T2^* = (\pi \delta\nu_{1/2})^{-1}$). To determine whether sucrose shows repetitive behavior toward starch or water, we followed the retrogradation of four sets of samples corresponding to four starch and water ratios, with 0-42% sucrose added by low-resolution ^1H NMR spectroscopy.

METHODS

One of the advantages of low-resolution NMR spectroscopy is that it enables the study of heterogeneous substrates, such as starch suspensions or gels. However, the low homogeneity of the magnetic field does not allow discrimination between protons according to chemical shifts, but only according to their relaxation times spin-lattice ($T1$) and spin-spin ($T2$). After a single 90° pulse, the free induction decay (FID) shows first a fast-decreasing signal coming from the solid part of the sample (CH, CH_2 , and a part of $-\text{OH}$ of crystalline or amorphous starch) according to a gaussian law:

$$S(t, t') = \sum_i S_{0i}(t') \exp\left(-\left(\frac{t}{T_{2i}}\right)^2\right) \quad (1)$$

where t and t' are times in relation to the 90° pulse and the gelatinization start, respectively; $S_{0i}(t')$ and T_{2i} are signal and relaxation times of the component i , and a slow-decreasing signal coming from the "liquid" part (CH, CH_2 of gelatinized starch, H_2O , and exchangeable starch protons).

MATERIALS

NMR

A low-resolution NMR spectrometer (Minispec PC120, Bruker) equipped with a 10 VTS probe was used for measurements. Conditions were: temperature 20°C , tube diameter 10 mm, and 90° pulse width = 1.1 μsec . The FID signal was sampled at 1 MHz with an acquisition card from Bakker Electronics, Team 490. The initial signal of the solid phase S_i was determined by fitting the FID curve, sampled every 1 μsec from 11 μsec (dead time), by a bi-gaussian function. The calculated curve (Fig. 1) is in good agreement with the experimental one.

Samples

Gels were prepared with wheat starch (CereStar) in NMR-sealed tubes. The appropriate aqueous sucrose solutions were added to neat starch (12.5% water content) in the NMR tube to get the correct water and starch ratio with the required sucrose per-

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centage. The mixture was then made in the tube and kept for 15 min at room temperature before gelatinization in an oven at 150°C for 1 hr; the gelatinization temperature was chosen to be in the range used for industrial processing.

RESULTS AND DISCUSSION

We followed the evolution of starch-water and starch-water-sucrose gels by measuring the increase in the NMR signal coming from the solid phase for a period of 15 days. To obtain the true total signal

$$S_0(t') = \sum S_{0i}(t')$$

coming from the solid phase, and not the partial signal measured after the dead time of the probe (10 μ sec), a bi-gaussian fitting was performed on the FID:

$$S(t, t') = S_0(t') \left[P_a \exp\left(-\frac{t}{T_{2a}}\right)^2 + P_b \exp\left(-\frac{t}{T_{2b}}\right)^2 \right] \quad (2)$$

The evolution of the intensity of the two components $T_{2a} = 20.6 \pm 0.5 \mu$ sec and $T_{2b} = 112 \pm 6 \mu$ sec, as well as that of

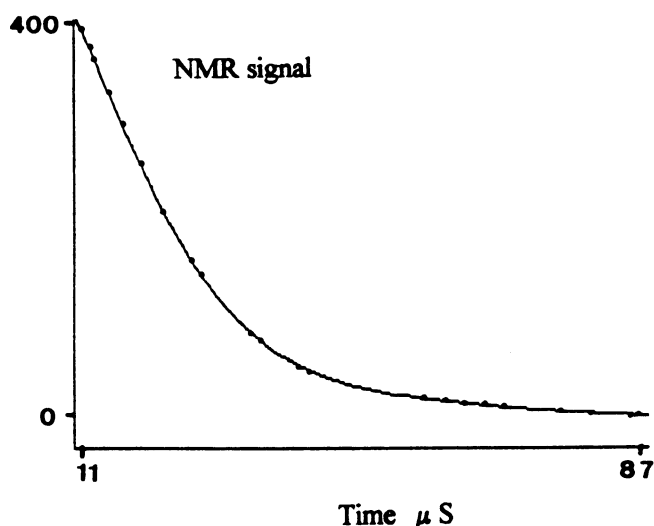


Fig. 1. Nuclear magnetic resonance (NMR) signal (arbitrary unit) of the solid phase of a starch water gel (starch/water = 1.0), sampled at 1 MHz, and the bi-gaussian fitting curve. • = major part of the 76 experimental points.

