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Assignments of Proton Populations in Dough and Bread Using NMR Relaxometry of Starch, Gluten, and Flour Model Systems

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ABSTRACT: Starch−water, gluten−water, and flour−water model systems as well as straight-dough bread were investigated with ^{1}H NMR relaxometry using free induction decay and Carr−Purcell−Meiboom−Gill pulse sequences. Depending on the degree of interaction between polymers and water, different proton populations could be distinguished. The starch protons in the starch−water model gain mobility owing to amylopectin crystal melting, granule swelling, and amylose leaching, whereas water protons lose mobility due to increased interaction with starch polymers. Heating of the gluten−water sample induces no pronounced changes in proton distributions. Heating changes the proton distributions of the flour−water and starch−water models in a similar way, implying that the changes are primarily attributable to starch gelatinization. Proton distributions of the heated flour−water model system and those of fresh bread crumb are very similar. This allows identifying the different proton populations in bread on the basis of the results from the model systems.

KEYWORDS: *proton mobility, low-resolution proton nuclear magnetic resonance, starch gelatinization, gluten polymerization, dough, bread*

INTRODUCTION

The transition from wheat flour to bread is a complex process in which several transformations take place, including those associated with changes in water distribution.[1] At the dough stage, flour particles are hydrated, constituents gain mobility, and a continuous cohesive viscoelastic gluten protein network is formed. Starch can absorb up to 46% of the total water in bread dough, but further acts as inert filler in the continuous dough protein matrix.[2] During baking, dough undergoes irreversible changes and water is redistributed. At about 65 °C, starch granules gelatinize and swell.[1,3] However, because the amount of water in dough is limited, the starch granular identity is largely retained.[4] Gluten polymerization during baking gives rise to a continuous, permanent gluten network.[1] During cooling of bread, changes in the amylose fraction of starch result in a permanent, partially crystalline network. This network and the thermoset gluten network are responsible for the resilience of freshly baked bread.[1]

The interactions of the flour polymers with water reduce water mobility and result in different molecular mobilities in both dough and bread.[5−9] The molecular mobility of water and biopolymers in food products can be studied with proton nuclear magnetic resonance (^{1}H NMR). ^{1}H NMR detects both longitudinal or spin−lattice relaxation times (T_1) and transverse or spin−spin relaxation times (T_2) of protons in a magnetic field. The longitudinal and transverse components occur simultaneously in a relaxation process. Depending on the water level in the system, the used resonance frequency and the type of NMR measurements [free induction decay (FID) and/or Carr−Purcell−Meiboom−Gill (CPMG) pulse sequence], different proton populations can be distinguished.

Low-resolution (LR) ^{1}H NMR has been mainly used to measure T_2 relaxation times in flour polymer model systems, dough, and bread. Different proton populations arise due to the different environments the protons occur in. For instance, in a starch−water system, protons inside a starch granule have a lower mobility and, therefore, lower relaxation times than protons outside the granule.

Starch is the main constituent of wheat flour. A number of LR ^{1}H NMR studies have dealt with changes in *starch model systems* during thermal treatment. Tang et al.[10] studied the molecular distribution of water in potato starch granules (water content of ca. 55%) with ^{1}H NMR. By using the CPMG pulse sequence, they identified four proton populations with different T_2 values, which they assigned to water populations of various mobilities. When the temperature was raised, the relative areas of the proton populations with intermediate mobility changed due to granule swelling and amylose leaching.[11] Similar results were described for cassava,[12] maize,[13] and rice[14] starches. Choi and Kerr[15] studied wheat starch gels (water content = 60−75%) and, using the CPMG pulse sequence, detected two different water fractions. The fraction of lower mobility was assigned to granule remnants, whereas that of higher mobility was assigned to the intergranular starch gel. Similar results were obtained and interpretations given by Wang et al.[9] for wheat starch−gluten model systems (water content = 62−72%) of different gluten contents. Wheat starch−gluten gels had higher relaxation times than wheat starch gels, which indicated less

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swelling and water absorption by the starch granules in the presence of gluten as a result of competition for water.

Whereas much research has been performed on starch, studies on the molecular mobility in *gluten−water systems* are less abundant. In one study, Ablett et al.[16] postulated that the proton mobility of gluten is retained after heat treatment, even though its rheological properties had changed. Wynne-Jones and Blanshard[17] reported that a heated gluten gel has a higher water-binding capacity than a starch gel.

