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Carbohydrate Research 337 (2002) 147–153

CARBOHYDRATE RESEARCH

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Use of ^{1}H cross-relaxation nuclear magnetic resonance spectroscopy to probe the changes in bread and its components during aging

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Received 27 July 2001; accepted 29 October 2001

Abstract

^{1}H nuclear magnetic cross-relaxation spectroscopy was used to probe the molecular mobility/rigidity in bread and its components during storage. The Z-spectra lineshapes, attributed to the solid-like polymer fractions of the samples, differed for the bread, gelatinized waxy starch (GX), gelatinized wheat starch (GW), heated flour (HF), and heated gluten (HG). Upon storage, no change was observed in the Z-spectrum of the bread sample, while the Z-spectra for GX, GW, and HG increased in the width at half height of the decomposed broad component (increased rigidity). These trends in the Z-spectra detected by NMR were contradictory to the DSC results that showed an increase in amylopectin retrogradation enthalpy for all samples containing starch, including bread. These trends in the Z-spectra detected by NMR were not reflected by the DSC results that showed an increase in amylopectin retrogradation enthalpy for all samples, including bread. The differences in molecular mobility could not be therefore, due to recrystallized amylopectin and may be attributed to the role of gluten and/or redistribution of water in the amorphous regions of the samples.

Keywords: Proton cross-relaxation NMR; Bread staling; Molecular mobility; Starch retrogradation

1. Introduction

Bread staling is a complex process involving a combination of physical, chemical and sensory changes resulting in an unacceptable product.[1] Formulation and processing technologies designed to control the staling rate have long been investigated, especially those designed to curtail the increase in firmness of the product. Such textural changes occurring in bread during storage have been attributed to several factors, including recrystallization of amylopectin, water redistribution, gluten functionality changes and the state of the amorphous domains.[2–5] Properties of bread observed at a macroscopic level (e.g., texture) are the tangible manifestation of changes that take place in the product at both structural and molecular levels. Consequently, the state of the bread polymers (e.g., crystalline, amorphous) and availability of water are among the factors that influence bread's textural properties.

Nuclear magnetic resonance (NMR) spectroscopy provides a means for studying molecular motions of polymers and solutes such as water. Cross-relaxation (CR) NMR spectroscopy is a high-resolution proton (^{1}H) method for determining the information on the relaxation of the solid component via the observable liquid spin system.[6] The ^{1}H CR NMR method has been shown to be a beneficial tool for probing solid components in gels using a simple and economical NMR spectrometer, without the need for a solid-state instrument.[7] A sample is irradiated with a radio frequency

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0008-6215/02/$ - see front matter
PII: S0008-6215(01)00293-2

pulse that is off-resonance from the liquid signal. Due to the dipolar coupling between the liquid and solid protons, the amplitude of the liquid spectrum will change with the offset frequency, and a Z-spectrum is obtained.[6–8]

The Z-spectrum is a reflection of the solid-like protons in the sample. Wu and Eads[9] found an increase in the area and width of the Z-spectral line shape for waxy starch gels during aging that was dependent on concentration and storage time.[8] These researchers further decomposed the complex Z-spectra for waxy starch gels into a Lorentzian (more mobile, solid protons) and a Gaussian (more rigid, solid protons) component. The Gaussian component was found to increase with storage time and was attributed to the starch chains restricted motions such as in aggregates or crystallites. Additionally, differential scanning calorimetry (DSC) peak area at 60 °C (reflecting amylopectin recrystallization) increased during storage and was paralleled by an increase in the Gaussian component area.[9]

Although there exists a large volume of work correlating the recrystallization of starch (mainly amylopectin retrogradation) with bread staling, the subject remains controversial. The objective of this work was to probe the molecular mobility/rigidity using ^{1}H CR NMR in amylopectin and other bread components that may play a role in the firming process over time.