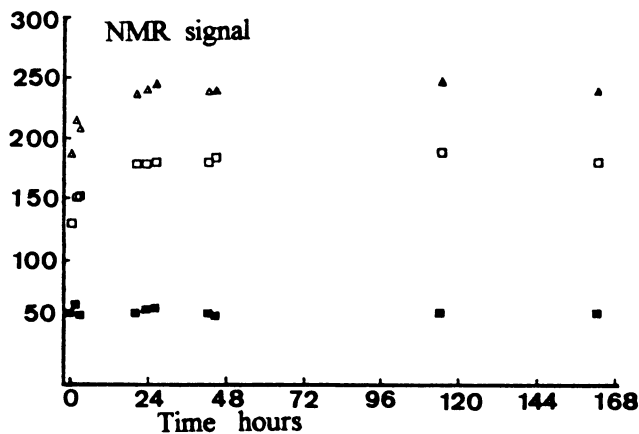


Fig. 2. Evolution of the intensity of the two solid components and of their sum (▲) obtained by a bi-gaussian fitting for a starch-water-sucrose gel (37.5%, 33.8%, 28.5%, respectively) corresponding to the spin-spin relaxation times: $T_{2a} = 20.6 \pm 0.5 \mu$ sec (■) and $T_{2b} = 112 \pm 6 \mu$ sec (□). Measurements: $n = 11$.

the total signal are presented in Figure 2. The variation of the "solid" signal ΔS caused by the retrogradation has thus been obtained by calculating the difference between the signal S_{00} (30 min after the gelatinization) and the asymptotic value of the curve $S_{0\infty} = S_0(t')$ ($t' = 15$ days). The evolution of gels with and without sucrose are shown on Figure 3.

First, the $S_0(t')$ values, measured during the retrogradation process, have been treated by the Avrami model, which gives the fraction of uncrystallized starch at t' :

$$U_i = \exp(-kt^n) \quad (3)$$

where:

$$U_i = [S_{0\infty} - S_0(t')]/[S_{0\infty} - S_{00}] \quad (4)$$

where k = a constant, and n = the Avrami exponent, which can be expressed as:

$$\ln[-\ln(U_i)] = \ln(k) + n \ln(t') \quad (5)$$

A linear analysis of $\ln[-\ln(U_i)]$ versus $\ln(t')$ gave a satisfactory straight line (correlation coefficient $r^2 = 0.968$) as shown Figure 4, for a starch and water ratio equal to 0.54, with the parameters:

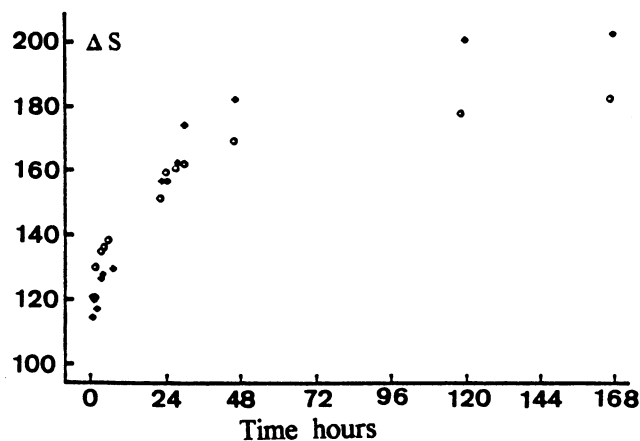


Fig. 3. Evolution of the solid nuclear magnetic resonance (NMR) signal (ΔS) for a starch-water gel (starch/water = 0.54) (◊) and a starch-water-sucrose gel (29.2%, 54.1%, and 16.7%, respectively) (○) with the same starch-water ratio.