NMR has also been used for analyzing water mobility in *dough*,[8,18,19] during the different stages of the *breadmaking process*,[5] and in *bread crumb*,[7,9,17,20−23] but the complex matrix with its different biopolymers makes discrimination between different proton populations challenging.

Engelsen et al.[5] and Curti et al.[7] found three proton populations in bread crumb, which they tentatively assigned to protons of water in association with gluten and gelatinized starch and to more mobile exchanging protons (observed with a FID−CPMG pulse sequence and CPMG pulse sequence, respectively). Wang et al.[9] observed two proton populations in bread crumb using the CPMG pulse sequence and assigned them to water protons of different mobilities. Sereno et al.[23] found only one main proton population (observed with the CPMG pulse sequence) in the crumb of baguettes, reflecting the fast exchange between the hydroxyl protons of water and those of starch and the restricted water mobility within the gluten−starch polymer matrix.

To deepen our understanding of the breadmaking process, it is essential to understand the changes in proton mobility of the major flour biopolymers starch and gluten during baking. However, despite much research already done in the field, the microstructural complexity of starch granules and starch gels makes it difficult to fully assign the NMR signals to subgranular compartments or to either amylose or amylopectin. In addition, the proton distribution and changes in proton mobility of a gluten gel during thermal treatment remain to be elucidated.

Against this background, the aim of this study was to further refine the identification of the proton populations in unheated and heated starch, gluten, and flour model systems. Both the FID and CPMG pulse sequence were used to study the fast and more slowly relaxing protons, respectively. On the basis of the obtained information, the complex spectra of crumb and crust from fresh bread were interpreted.

MATERIALS AND METHODS

Materials. Wheat flour (Surbi) (13.4% moisture, 71.5% starch, 11.1% protein) was provided by Dossche Mills (Deinze, Belgium). Wheat starch (11.5% moisture, 25% amylose, 0.2% proteins) and wheat gluten (8.0% moisture, 78.0% protein, 10.6% starch, 0.5% arabinoxylan) were from Tereos Syral (Aalst, Belgium). Normal maize starch (11.8% moisture, 22% amylose), waxy maize starch (11.6% moisture, 1.0% amylose), and high-amylose maize starch (12.1% moisture, 66% amylose) were from Cargill (Vilvoorde, Belgium). Moisture content was determined according to AACC Method 44-15.02.[24] Starch and arabinoxylan contents were determined by the gas−liquid chromatographic procedure described by Courtin and Delcour.[25] Protein content was determined using an adaptation of the AOAC Official Method 990.03[26] to an automated Dumas protein analysis system (EAS VarioMax N/CN, Elt, Gouda, The Netherlands), with 5.7 as the nitrogen to protein conversion factor. Amylose content was determined according to the Megazyme (Bray, Ireland) procedure, which itself is based on that of Yun and Matheson.[27] All reagents, solvents, and chemicals were of analytical grade and obtained from Sigma-Aldrich (Bornem, Belgium) unless indicated otherwise.

Preparation of Model Systems. Wheat and normal, waxy, and high-amylose maize starches, gluten, wheat flour, and dough model systems with a water content of 47% (representing a typical bread dough moisture content) were analyzed with LR ^{1}H NMR before and after heat treatment (10 min at 110 °C) in an oil bath. The dough model system contained flour, sugar, salt, and water in the same proportions as in the bread recipe described below. A starch−gluten (85:15 w/w) model system with a water content of 47% was also produced. The samples were heated in NMR tubes. Three sample tubes per model system were analyzed. The heated samples were allowed to cool for at least 120 min prior to analysis.

To distinguish between exchanging protons and nonexchanging (CH) protons, dideuterium oxide (D_2O) experiments were carried out. Because ^{2}H cannot be analyzed by ^{1}H NMR, only the nonexchanging CH protons give a signal.[10,12] Wheat starch−, gluten−, and flour−D_2O mixtures were made in the same proportions as in the model systems with a water content of 47%. Deuterium-exchanged starch, gluten, and flour were prepared by adding excess D_2O, stirring the samples overnight, and subsequent freeze-drying. This cycle was repeated three times. To correct for the impact of the exchange method on the proton distribution of the sample, a control experiment was performed with water instead of D_2O.