2. Results and discussion

Fig. 1(a) and (b) show the results of a cross-relaxation ^{1}H NMR experiment for water and bread. Fig. 1(a) shows the liquid ^{1}H signal (single resonance) observed at various offset frequencies, and Fig. 1(b) represents the Z-spectrum that is obtained from the amplitude of these signals. The Z-spectrum for water shows a very narrow curve reflecting only the saturation pulse due to absence of solid in the water sample. The Z-spectrum for the bread sample is significantly wider than the one of water. In the bread sample, the solid protons excited by the NMR pulse, transfer magnetization to liquid protons, resulting in a NMR liquid signal of lower intensity due to the cross-relaxation between the spins of restricted mobility and those of the mobile bulk-water pool.[10]

Fresh (day 0) samples.—To better understand the effect of the different bread components on the interaction between liquid and solid protons (visualized by the Z-spectrum lineshape), the results of the cross-relaxation experiment for gelatinized wheat starch (GW), gelatinized waxy starch (GX), heated gluten (HG), and heated flour (HF) at day 0 are plotted with those of bread in Fig. 2. The Z-spectra have lineshapes similar to those found by Wu and Eads[9] and were attributed to the solid-like polymer fractions of the samples. The different samples resulted in variable Z-spectra, although they contained the same amount of moisture ($\sim$50% moisture, total basis) except for the bread samples (36% moisture, total basis). Different lineshapes are indicative of different mobilities of the solid–proton fraction. All samples except the bread showed similar normalized intensities at frequency offsets >30 kHz but deviated at lower frequencies (Fig. 2). Such deviations are indicative of proton rigidity differences in the solid. For example, a narrower line shape (e.g., waxy starch) indicates the presence of more-mobile solid protons.

Various models have been used to characterize the lineshape of the cross-relaxation spectra.[6,10–13] The details of these are summarized elsewhere.[7] Wu and Eads[9] used the equation derived by Grad and Bryant[6] to fit

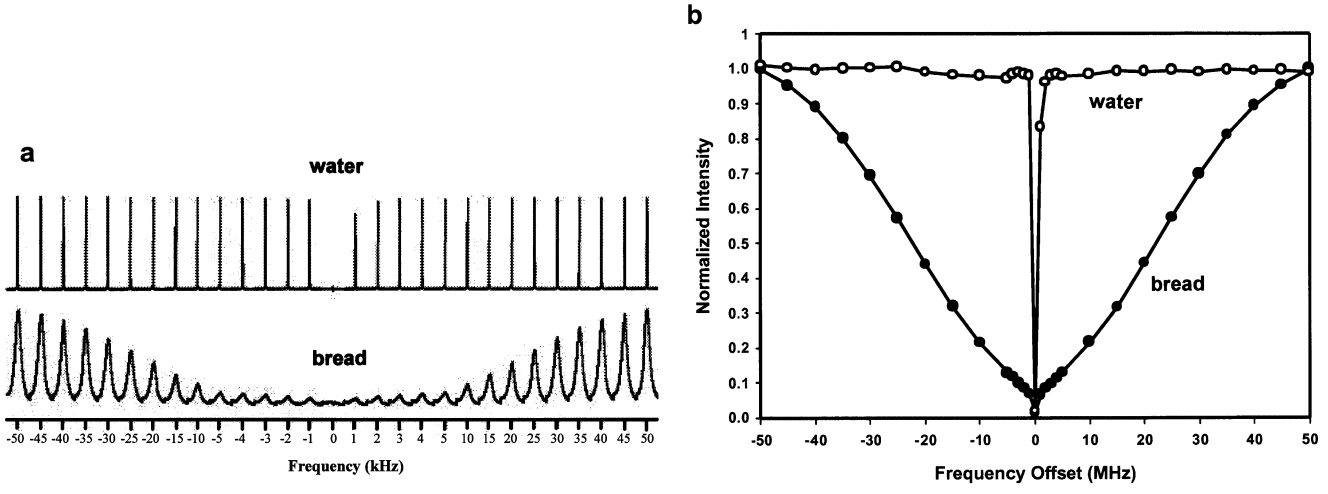

Fig. 1. Results of a cross-relaxation ^{1}H NMR experiment for water and bread. (a) Liquid ^{1}H signal (single resonance) observed at various offset frequencies; (b) Z-spectrum obtained from the normalized amplitude of Fig. 1(a) signals.