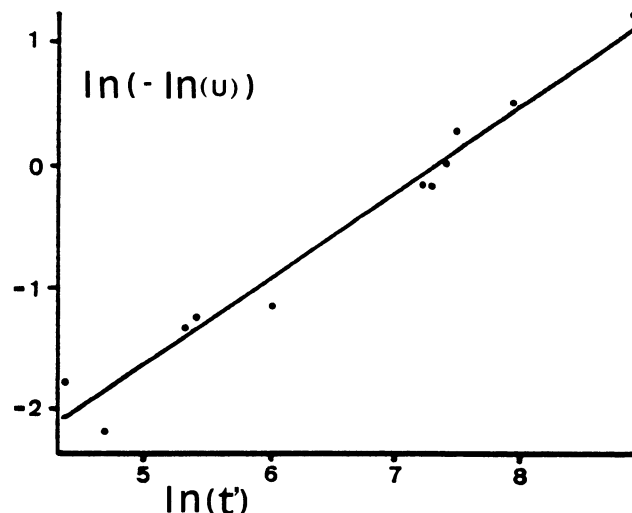


Fig. 4. Evolution of the solid nuclear magnetic resonance (NMR) signal (ΔS) of a starch-water gel (starch/water = 0.54) according to the Avrami relation $\ln[-\ln(U)] = \ln(k) + n \ln(t')$. Avrami exponent $n = 0.79$; constant $k = 2.55 \cdot 10^{-3} \text{ mn}^{-1}$, correlation coefficient $r^2 = 0.96$.

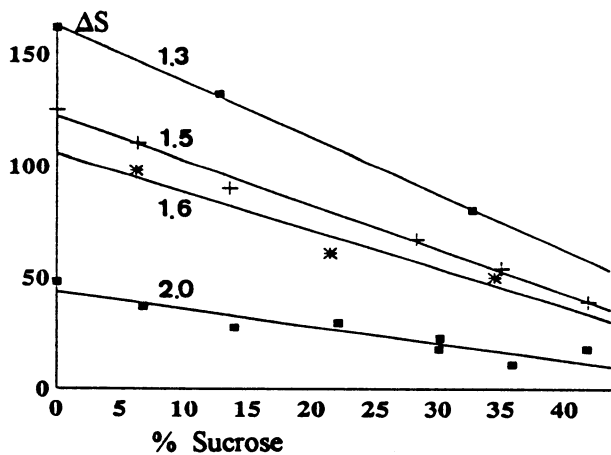


Fig. 5. Evolution of the nuclear magnetic resonance (NMR) signal variation ΔS (arbitrary unit) during the retrogradation process, according to the percentage of sucrose for four starch-water ratios: 1.3, 1.5, 1.6, and 2.0.

TABLE I
Composition of Starch-Water-Sucrose Gels^a

St/W ^b	$\Delta S = 0^c$		Corresponding Composition of Gels			
	Sucrose %	<i>a</i>	<i>b</i>	Water	Sucrose	Water-Sucrose
1.3	64.8	162.7	-2.51	0.77	3.25	0.24
1.5	61.6	172.6	-2.80	0.67	2.7	0.25
1.6	61.5	105.5	-1.71	0.62	2.6	0.24
2.0	56.9	43.6	-0.77	0.5	1.98	0.25

^aObtained by extrapolating at $\Delta S = 0$ of the four straight lines in Figure 2. Amount of starch has been fixed at 1 unit.

^bStarch-water ratio (w/w) of the starch-water-sucrose gels.

^cPercentage of sucrose to obtain a null evolution of the solid nuclear magnetic resonance signal after the gelatinization.

$n = 0.79 \pm 0.1$, $k = 2.55 \cdot 10^{-3} \text{ mn}^{-1}$. This Avrami exponent value is in good agreement with the results obtained by DSC (Longton and Legrys 1981, Russell 1983). The evolution of the retrogradation process is therefore well monitored by the $S_0(t')$ parameter. This NMR parameter has already been used, but in a simpler way by purely exploiting the signal intensity difference between 11 μsec and 70 μsec (Teo and Seow 1992). The ΔS values obtained by gels containing up to 42% sucrose by weight and four different starch and water ratios (1.3, 1.5, 1.6, and 2.0) are presented in Figure 5. We note that in these four cases, a linear fitting of the points is satisfactory. From these results, we have assumed that for a null value of ΔS , the retrogradation and, therefore, the gelatinization could not occur. The extrapolation of the four correlated straight lines $\Delta S = f(\% \text{ sucrose})$ allows us to determine the percentage of sucrose necessary to reach the threshold $\Delta S = 0$. As for the four starch and water ratios studied, this threshold is reached for various sucrose-starch ratios, whereas the water and sucrose ratio, obtained from:

$$\frac{\text{water}}{\text{sucrose}} = \frac{a + 100b}{\left(1 + \frac{\text{starch}}{\text{water}}\right) * a} \quad (6)$$

where a and b are the parameters of the straight line $\Delta S = a + b (\% \text{ sucrose})$ is almost stable (water/sucrose = 0.244 ± 0.006) (2.4%) (Table I).