Breadmaking Procedure. Bread was made according to a straight-dough method[28] for 100 g of flour. Dough ingredients [100.0 g of wheat flour (14.0% moisture), 5.3 g of compressed yeast (Bruggeman, Ghent, Belgium), 6.0 g of sucrose, 1.5 g of NaCl, 57.0 mL of water] were mixed for 4 min in a 100 g pin mixer (National Manufacturing, Lincoln, NE, USA) at 25 °C and fermented in a fermentation cabinet (National Manufacturing) for 90 min (30 °C, 90% relative humidity) with intermediate punching at 52 and 77 min and final punching at 90 min using a dough sheeter (National Manufacturing). Subsequent to molding and proofing (36 min at 30 °C, 90% relative humidity), dough was baked at 215 °C for 24 min in a National Manufacturing rotary hearth oven. After cooling for 120 min, three samples, each from the crumb center of a different bread, and three samples from the top center crust of different breads were placed in separate NMR tubes.

^{1}H NMR Measurements. Measurements of proton distributions in model systems and fresh bread were performed with a Minispec mq 20 low-field pulsed NMR spectrometer (Bruker, Ettlingen, Germany), with an operating resonance frequency of 20 MHz for ^{1}H (magnetic field strength of 0.47 T). The probe head was kept at 25 ± 1 °C. An external water bath was used to maintain the desired temperature. In this study, only T_2 values were studied. The transverse relaxation curves were acquired by using a single-pulse (signal after a 90° pulse, FID) and the CPMG pulse sequence.[29,30] The lengths of the pulses were 2.86 and 5.42 μs, respectively. For the FID signal, an acquisition period of 0.5 ms was used and 500 data points were acquired. The CPMG sequence had a pulse separation of 0.1 ms and 2500 data points were collected. For both measurements, a recycle delay of 3.0 s was used and 32 scans were accumulated to increase the signal-to-noise ratio.

Samples of the unheated model systems, crumb, and crust (approximately 0.3 g) were placed in three Bruker NMR tubes (10 mm external diameter), tightly compressed to a height of 8 mm to avoid large air holes and sealed to prevent moisture loss. Each tube was analyzed three times to confirm the reproducibility of the measurements, and the exact sample weight of each tube was taken into account. Following heat treatment of the model systems (10 min at 110 °C in an oil bath and cooling for at least 120 min), the same tubes were analyzed again in triplicate.

The CONTIN algorithm of Provencher[31] (software provided by Bruker) was used to transform the transverse relaxation curves with an inverse Laplace transformation to continuous distributions of T_2 values. With the FID experiments, less mobile protons are analyzed (FID proton populations), whereas the more mobile protons are detected with the CPMG pulse sequence (CPMG proton populations). The areas under the curves of the populations with certain T_2 values are proportional to the number of protons in the population. For the model systems and bread, the inhomogeneity of

