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Note

A Fourier-transform infrared study of wheat starch gels

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The retrogradation of starch and starch components has traditionally been studied by X-ray diffraction, differential scanning calorimetry, and rheological methods; more recently, Raman[1] and Fourier-transform infrared (F.t.-i.r.)[2] have been used. The region of the i.r. spectrum between 1400–800 cm^{-1} is sensitive to the conformation of polysaccharides in aqueous solution[3], even though most bands arise from highly coupled C–O and C–C vibrations[4], and precise assignment is difficult. However, the intensity of the bands is such that the majority are likely to be associated with C–O dipoles, probably from primary or secondary alcohols. In the i.r. spectrum of waxy-maize starch[2], a band-narrowing process was observed in the region 950–1050 cm^{-1} during storage, leading to the gradual development of distinct bands at 1046 and 1019 cm^{-1} from an initial broad band centred at 1020 cm^{-1} in the freshly gelatinised material. The extent of the line narrowing was quantified by taking the ratio (R) of the absorbances at 1019 and 1037 cm^{-1} (the valley minimum in the fully retrograded material) as a function of time. The time course of the F.t.-i.r. measurements was similar to that for shear modulus measurements, indicating a direct correlation between the spectroscopic measurement and a functional property. We now report the extension of these studies to wheat starch gels, where the same process can be seen although the overall effect is not so marked as for waxy-maize gels, and at no time during retrogradation do two distinct bands occur.

The i.r. spectrum of wheat starch granules in water (Fig. 1a) has bands at 1019 (II) and 1006 cm^{-1} (III), and a distinct shoulder at 1046 cm^{-1}. Band III is not seen in the corresponding spectrum of waxy-maize starch. After gelatinisation, band III was lost and band I became less distinct (Fig. 1b). During storage for 21 days at 1° in a sealed cell, the shoulder at 1046 cm^{-1} became more pronounced (Fig. 1c) as band I gradually re-appeared. The development of a more distinct shoulder is due to the narrowing of the line widths probably because, in the gel state, there is a wide distribution of possible molecular conformations and, hence,

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bond energies. As retrogradation takes place, the more ordered crystalline state appears, which has fewer possible conformations and thus a smaller distribution of bond energies. Another possible cause of broadening in these systems, particularly of alcoholic C–O related modes, is interaction with water. A range of O···H···O distances would lead to a spread of C–O mode frequencies. However, in the i.r. spectra of dry amorphous starches, the lines are also broad. In such a system, broadening cannot result from interaction with water but must be due to a spread of bond energies resulting from a range of conformations. On the addition of water to the samples, the lines sharpen due to increased crystallinity[5]. This effect has been also observed in solid-state ^{13}C-n.m.r. spectra of starches[6] where the spread of conformations causes a spread of chemical shifts which is reduced on the addition of water.

In order to better observe and quantify the effects, the resolution of the i.r. spectrum was enhanced by the process of Fourier self deconvolution. When the ratio (R) of the absorbances at 1046 and 1022 cm^{-1} in the deconvoluted spectra are plotted against time, the curve shown in Fig. 2 resulted; this is similar to that for waxy-maize starch, but the magnitude of the change is somewhat less. Unlike waxy-maize starch, which is almost pure amylopectin, wheat starch contains significant amounts of amylose. However, since the crystallisation of amylose is a relatively fast process[7], the changes observed in the F.t.-i.r. spectrum during several days are due solely to changes in the amylopectin. Therefore, the differences between the changes in the spectra of waxy-maize starch and wheat starch do not arise because of the presence of amylose in one sample. Comparison of the rigidity of amylopectin gels from various botanic sources[8] after 21 days reveals considerable variation. In particular, the rigidity of wheat starch gels was less than that from waxy maize and hence it may be assumed that the corresponding crystallinity was less.

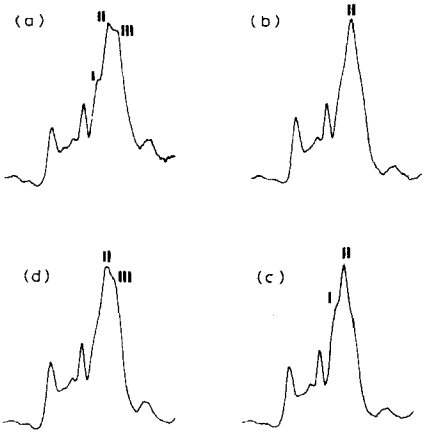

Fig. 1. I.r. spectra (1250–850 cm^{-1}) of wheat starch (a) in cold water, (b) after gelatinisation at 90°, (c) gel stored at 1° in sealed cell for 21 days, (d) gel stored under drying conditions. Vertical axes in absorbance.

