NOTE

^{13}C Nuclear Magnetic Resonance Spectroscopic Methods for Investigating Sucrose-Starch Interactions with Increasing Temperature[1]

L. M. HANSEN,[2] J. V. PAUKSTELIS,[3] and C. S. SETSER[2]

Many techniques have been used to study starch gelatinization and effects of different sugars on the thermal properties of starch. Methods include amylograph measurements of changes in viscosity (Lund 1984), microscopic observations of loss of birefringence and changes in the swelling of the granule (Watson 1964, Bean and Osman 1959, Bean and Yamazaki 1978, Bean et al 1978), and differential scanning calorimetry (DSC) to determine melting temperature and the enthalpies of melting as modified by sugar solutions (Spies and Hoseney 1982, Wootton and Bamunuarachchi 1980). Results of several studies agree that sugars delay the onset of starch gelatinization (Bean and Osman 1959, Miller and Trimbo 1965, Bean and Yamazaki 1978, Evans and Haisman 1982, Savage and Osman 1978, Koepsel and Hoseney 1980). In general, disaccharides delay the gelatinization more than monosaccharides (Savage and Osman 1978, Spies and Hoseney 1982) but the mechanism through which sugars delay starch gelatinization remains unclear. One theory is that the sugars and starch interact in the amorphous areas of the starch granule, stabilizing this region and, thus, delaying gelatinization onset (Spies and Hoseney 1982). Current methods to investigate starch-sugar systems measure physical changes of the starch. If an interaction actually is occurring, this needs to be determined at the molecular level, which might be possible using nuclear magnetic resonance (NMR) spectroscopy techniques.

Some research using NMR has been done with starch-water systems. These studies investigated granule hydration using proton NMR (Lelievre and Mitchell 1975, Jaska 1971, Hennig and Lechert 1977, Callaghan et al 1983) and ^{13}C-NMR (Callaghan et al 1983, Fuke and Matsuoka 1984). We found no studies found using ^{13}C-NMR to investigate sugar-starch interactions as a function of increasing temperature. The objective of this study was to develop methodology and determine the feasibility of investigating the starch-sugar interaction using variable temperature ^{13}C-NMR techniques.

MATERIALS AND METHODS

Solutions

Wheat starch (Aytex P, prime wheat starch, Ogilvie Mills, Inc.) was used in all investigations. Reagent grade sucrose (Mallinckrodt, Inc.) was used to study sugar-starch interactions. Starch and sugar dispersions were suspended in a 0.15% xanthan gum (Keltrol T, Kelco) solution to prevent settling of the starch. Sugar solutions of 0.5*M*, 1.0*M*, 1.5*M*, and 2.0*M* were prepared using the 0.15% xanthan gum solution as the solvent. Sugar-starch dispersions were prepared on 30% starch, 70% sugar solution (w/w) basis. This ratio was established because enough carbon atoms (^{13}C) of both sucrose and starch were provided for strong NMR signals; the dispersion remained sufficiently fluid to be injected into a 5-mm NMR sample tube; and gelatinization of starch, as evidenced by use of DSC techniques, was apparent during the temperature range used at the different molar sugar concentrations studied. Dispersions were prepared and allowed to equilibrate at room temperature for approximately 8 hr before measurement in the NMR spectrometer.

Differential Scanning Calorimetry

The technique utilized to verify starch gelatinization was DSC. Measurements were made using the Perkin Elmer DSC-2, FTS Systems Flexicooler with temperature controller according to Kim et al (1986). The instrument was calibrated with indium.