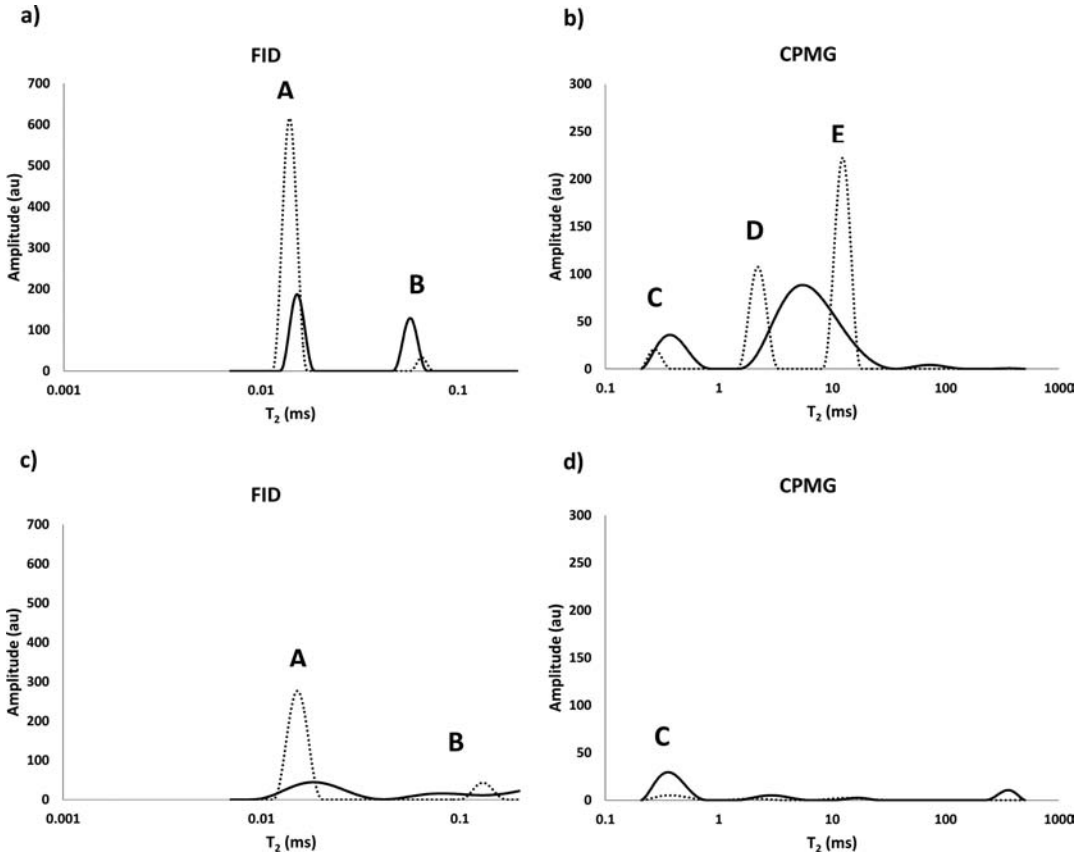

Figure 1. Free induction decay (FID) (a) and Carr–Purcell–Meiboom–Gill (CPMG) (b) proton distributions of unheated (···) and heated (——) wheat starch–water model systems (water content = 47%) and FID (c) and CPMG (d) proton distributions of unheated (···) and heated (——) wheat starch–D_2O model systems (D_2O content = 47%) obtained by the inverse Laplace transformation of the FID and CPMG pulse sequence. Amplitudes are given in arbitrary units (au). The different proton populations are indicated with capital letters in order of their increasing mobility.

the static magnetic field affected the output for the most mobile FID population (around 0.5 ms). Therefore, this fraction was omitted in the following analyses.[11,30,32]

Differential Scanning Calorimetry (DSC) Measurements. DSC was performed with a Q1000 DSC (TA Instruments, New Castle, DE, USA). Unheated and heated model systems and bread crumb were accurately weighed (2.5–4.0 mg) in aluminum pans (Perkin-Elmer, Waltham, MA, USA). Deionized water was added in a ratio of 1:3 w/w dry matter sample/water. The pans were hermetically sealed and equilibrated at 0 °C before heating from 0 to 120 °C at 4 °C/min (together with an empty reference pan). Before analysis, the system was calibrated with indium. The temperatures and enthalpies corresponding to the melting of amylopectin crystals (gelatinization) were evaluated from the thermograms using Universal Analysis software (provided by TA Instruments). Enthalpies were expressed in joules per gram of sample (on dry matter basis). All samples were analyzed in triplicate.

RESULTS AND DISCUSSION

Model Systems. *Starch–H_2O.* For the unheated starch–water sample, the presence of two FID (Figure 1a) and three CPMG (Figure 1b) proton populations was deduced from the relaxation signals. Of these, two populations predominated. The first one, that is, the FID population with lowest T_2 (population A in Figure 1a), consisted of rigid nonexchanging starch CH protons, because it was still present after water was exchanged with D_2O (population A in Figure 1c). We reasonably assume that CH protons in a rigid crystal structure and in amorphous starch not in contact with water have the lowest mobility and are represented in this FID peak. The second abundant proton population was the one represented in the third CPMG peak (population E in Figure 1b). These protons had the highest mobility and included hydroxyl protons of water and starch (as this population was of very low intensity in the starch–D_2O sample, Figure 1d) present in a mobile environment. The T_2 of this population was much smaller than that of free water, which has a T_2 of 2–3 s. We therefore suggest this population to consist of water protons that exchange with starch hydroxyl protons in the extragranular space (Figure 1b). The second and less abundant CPMG population (population D) can be attributed to protons in a less mobile environment, such as confined water protons that exchange with starch hydroxyl protons in the intragranular space. Similar differences in properties between protons inside and outside the starch granule in unheated starch–water samples have been suggested earlier for rice and potato starches.[10,11,14] In contrast to Tang et al.,[10,11] who studied starch–D_2O model systems and assigned the populations to starch CH protons, we are of the opinion that exchanging OH protons and nonexchanging CH protons are detected simultaneously in starch–water samples. In this context, we compared the starch–D_2O model systems with the starch–water model systems to assign the peaks to different CH and OH proton populations, depending on the environment the protons occur in. Ritota et al.[14] assigned the proton populations in rice starch–water model systems to both OH and CH protons, but they used only the CPMG pulse sequence to analyze the proton populations.