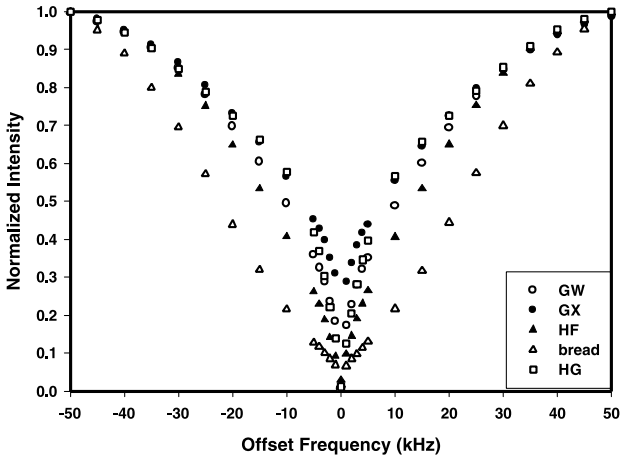

Fig. 2. Results of cross-relaxation ^{1}H NMR experiments for bread and its components at day 0 storage. Abbreviations are for wheat starch (GW), gelatinized waxy starch (GX), heated gluten (HG), and heated flour (HF).

Table 1
Calculated probability of curve fitting the ^{1}H cross-relaxation NMR Z-spectra with a combination of Lorentzian and Gaussian curves

Sample	Ratio	Curve fit
Wheat	2.7×10^{-21}	2 Lorentzians
Waxy	1.0×10^{-18}	2 Lorentzians
Gluten	7.2×10^{-7}	2 Lorentzians
Dough	8.7×10^{-11}	2 Lorentzians
Bread	2.3×10^{3}	Lorentzian + Gaussian

A ratio >1 implies that the data are more appropriately fitted with a Lorentz–Gaussian curve, while a ratio <1 implies that the data are more appropriately fitted with two Lorentzian curves.

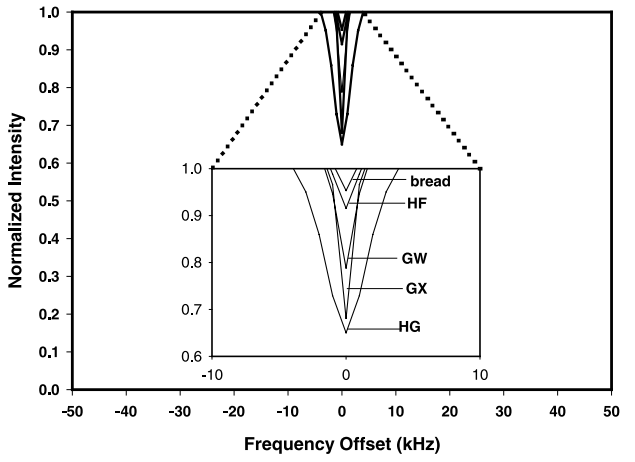

Fig. 3. Results for the narrow components obtained from the decomposition of the Z-spectra of bread, wheat starch (GW), gelatinized waxy starch (GX), heated gluten (HG), and heated flour (HF). Inset represents a magnification of the results.