NMR Measurements

Carbon-13 (^{13}C) NMR spectra were recorded on a Bruker WM-400 NMR spectrometer with variable temperature control. Sugar-starch solutions were placed in a 5-mm sample tube. A concentric tube (1.5 mm) containing the external standard of dimethylsulfoxide ($Me_2SO:D_2O$) was inserted into the 5-mm sample tube, which was then placed into the NMR spectrometer. Data were acquired with a 0.5-sec delay between pulses to ensure full recovery of signal after a 6-μsec (60°) pulse. Data points (16K) were zero filled to 32K, line broadened 5 hertz (Hz), and Fourier transformed. Adequate signal-to-noise ratio was obtained after 256–512 pulses. Chemical shifts of the sugars and starch were measured at 40, 50, 60, 70, 80, and 90° C. Samples were held 5 min at each temperature before data acquisition was started to insure temperature equilibrium and to obtain reproducible results. The amount of time needed to equilibrate the spectrometer at any temperature was determined by raising the temperature to the desired value and holding 5 min, then acquiring data every 3 min for 1 hr. No change in the chemical shifts of sucrose or starch as a function of time was noted, indicating that the temperature of the instrument and the starch dispersion had equilibrated. Duplicate samples were measured and the results were averaged.

To observe the behavior of sugar and starch before they were mixed together, the chemical shifts of reference sugar solutions and reference starch solutions were determined at each temperature individually before the chemical shifts of the sugar-starch dispersions were determined. Chemical shifts of sucrose-water and sucrose-xanthan gum solutions were done to determine if the xanthan gum added to the solution affected the chemical shifts of sucrose. No differences (e.g., < 0.25 Hz) in chemical shifts were noted between the two solutions. Without xanthan gum, starch does not remain dispersed, which leads to irreproducible results using NMR spectroscopy. To ascertain effects of xanthan gum on starch gelatinization, onset temperatures were determined using DSC for starch-xanthan and starch-water (2:1) dispersions. Both systems had the same onset temperature (55.6° C), as well as the same range and slope of endotherms. If xanthan gum is interacting with starch, the interaction is not altering the onset temperature of gelatinization. All NMR chemical shifts were determined relative to the external Me_2SO standard. The standard was not affected by the solutions, and the same standard was used for every sample.

At each temperature, the differences were determined between the chemical shifts of the sugar in solution alone and the chemical shifts of the carbon atoms of the sugar in the sugar-starch dispersions. If an interaction between the sugar and the starch were

---

[1] Contribution 87-208-J from the Department of Foods and Nutrition, Kansas Agricultural Experiment Station. Presented at the AACC 71st Annual Meeting, Toronto, ON, Canada, October 1986.

[2] Graduate research assistant and professor, respectively, Department of Foods and Nutrition, Kansas State University, Manhattan 66506.

[3] Professor, Department of Chemistry, Kansas State University.

occurring, the environment near the sugar carbon atoms would be altered, and the chemical shift of the carbon atoms of the sugar-starch dispersions would differ from that of the carbon atoms in the sugar solution alone. A plot of the differences in chemical shifts as a function of temperature should produce a line with approximately zero, or constant, slope if no interactions occur. Deviations in slope for a particular carbon would indicate possible interaction sites; the larger the deviation, the stronger the interaction. Deviations of less than 1 Hz were not thought to be significant and were considered within the experimental error of the measurements.