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Heating and cooling of the sample decreased the area of population A by an amount of 9600 arbitrary units (au) (Figure 1a), whereas the areas of population B (Figure 1a) and population C (Figure 1b) increased by amount of ca. 2300 and 870 au, respectively. D_2O measurements showed these last two populations to also contain nonexchanging CH protons (Figure 1c,d). We assume they represent CH protons in amorphous parts of the granule, as will be discussed further. The fact that population B (FID) and population C (CPMG) were affected in the same way by heat treatment indicates that they represented the same protons. As a result of heat treatment, the starch sample was fully gelatinized, as shown with DSC (results not shown). Starch gelatinization involves melting of amylopectin crystals and amylose leaching to the extragranular space.[3,33,34] We observed a linear relationship between the decrease in DSC gelatinization enthalpy and the decrease in area of population A as a result of the heat treatment (Figure 2). The smaller area of population A and the larger area of

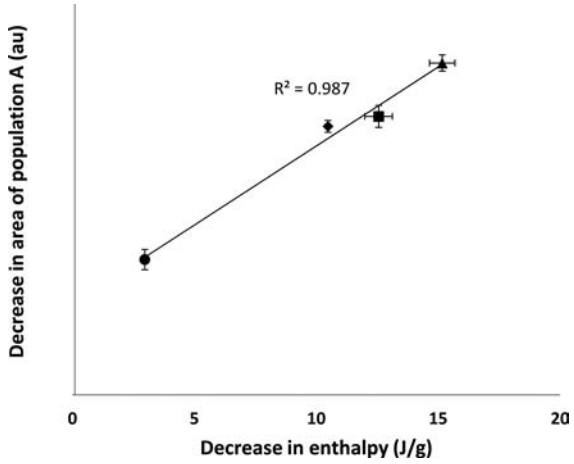

Figure 2. Decrease in area of the first free induction decay population (population A), representing solid CH protons of amylopectin crystals and densely packed amorphous starch not in contact with water (measured with nuclear magnetic resonance) and decrease in gelatinization enthalpy (measured with differential scanning calorimetry) during heating (10 min at 110 °C) are linearly related (R^2 = 0.987) for a high-amylose maize starch (●), a normal wheat starch (◆), a normal maize starch (■), and a waxy maize starch (▲) model system (water content = 47%). Amplitudes are given in arbitrary units (au).

population B (Figure 1a) and, thus, population C (Figure 1b) in the heated sample, therefore, likely resulted from melting of amylopectin crystals, on the one hand (Figure 2), and the increased portion of more mobile amorphous starch (population B or C in Figure 1) in stronger interaction with water molecules, on the other hand.

Figure 1b shows that, after heating and subsequent cooling, a distinction between the two mobile CPMG populations (populations D and E) can no longer be made. Gelatinization-associated phenomena, which include water uptake by the granules, destroy their original structure and lead to a faster exchange of water protons with those of starch. In this view, the large and broad CPMG population (merging of populations D and E) can be assigned to hydroxyl protons of starch and water in the gel network containing the granule remnants. Their reduced mobility was caused by more intense contact between water and starch and, thus, by the increased viscosity[3,35] as a result of the heating process. At the same time, starch gelatinization increased the mobility of at least a substantial portion of the rigid CH protons. After heat treatment, a rigid starch fraction was detected (population A, Figure 1a), which may well represent CH protons of amorphous starch that remained immobile after heating and/or those of newly formed amylose crystals. Indeed, because the samples were allowed to cool for at least 120 min before NMR measurements were carried out, because the used starch concentration was relatively high, and because the starch had been fully gelatinized as shown with DSC, it is very well possible that some amylose had already crystallized during cooling. Our results on wheat starch are generally in agreement with the findings concerning proton mobilities in the case of potato,[11] cassava,[12] and rice[14] starches.