the cross-relaxation spectra of starch gels with a combination of Gaussian and Lorentzian curves. Changes in the parameters of these decomposed curves (such as area and linewidth) were attributed to certain factors in the original cross-relaxation equation. A similar approach was used in this work. All samples, except bread, were best fitted with two Lorentzian curves representing broad and narrow components. Bread samples were best fitted with a combination of Lorentzian (narrow) and Gaussian (broad) curves (Table 1). Wu and Eads[9] found a Lorentzian–Gaussian combination as best fit for their waxy starch gels with a standard deviation of 7% or less. A similar standard deviation was also found in this study when Lorentzian–Gaussian combinations were used. However, a lower standard deviation (2–3%) was obtained for a two Lorentzian fit. The narrow and broad decomposed curves for all samples are shown in Figs. 3 and 4, respectively. A larger narrow component (mobile solid ^{1}H fraction) was found in HG, followed by GX, GW, and HF, and the smallest mobile solid ^{1}H fraction was found in the bread (Fig. 3). This sequence was opposite when the broad (less mobile) solid ^{1}H fraction is considered (Fig. 4). The normalized peak area for each of the decomposed curves was calculated since it has been attributed to the relative mass fraction of the more-mobile and more rigid solid protons. For day 0, the area of the wider component was 99% of the total for bread, 97% for the GX, GW and HF samples, and 88% for the HG. These broader curves represent the more rigid-solid protons. To quantify the differences in rigidity of these curves, the width at half height was obtained.[7,9] Table 2 shows the differences in rigidity for both the more rigid-solid protons (broad component) and the more mobile solid protons (narrow component). Al-

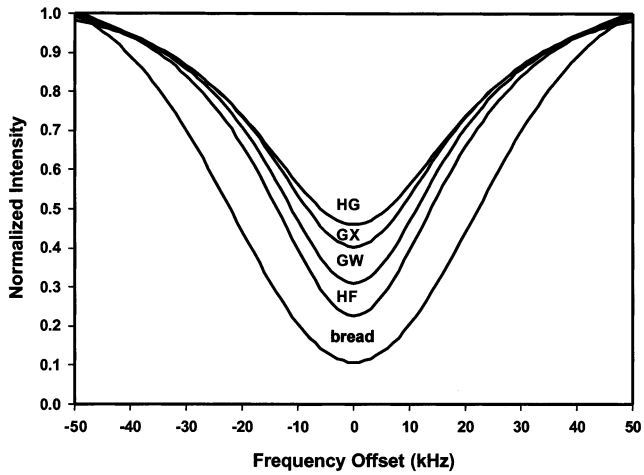

Fig. 4. Results for the broad components obtained from the decomposition of the Z-spectra of bread, wheat starch (GW), gelatinized waxy starch (GX), heated gluten (HG), and heated flour (HF).

Table 2
Width at half height of the decomposed curves (broad and narrow components) obtained from the Z-spectra of the various samples at day 0

Sample	Broad component (kHz)	Narrow component (kHz)
Bread	32.7	0.91
Gluten	23.2	2.19
Flour	21.1	1.45
Waxy starch	20.4	0.64
Wheat starch	19.8	0.97

though bread had a comparable mass fraction to the GW, HF, and GX samples, its proton rigidity was much greater (Table 2). This increased rigidity may be partially attributed to the differences in moisture (moisture content, mc) of these samples (36% mc for bread vs. 50% mc for the other samples). Wu and Eads[9] found an increase in the width at half height (rigidity) of the Z-spectra with increased concentration of waxy starch but no change in lineshape. In this case, however, the decomposition of the Z-spectra required Gaussian and Lorentzian curves for bread and two Lorentzian curves in the other samples; therefore, other factors (such as effect of rigid protons from other bread components) may also be involved in the bread's increased rigidity.

The more mobile solid protons described by the narrow components had a mass fraction of 1% for bread, 3% for GW, GX, and HF and 12% for gluten. Although gluten had the largest mass fraction of the more mobile solid protons (Table 2, narrow component), the lineshape was the widest indicating a greater rigidity. This increased rigidity of the protons may be due to a greater affinity of gluten for water, thereby restricting proton mobility as was found for low moisture samples.[14]

Stored samples.—Changes in the Z-spectra for all samples were followed during storage at 4 °C for 11 days. Since the NMR tubes were sealed during storage and no condensation was observed in the samples, it was assumed that little to no moisture was lost during storage. Fig. 5(a) shows such changes for GX (a similar trend was also found for GW and HF). A widening of the Z-spectra lineshape was observed with increased storage time as previously reported by Wu and Eads[9] for a 40% waxy starch sample stored at 5 °C for 6 days. In contrast, the Z-spectra for the bread sample (Fig. 5(b)) did not change with storage time.