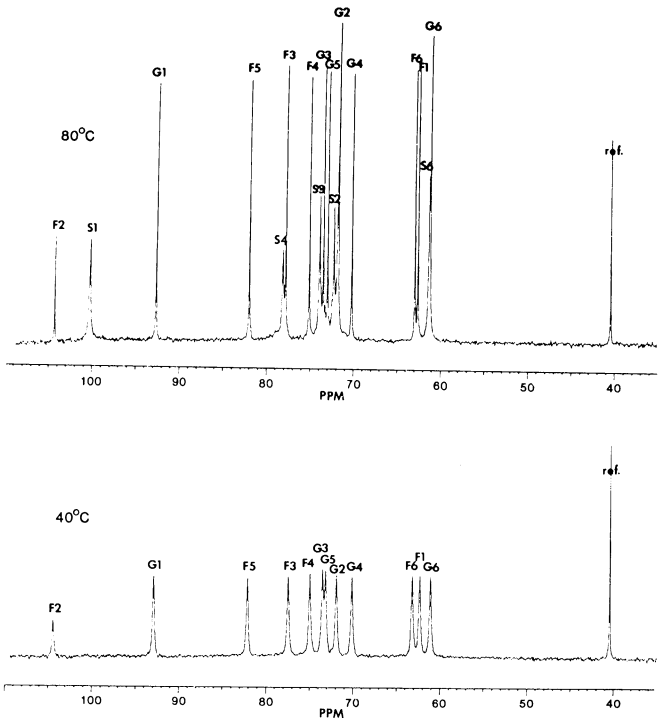

Fig. 1. ^{13}C-Nuclear magnetic resonance spectra of sucrose-starch dispersions at 40 and 80° C (512 pulses, pulse width 6.0 μsec [60°], 0.49-sec delay between pulses; line broadened 5.0 Hz). **PPM** refers to parts per million; 40.4 ppm is equal to the external dimethylsulfoxide reference. Glucose carbon atoms of the sucrose are labelled **G1** through **G6**, fructose carbon atoms are **F1** through **F6**, and starch carbon atoms are **S1** through **S6**. The S5 is not seen in the spectra because of overlap with a sucrose carbon.

RESULTS AND DISCUSSION

Sucrose-Starch Interactions

Illustrated in Figure 1 are examples of the NMR spectra of sucrose-starch solutions at 40 and 80° C. Chemical shifts of sucrose carbon atoms were previously determined (Hull 1982, Bock and Lemieux 1982). Chemical shift positions are indicated on the figure. To clarify whether a carbon atom is associated with the fructose or glucose portion of the sucrose molecule, the carbon atoms are labelled F1 through F6 and G1 through G6. For example, F1 represents the C1 carbon of fructose and G1 represents the C1 carbon of glucose. Chemical shifts of starch carbon atoms, previously assigned by Jane et al (1985), are labelled S1 through S6, representing the C1 through C6 positions of the carbon atoms of starch. Not all carbon atoms of the starch can be seen because of the overlap with some of the sucrose carbon atoms. Intensity of peaks was not important for these investigations, because only the relative positions (the chemical shifts) are used for determining and interpreting if interactions are occurring. Furthermore, the intensity of the peaks can change as a function of temperature, whereas the area of the peak will remain constant. Chemical shifts for the starch carbon atoms were not evident in the 40° C spectra and were observable only above 68° C, onset of gelatinization, of the 1.0*M* sucrose-starch dispersions, according to the DSC studies.