To further elucidate the role of the starch polymers amylose and amylopectin, NMR spectra of waxy maize starch (containing no amylose) and high-amylose maize starch (containing 66% amylose) were analyzed.

The proton distributions before and after heat treatment for a normal maize starch−water model system with the same concentration were similar to those observed for wheat starch (Figure 3a,b). Panels c and d of Figure 3 show the proton distributions of an unheated and heated waxy maize starch−water model system (water content = 47%). The changes in CH proton populations due to gelatinization and gelation were more pronounced in waxy maize than in normal maize starch (Figure 3a,b). This can be related to the virtual absence of amylose in waxy maize starch, which resulted in more amylopectin crystal melting. These differences led to a stronger decrease in the area of population A (by an amount of 11900 au compared to 10000 au for normal maize starch, Figure 2) and a stronger increase in that of population B (by an amount of 2800 au compared to 2200 au for normal maize starch) or population C (by an amount of 1600 au compared to 1300 au for normal maize starch). In contrast to what had been observed with normal starch, the two mobile hydroxyl proton populations D and E were not detected as one broad merged population after heating (Figure 3d). Waxy maize starch granules have a higher swelling power and disrupt more easily during heating[36] than those of normal maize starch. The gelatinized and well-dispersed amylopectin had hydroxyl protons of both high (population E) and low mobility (population D) (Figure 3d). Remarkably, the bulk of the hydroxyl protons in gelatinized waxy maize starch had higher mobility than the bulk of the corresponding protons in gelatinized normal maize starch. This points to a clear impact of amylose as a structure-building and, hence, mobility-reducing entity in the gelatinized normal starch system. Panels e and f of Figure 3 show the proton distributions of the unheated and heated high-amylose starch−water model system (water content = 47%). The applied heat treatment and subsequent cooling resulted in a decreased area of population A (Figure 2) and a concomitant increased area of population B and, thus, population C. It is of note that the proton profiles of the heat-treated and cooled high-amylose starch−water system resembled more closely those of normal starch than those of the waxy starch−water system, again pointing to a crucial role of amylose as a structure builder. Indeed, as for normal starch, the two mobile CPMG populations (populations D and E) were no longer separated after heating. The mobility of the broad merged hydroxyl proton population after heating was lower than that for normal starch (Figure 3b). Apart from the above, the different amylose/amylopectin ratio clearly affects