The Z-spectra lineshapes of all samples were decomposed into their two constituent peaks, as described above. Little change ($< 1\%$) was found in the % area of each of the components during storage, indicating that the mass fraction of the more mobile and more rigid solid protons in the system did not change (data not shown). Additionally, the linewidth of the more mobile (narrow) solid-proton component did not change during storage for any of the samples (Fig. 6(a)), indicating that this proton population did not change rigidity during storage. On the other hand, GX and GW, as well as HF, showed an increase in stiffness (width at half height) in the more solid-like (wide) proton component during the first two days of storage (Fig. 6(b)) and then remained constant. In contrast, the gluten sample showed a slight decrease in stiffness only in the first day of storage, and the bread sample did not change in stiffness throughout the storage period.

Wu and Eads[9] attributed the increase in the broad component width during storage of waxy starch gels to restriction of chain conformation or crystallinity measured by DSC. Fig. 7 shows the enthalpy of amylopectin retrogradation over time for the various samples. It is interesting to note that the width at half height in the bread samples showed no change upon storage (Fig. 6(b)), despite an increase in enthalpy detected by DSC (Fig. 7). These differences may be

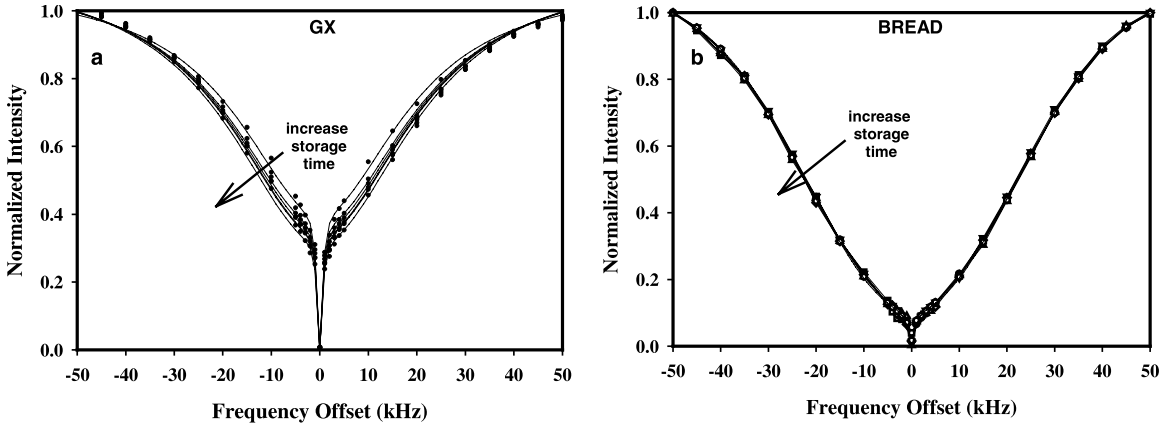

Fig. 5. Z-spectra results for samples stored at 4 °C for 11 days. (a) Gelatinized waxy starch, GX and (b) bread. Lines represent the fit of the Z-spectra to two Lorentzian curves (waxy starch) and a combination of Gaussian and Lorentzian curves (bread).