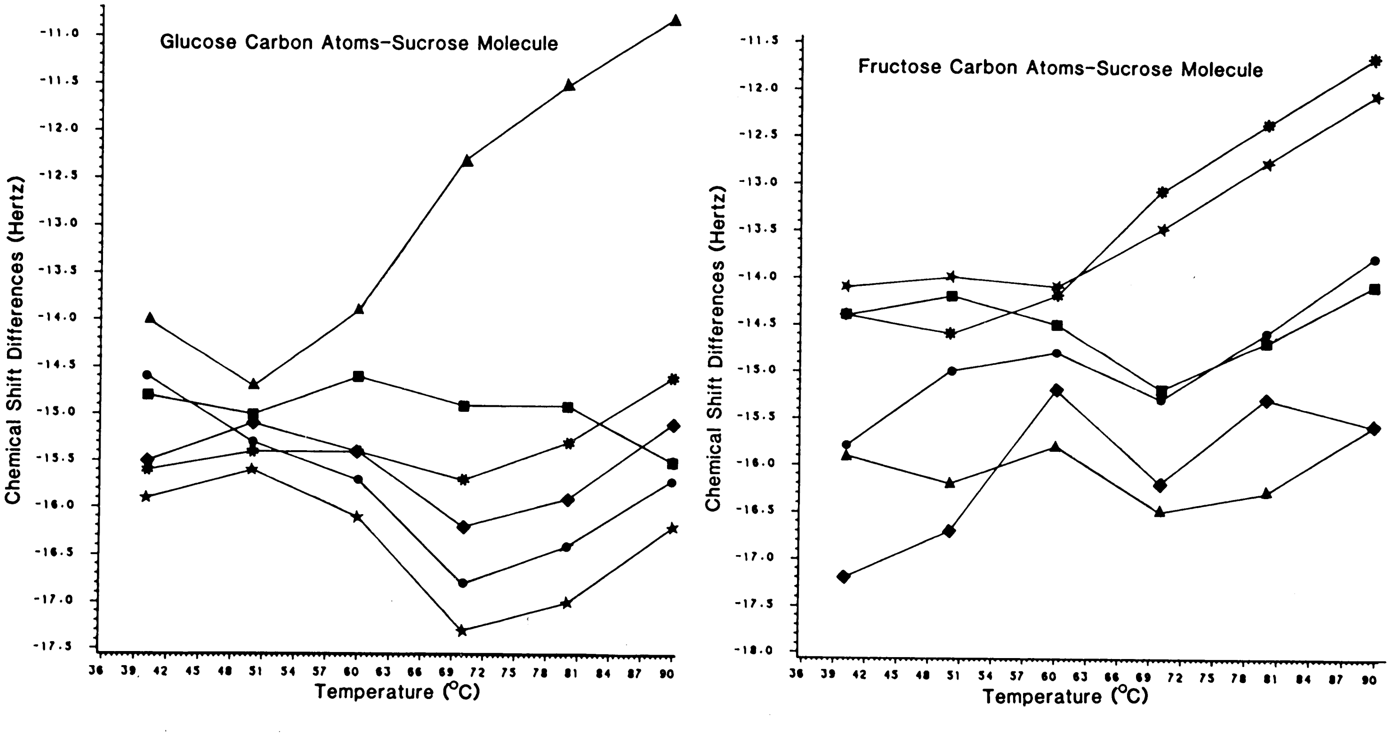

Fig. 2. Chemical shift differences between sucrose carbon atoms in a sucrose-starch solution and in a sucrose solution as a function of increasing temperature (512 pulses, pulse width 6.0 μsec [60°], 0.49-sec delay between pulses, line broadened 5 Hz). Chemical shifts of the sucrose-starch dispersions are all upfield relative to reference sucrose. The left hand portion of the figure shows the glucose carbon atoms of the sucrose molecule; codes are as follows: G1 ★, G2◆, G3 *, G4■, G5 ●, and G6 ▲. The right hand portion of the figure illustrates the fructose carbon atoms of the sucrose molecule; codes are F1 *, F2◆, F3★, F4 ■, F5 ●, and F6 ▲.

The differences between chemical shifts of the sucrose carbon atoms in solutions and the sucrose carbon atoms in the sucrose-starch dispersions are shown in Figure 2. All chemical shifts of sucrose in a sucrose-starch dispersion are upfield relative to reference sucrose. Although these data represent changes in slope with 1.0*M* sucrose-starch dispersions, similar trends in slope were noted when different molar concentrations were used. Deviations occur at higher or lower temperatures with different molar concentrations because sucrose concentration alters onset temperature. Significant changes in the chemical shift of G6, G5, G1, F3, F1, and possibly the G2 atoms of the sucrose molecule were observed. The F2 atom shows inconsistent changes; however, F2 gives a weak signal. The signal-to-noise ratio was less for F2 than for any of the other carbon atoms, making interpretation more difficult for this carbon than for the others. Onset of starch gelatinization in a 1.0*M* sucrose solution (70% solution, 30% starch) as measured by DSC occurred at 68° C. Major deviations in slope were apparent between 60 and 70° C, which corresponds to onset temperature of gelatinization.