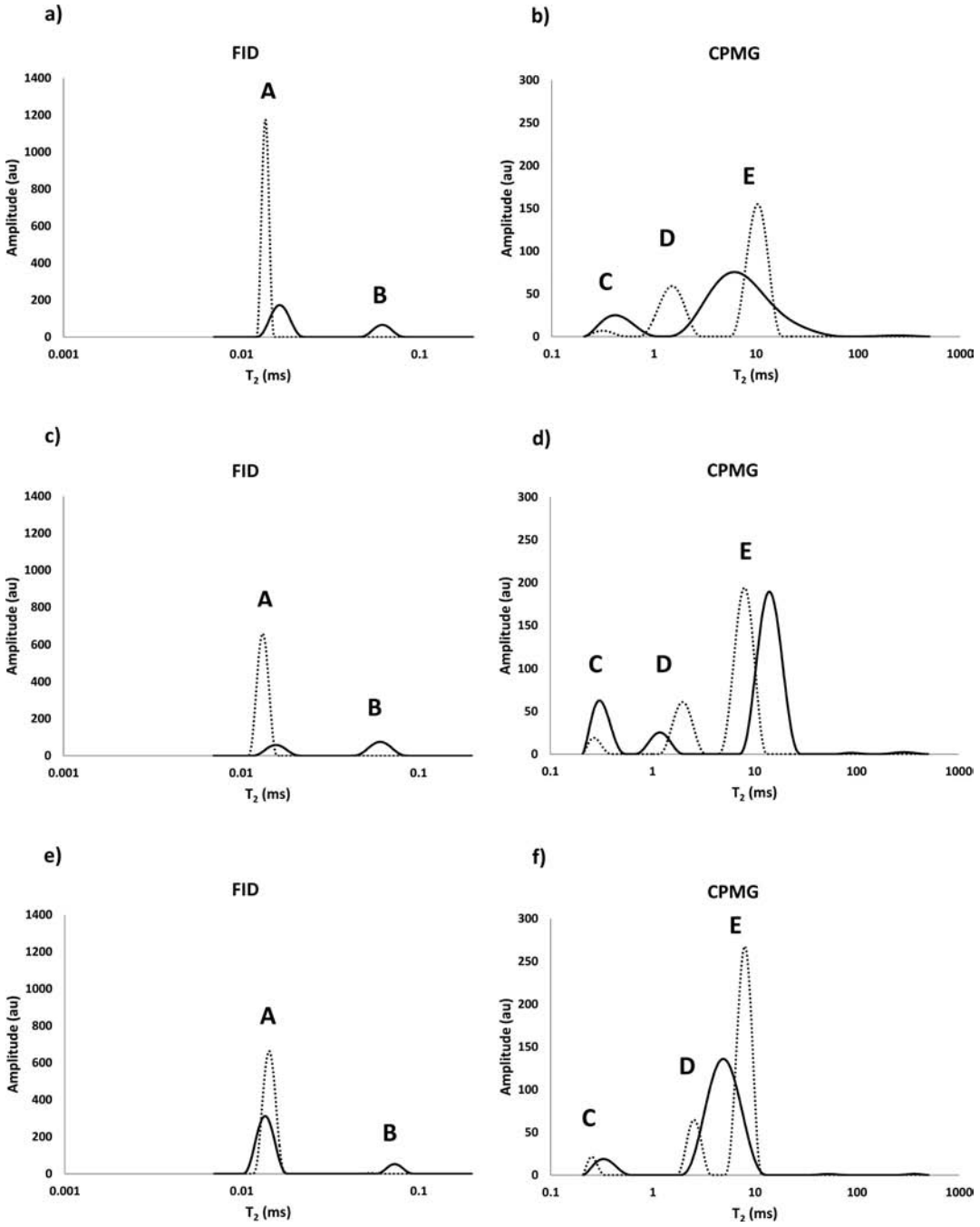

Figure 3. Free induction decay (FID) (a) and Carr–Purcell–Meiboom–Gill (CPMG) (b) proton distributions of unheated (···) and heated (——) normal maize starch–water model systems, FID (c) and CPMG (d) proton distributions of unheated (···) and heated (——) waxy maize starch–water model systems, and FID (e) and CPMG (f) proton distributions of unheated (···) and heated (——) high-amylose maize starch–water model systems (water content = 47%) obtained by the inverse Laplace transformation of the FID and CPMG pulse sequence. Amplitudes are given in arbitrary units (au). The different proton populations are indicated with capital letters in order of their increasing mobility.

the gelatinization process.[37,38] Nevertheless, the lower amylopectin level resulted both in a smaller decrease in area of proton population A (by 4800 au compared to 10000 au for normal maize starch, Figure 2) and in a less pronounced increase in area of population B (by 1500 au compared to 2200 au for normal maize starch) or population C (by 260 au instead of 1300 au for normal maize starch) (Figure 3a,b). The higher level of ordered amylose that could be formed during cooling also contributed to the higher area of population A after cooling (68% of the population remained instead of 36% for normal maize starch).

Gluten–H_2O. The unheated gluten–water sample had two distinct proton populations in the FID spectrum (populations A and B, Figure 4a) and three distinct proton populations in the CPMG spectrum (populations C, D, and E, Figure 4b). These included two predominant proton populations, one with rigid protons not in contact with water (population A) and one with mobile protons in strong interaction with water (population E). Exchange experiments with D_2O suggested that population A contained rigid nonexchanging CH protons (Figure 4c). These CH protons were possibly present in gluten chains in strong interaction with each other (possibly