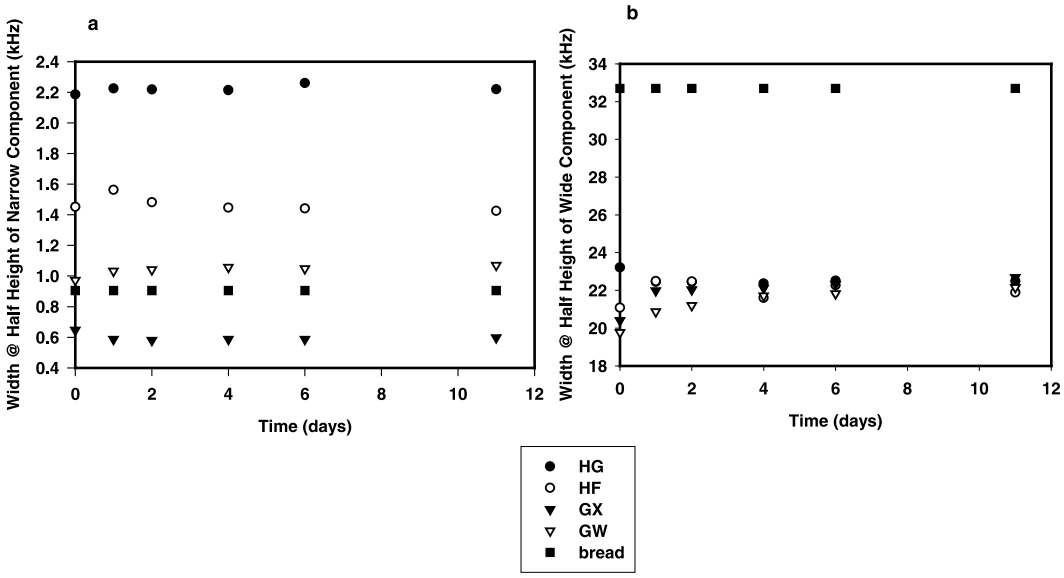

Fig. 6. Width at half height obtained from decomposed Z-spectra curves for bread, wheat starch (GW), gelatinized waxy starch (GX), heated gluten (HG), and heated flour (HF). (a) Narrow component; (b) and broad component.

attributed to the role of gluten in these samples, since the width at half height of stored gluten samples decreased in the first day with little change after that (Fig. 6(b)). Additionally, Kim-Shin and co-workers[15] found a decrease in water mobility in bread during storage and attributed this phenomenon to a redistribution of water in the amorphous phase of amylopectin or gluten, and not the incorporation of water into crystalline regions. Therefore, a redistribution of moisture among the bread components could yield a constant solid-proton Z-spectra for bread during storage. Further work is required to better understand this phenomenon.

All samples showed a sharp increase in enthalpy (proportional to the amount of retrograded amylopectin) in the first day, followed by a slower increase up to day 4 (or day 6 for wheat starch) with no further increase thereafter (Fig. 7). These trends are similar to those previously reported.[16–18] Unlike in Wu and Eads[9] work, no correlation between the DSC enthalpy of amylopectin retrogradation and the increased stiffness observed by the wide component in NMR (Fig. 8) was found in our work. The GW did show a straight-line relationship ($r^2 = 0.99$) between the enthalpy of retrograded amylopectin and GW wide component up to day 6 as observed by Wu and Eads[9] for the 40% starch gels, but not upon further storage. A possible explanation for these discrepancies may reside in the event being detected by each method. For example, Cooke and Gidley[19] showed that ^{13}C NMR is sensitive to the double-helix content in starch and that the DSC endotherm reflects the loss of these double helices, not crystallinity. It is hypothesized that the Z-spectra obtained in this work may reflect solid protons associated with crystalline order and therefore deviate from DSC results.

Proton cross-relaxation NMR proved a viable technique to probe differences in bread and its components upon storage. The lack of change in the bread Z-spectrum with increased storage time suggests that this NMR technique does not probe the same phenomenon as DSC. Previous work has shown that NMR spectroscopy can detect molecular motions in the picosecond–millisecond range, while DSC detects events over a milliseconds–second range.[20] Thus, further work in this area is needed to better understand the origins of the solid protons contributing to the Z-spectrum.