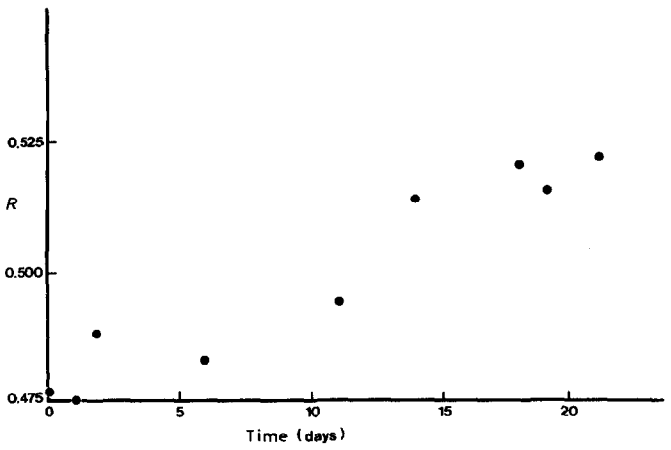

Fig. 2. Plot of F.t.-i.r. band ratio, R, versus time (days) for retrogradation of stored wheat-starch gel. See text for the definition of R.

The general structure of the spectrum of wheat starch is therefore likely to be broader than that of waxy maize due to lack of crystallinity. Even so, the results reported here do indicate that the retrogradation process may be followed by F.t.-i.r. spectroscopy.

During storage of a wheat starch gel in a sealed cell, no changes were observed at 1006 cm^{-1}, but, on storage under conditions where gradual drying occurs, the dominant process is no longer as described above. Instead, band III grows until it returns to its original intensity in the fully dried material (Fig. 1d). In order to study this effect, a gel was applied to an exposed ATR plate and allowed

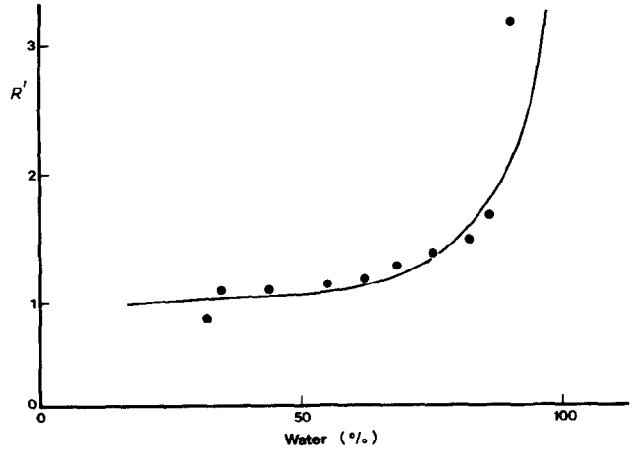

Fig. 3. Plot of F.t.-i.r. band ratio, R', versus water content for drying wheat-starch gel. See text for the definition of R'.