CONCLUSION

Methodology presented using ^{13}C-NMR indicate sugar-starch interactions are observable using ^{13}C-NMR spectroscopy, and these occur within the temperature range of starch gelatinization as measured by DSC. Interactions are not uniform, and some carbon atoms of the sucrose appear to be involved more than others. Suspension of the starch in xanthan gum and equilibration of the dispersion at each temperature studied were crucial factors to obtaining reproducible results. Using this method, further studies are underway to observe similarities and differences between various sugar-starch interactions to gain insight into the those interactions. Similar investigations using relaxation measurements, T1 and T2, are been planned.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support provided by Nabisco Brands, Inc.

LITERATURE CITED

BEAN, M. M., and OSMAN, E. M. 1959. Behavior of starch during food preparation. I. Effects of different sugars on the viscosity and gel strength of pastes. Food Res. 24:665.

BEAN, M. M., and YAMAZAKI, W. T. 1978. Wheat starch gelatinization in sugar solutions. I. Sucrose: Microscopy and viscosity effects. Cereal Chem. 55:936.

BEAN, M. M., YAMAZAKI, W. T., and DONELSON, D. H. 1978. Wheat starch gelatinization in sugar solution. II. Fructose, glucose, and sucrose cake performance. Cereal Chem. 55:945.

BOCK, K., and LEMIEUX, R. U. 1982. The conformational properties of sucrose in aqueous solution: Intramolecular hydrogen-bonding. Carbohydr. Res. 100:63.

CALLAGHAN, P. T., JOLLEY, K. W., LELIEVRE, J., and WONG, R. B. K. 1983. Nuclear magnetic resonance studies of wheat starch pastes. J. Colloid Interface Sci. 92:332.

EVANS, D. D., and HAISMAN, D. R. 1982. The effect of solutes on the gelatinization temperature range of potato starch. Staerke 34:224.

FUKE, Y., and MATSUOKA, H. 1984. Studies on the physical and chemical properties of kiwi fruit starch. J. Food Sci. 49:620.

HENNIG, H. J., and LECHERT, H. 1977. NMR study of D_{20} in native starches of different origins and amylose of type-B. J. Colloid Interface Sci. 62:199.

HULL, W. E. 1982. Two-Dimensional NMR. Bruker Analytische Messtechnik: Karlsruhe, FRG.

JANE, J. L., ROBYT, J. F., and HUANG, D. E. 1985. ^{13}C-N.M.R. study of the conformation of helical complexes of amylodextrin and amylose in solution. Carbohydr. Res. 140:21.

JASKA, E. 1971. Starch gelatinization as detected by proton magnetic resonance. Cereal Chem. 48:437.

KIM, K. O., HANSEN, L., and SETSER, C. 1986. Phase transitions of wheat starch-water systems containing polydextrose. J. Food Sci. 51:1095.

KOEPSEL, K., and HOSENEY, R. C. 1980. Effects of corn syrups in layer cakes. Cereal Chem. 57:49.

LELIEVRE, J., and MITCHELL, J. 1975. A pulsed NMR study of some aspects of starch gelatinization. Staerke 27:113.

LUND, D. 1984. Influence of time, temperature, moisture, ingredients, and processing conditions on starch gelatinization. CRC Crit. Rev. Food Sci. Nutr. 20(4):249.

MILLER, B. S., and TRIMBO, H. B. 1965. Gelatinization of starch and white layer cake quality. Food Technol. 19:640.

SAVAGE, H. L., and OSMAN, E. M. 1978. Effects of certain sugars and sugar alcohols on the swelling of cornstarch granules. Cereal Chem. 55:447.

SPIES, R. D., and HOSENEY, R. C. 1982. Effects of sugars on starch gelatinization. Cereal Chem. 59:128.

WATSON, S. A. 1964. Determination of starch gelatinization temperature. In: Methods in Carbohydrate Chemistry, Vol. 4. R. L. Whistler, R. J. Smith, and M.L. Wolfrim eds. Academic Press: New York.

WOOTTON, M., and BAMUNUARACHCHI, A. 1980. Application of differential scanning calorimetry to starch gelatinization. III. Effect of sucrose and sodium chloride. Staerke 32:126.

[Received May 6, 1987. Revision received August 17, 1987. Accepted August 18, 1987.]