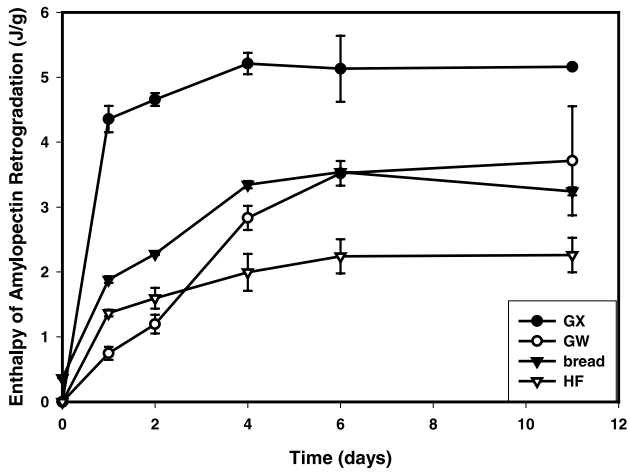

Fig. 7. Changes in enthalpy of amylopectin retrogradation (area of endothermic peak around 60 °C) during storage at 4 °C for 11 days obtained from differential scanning calorimetry. Abbreviations are for wheat starch (GW), gelatinized waxy starch (GX), and heated flour (HF).

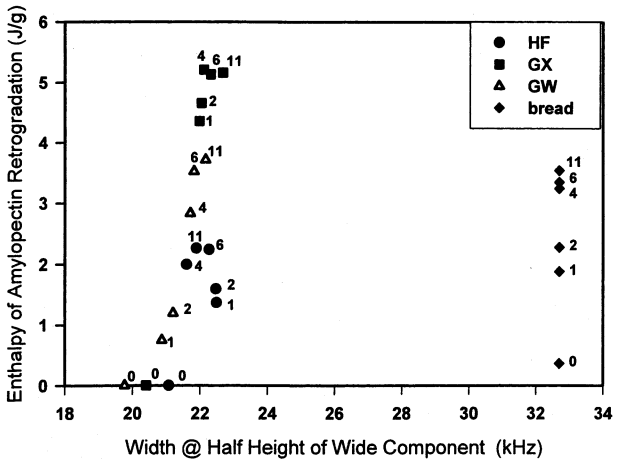

Fig. 8. Relationship between width at half height of the wide component obtained from decomposition of the NMR Z-spectra and amylopectin retrogradation obtained from DSC, for bread, wheat starch (GW), gelatinized waxy starch (GX), and heated flour (HF). Numbers next to the symbols represent the days of storage of the sample.

3. Experimental

Sample preparation.—Wheat starch (Gemstar 100, Manildra Milling Corporation, Minneapolis, MN), flour (Gold Medal high-gluten bread flour, General Mills, Minneapolis, MN), waxy corn starch (Staley 7350 Waxy #1 starch, Staley Food Ingredients, Decatur, IL), and gluten (Sigma, St. Louis, MO) were weighed and placed inside a 5-mm i.d. NMR tube, and appropriate amounts of water were added to make 50% suspensions (total weight was ~0.5 g). Sample preparation followed that reported by Wu and co-workers.[8] The average initial moisture contents of the samples (total basis) as determined by thermogravimetric analysis (TGA 2950, TA Instruments, New Castle, DE) were wheat flour, 10.7%; wheat starch, 9.9%; waxy starch, 10.4%; and gluten, 7.3%. The samples were mixed both with a thin wire and with a vortex mixer and sealed to avoid moisture loss. Prior to heating at 90 °C for 15 min, samples were degassed with an aspiration pump to reduce trapped air in the mixture. These samples are referred to as gelatinized wheat (GW), gelatinized waxy (GX), heated gluten (HG), and heated flour (HF). Bread was prepared in a bread machine (Zojirushi model BBCC-V20, Zojirushi Corp. Osaka, Japan) using the following formula: all purpose flour, 55.8%; water, 34.9%; sugar, 4.6%; shortening, 2.6%; yeast, 1.1%; and salt, 1.0%. The bread was then cooled for 1 h at rt before inserting a crumb sample obtained from the center of the loaf into a 5-mm i.d. NMR tube. Final moisture content of the bread was 36% total basis. The NMR tubes containing the samples were sealed to prevent moisture loss, stored at 4 °C and analyzed at 22 °C after 0, 1, 2, 4, 6 and 11 days.