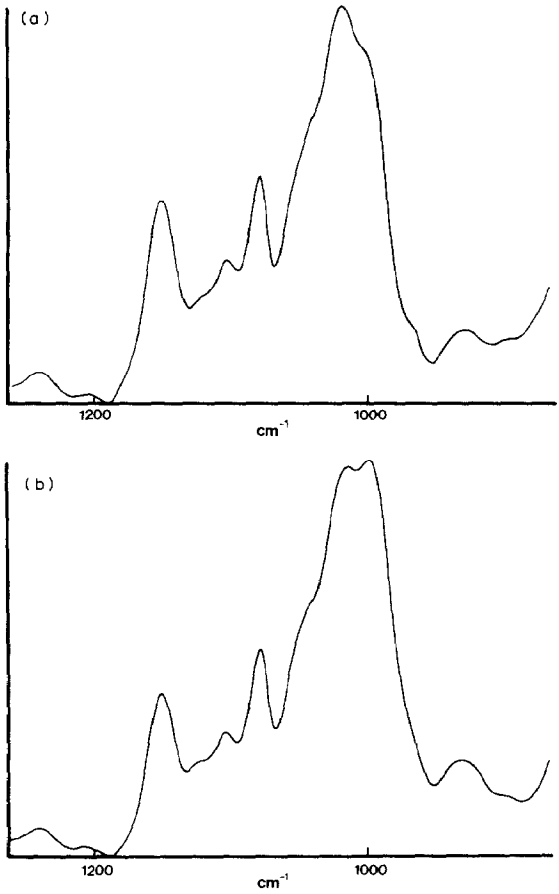

Fig. 4. I.r. (ATR) spectra of bread in the starch region (1250–850 cm^{-1}): (a) fresh bread, (b) partially dried, stale bread. Vertical axes in absorbance.

to dry out *in situ*. At regular intervals, the i.r. spectrum was recorded and the water content was determined from the ratio of the absorbances at 1640 (water O–H bend) and 1019 cm^{-1} by reference to data for wheat starch gels with various known water contents. The spectrum of water was then subtracted, and the ratio (R') of the absorbances at 1019 and 1006 cm^{-1} was obtained and plotted against water content (Fig. 3).

The ability to monitor these effects in wheat starch is important since it is the most widely used food starch and is present in a wide range of foodstuffs. The methods used for these model systems can also be applied to such whole products as bread. The quality of the ATR spectrum of the starch region of fresh broad shown in Fig. 4a is such that it is feasible to monitor retrogradation by following changes at 1046 cm^{-1} and also to investigate the distribution of water and drying effects (see Fig. 4b). F.t.-i.r. spectroscopy may prove to be a valuable method for quality control and the study of carbohydrates in whole food systems.

EXPERIMENTAL

Preparation of gels. — For the retrogradation studies, a 20% gel of wheat starch was prepared by dispersing the starch with gentle agitation in boiling water containing 0.02% of sodium azide as preservative. The hot solution was poured into the F.t.-i.r. cell and cooled rapidly to 1° to initiate gelation. A sample, in slurry form, was also prepared for F.t.-i.r. analysis before gelatinisation. A sample of gel, prepared in an identical manner, was used for the drying experiments. For calibration purposes, a series of wheat starch gels were prepared over the range 10–50%.

F.t.-i.r. measurements. — All measurements were carried out on a Digilab FTS60 spectrometer operating at 4 cm^{-1} resolution, using a TGS detector; 256 interferograms were co-added before Fourier transformation. Triangular apodisation was employed. Retrogradation was studied[2] using a cylindrical internal reflectance (CIR) cell (Spectra-Tech Inc.), with a ZnSe crystal. The gel was sealed into the CIR cell in order to prevent loss of water, and the cell was stored at 1°. At regular intervals, the cell was allowed to reach room temperature before F.t.-i.r. measurements were made. A background for the empty CIR cell was obtained and used for all subsequent samples. The spectrum of water was subtracted from all starch spectra in order to eliminate the distorting effect in the region 1100–800 cm^{-1}. Resolution enhancement was applied to all resulting spectra, using techniques described by Cameron *et al.*[9] modified for use on the Digilab FTS60. The assumed line shape was Lorentzian with a half-width of 15 cm^{-1}. The resolution enhancement factor (k) was optimised at 1.5. Ratio mesurements were made only on the enhanced spectra. The spectrum of the material before gelatinisation and all drying experiments were carried out was acquired by using a Spectra-Tech horizontal attenuated total reflectance (ATR) attachment with a ZnSe crytal. A background was acquired of the clean crystal and all starch samples were spread onto the crystal. A spectrum of water for substraction purposes was acquired in the same way. For drying experiments, the gels of known water content were applied to the ATR. A 20% wheat starch gel was then applied to the crystal and allowed to dry gradually, and spectra were obtained at regular intervals.

The spectra of fresh and dry bread were obtained using a Spectra-Tech continuously variable ATR cell with a ZnSe parallelogram crystal (50 × 20 × 3 mm, 45°) with the incident angle set at 45°. After collection of a background of the empty cell, the bread was applied to the ATR plate as a slice the same size as the crystal and clamped to ensure good optical contact.

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