This value represents 4.6 ± 0.1 water molecules per sucrose molecule, which corresponds to a 80.4% sucrose-water solution by weight. Whereas the saturation is obtained for 67% by weight at 20°C and atmospheric pressure, such a mixture prepared in similar conditions as gels gave a stable solution for over a year without recrystallization of sucrose. Considering that each sucrose molecule "links" 4.6 water molecules, we have compared the

retrogradation of a starch-water-sucrose gel (38.9%, 26.0%, and 35.1%, respectively) and a gel without sucrose, whose starch-water ratio was equal to the starch and "free water" ratio of the first one, which gave a starch-water ratio 2.25 instead of 1.5. The increase of the solid signal of the gel without sucrose (34 units) is lower than that of the gel with sucrose (54 units). This difference in the two gels behavior must be caused by the plastifying effect of the water-sucrose entity, which therefore must contribute to the system mobility. We determined that the solid signal for a gram of starch for the gel with sucrose is 21% higher than that for the gel without sucrose. This signal difference can be explained in two ways: 1) a part of the sucrose contributes to the solid signal, or 2) more likely, less starch gelatinized in the presence of sucrose, which finds itself totally in the liquid phase.

CONCLUSION

The evolution of the solid signal obtained by low-resolution NMR with starch-water-sucrose gels supports the idea of a water-sucrose interaction in gels. The water-sucrose entity finds itself in a liquid form towards NMR and plays a plastifying part in relation to the system. However, we must keep in mind that these gels were prepared in sealed NMR tubes, so there was no loss of water during gelatinization. These particular physical conditions may have modified the reaction mechanisms in comparison with those that occur during DSC experiments, so new measurements at a lower gelatinization temperature would have to be made.

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LITERATURE CITED

- CAMERON, R. E., and DONALD, A. M. 1993. A small-angle X-ray scattering study of starch gelatinization in excess and limiting water. *J. Polym. Sci., B Polym. Phys.* 31:1197-1203.
- CARRILLO, P. J., GILBERT, S. G., and DAUN, H. 1989. Starch/sucrose interactions by organic probe analysis: An inverse gas chromatography study. *J. Food Sci.* 54:162-165.
- CHINACHOTI, P., WHITE, V. A., LO, L., and STENGLE, T. R. 1991. Application of high-resolution carbon-13, oxygen-17, and sodium-23 nuclear magnetic resonance to study the influences of water, sucrose, and sodium chloride on starch gelatinization. *Cereal Chem.* 68:238-244.
- ELIASSON, A. C. 1992. A calorimetric investigation of the influence of sucrose on the gelatinization of starch. *Carbohydr. Polym.* 18:131-138.
- HANSEN, L. M., SESTER, C. S., and PAUKSTELIS, J. V. 1989. Investigations of sugar-starch interactions using carbon-13 nuclear magnetic resonance. I. Sucrose. *Cereal Chem.* 66:411-415.
- KIM, C. S., and WALKER, C. E. 1992. Effects of sugars and emulsifiers on starch gelatinization evaluated by differential scanning calorimetry. *Cereal Chem.* 69:212-217.
- LIM, H., SESTER, C. S., PAUKSTELIS, J. V., and SOBCZYNSKA, D. 1992. ¹⁷O Nuclear Magnetic Resonance studies on wheat starch-sucrose-water interactions with increasing temperature. *Cereal Chem.* 69:382-386.
- LONGTON, J., and LEGRYS, G. A., 1981. Differential scanning calorimetry studies on the crystallinity of ageing wheat starch gels. *Starch/Staerke* 33:410-414.
- RUSSELL, P. L. 1983. A kinetic study of bread staling by differential scanning calorimetry. *Starch/Staerke* 35:277-281.
- SLADE, L., and LEVINE, H. 1988. Non-equilibrium melting of native granular starch. I. Temperature location of the glass transition associated with gelatinization of A-type cereal starches. *Carbohydr. Polym.* 8:183-208.
- SOBCZYNSKA, D., SESTER, C., LIM, H., HANSEN, L., and PAUKSTELIS, J. 1990. Use of 13-C and 17-O NMR to study wheat starch-water-sugar interactions with increasing temperatures. In: *NMR Applications in Biopolymers*. J. W. Finley, ed. Plenum Press: New York.
- SPIES, R. D., and HOSENEY, R. C. 1982. Effect of sugars on starch gelatinization. *Cereal Chem.* 59:128-131.
- TEO, C. H., and SEOW, C. C. 1992. A pulsed NMR method for the study of starch retrogradation. *Starch/Staerke* 44:288-292.