Cross-relaxation ^1H nuclear magnetic resonance.—NMR spectra were acquired on a Bruker DMX 300 spectrometer (Bruker Instruments, Billerica, MA) using a 5-mm broad-band $^1H/X$ double resonance liquid-state probe operating at a ^1H frequency of 300.13 MHz. Z-spectra were obtained using a modified method based on the one described by Wu and co-workers.[8] The pulse sequence is schematically represented in Fig. 9. Notably, a Gaussian saturation pulse was used in order to narrow the bandwidth of the saturation pulse. Fig. 1(a) shows the selectivity of this modification. Insignificant water saturation occurs 1 kHz off-resonance from the Gaussian pulse. The power of the saturating pulse was adjusted between samples to ensure full saturation of the liquid peak, while its length was maintained at 600 ms. The directly observed water spectrum was acquired in four scans with 8 K complex data points over a sweep width of 25 kHz (acquisition time = 0.2 s). All spectra used a recycle delay of 1 s and a ^1H 90° pulse width 5 μs. The offset frequency from the water resonance of the saturation pulse was varied from −50 to 50 kHz. The offset frequencies were calculated from the difference between frequency of off-resonance preparation pulses used to irradiate the solid component and the frequency of on-resonance 90° pulse of the liquid component.

All cross-relaxation solid spectra were normalized (taking as reference the highest intensity ^1H peak, generally the one at the furthest frequency from the saturation pulse) and decomposed into either Lorentzian and/or Gaussian curves with SIGMAPLOT™ Version 6.0 (SPSS Science, Chicago, IL). All day 0 Z-spectra were fit to both Lorentzian–Gaussian and two Lorentzian curves. From the value of the χ^2 for the best fit to each type of curve, a hypothesis test was performed. The hypothesis test was performed by taking the ratio of the probabilities that the two fits describe the data: Pr(Lorentzian–Gaussian)/Pr(two Lorentzian). Since both models have the same number of parameters, no Occam factor (penalty factor for increasing the number of parameters) is needed. The probabilities were calculated using a maximum likelihood approach:[21] $Pr = Exp(-0.5\chi^2)$. The calculated ratio would be much greater than 1 if the Lorentzian–Gaussian describes the data better, and much less than 1 if the two Lorentzian is preferred. Table 1 shows the results of the hypothesis

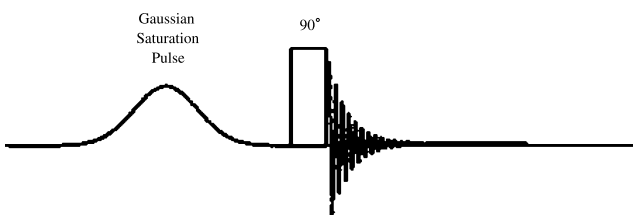

Fig. 9. Schematic representation of the ^1H cross-relaxation NMR pulse sequence.

test for all samples at day 0. We then analyzed all subsequent days using the model chosen by the day 0 result.

Differential scanning calorimetry (DSC).—Differential scanning calorimetry (DSC) was used to quantify the degree of amylopectin retrogradation. For starches and flour, enough water was added to make a 50% slurry. Slurry (10–15 mg) was placed in aluminum hermetic sample pans (TA Instruments, New Castle, DE) and sealed tightly to avoid moisture loss. The slurries were gelatinized in the aluminum pans by heating them from 25 to 90 °C and holding them at this temperature for 15 min. The heating was carried out in a TA Instruments DSC (DSC 2920, TA Instruments, New Castle, DE), and a heating rate of 5 °C/min was used. The instrument was initially calibrated using indium, and an empty pan was used as the reference. These samples were then cooled back to rt and stored at 4 °C for 0, 1, 2, 4, 6 and 11 days. After storage, samples were scanned in the DSC from −50 °C to 110 °C at 5 °C/min. Breadcrumb samples were obtained from the center of the loaf, and stored and analyzed under the same conditions as the other samples. The presence of retrograded amylopectin was measured from an endothermic melting peak at around 60 °C. Analyses were performed in three to four replicates. Standard deviations of the area of the recrystallized amylopectin peak were less than 10%.

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