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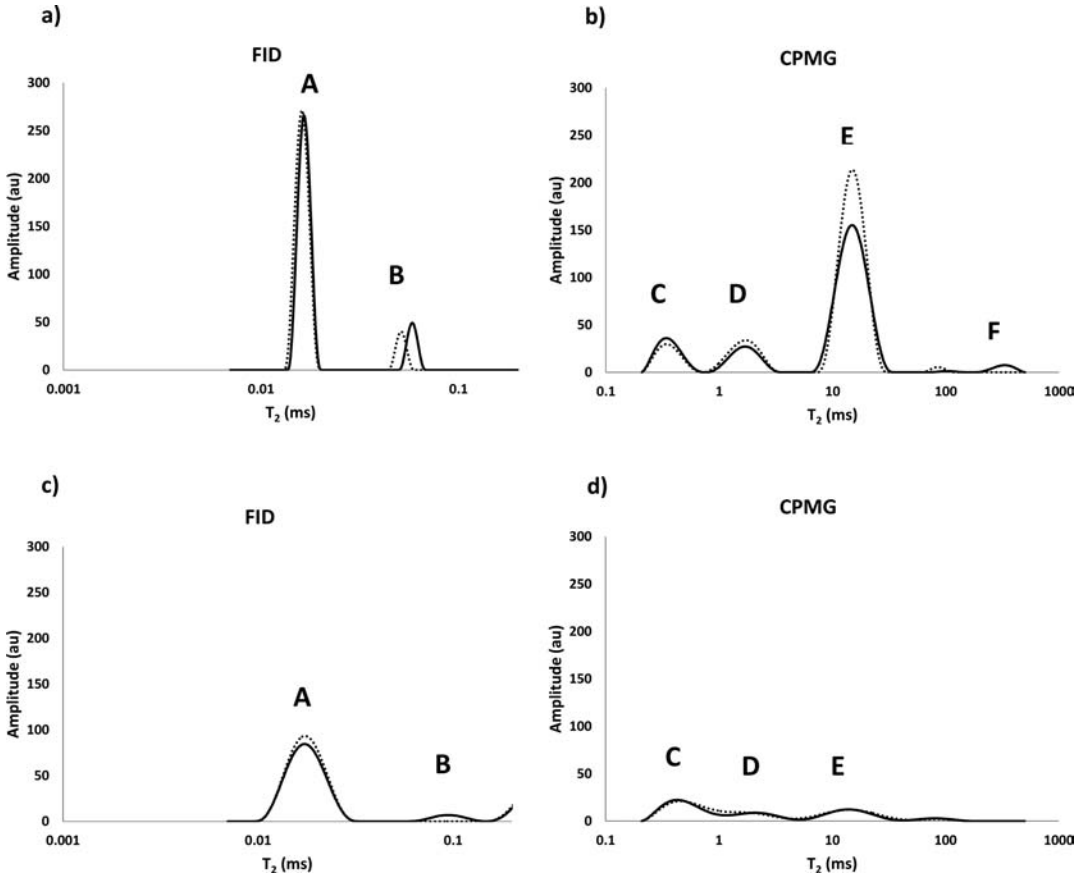

Figure 4. Free induction decay (FID) (a) and Carr–Purcell–Meiboom–Gill (CPMG) (b) proton distributions of unheated (···) and heated (——) gluten–water model systems (water content = 47%) and FID (c) and CPMG (d) proton distributions of unheated (···) and heated (——) gluten–D_2O model systems (D_2O content = 47%) obtained by the inverse Laplace transformation of the FID and CPMG pulse sequence. Amplitudes are given in arbitrary units (au). The different proton populations are indicated with capital letters in order of their increasing mobility.

hydrophobic parts of gluten and regions with many gluten–gluten interactions). Population E largely consisted of exchanging protons, as it was of very low intensity in the gluten–D_2O sample (Figure 4d). These exchanging protons originate from water and from gluten functional groups (such as –NH, –OH, –SH[9]) in a more mobile environment such as on the outside of the gluten network (bulk phase). D_2O measurements allowed the conclusion that population B (FID) and populations C and D (CPMG) also contained CH protons (Figure 4c,d). These protons probably interacted with water and therefore had a higher mobility than the CH protons not exposed to water in population A. Population D most likely contained not only a small population of nonexchanging CH protons but also exchanging protons, because its area in the gluten–D_2O sample was smaller than that in the gluten–water sample (Figure 4d).

The molecular interactions of gluten with itself and with water have been described in a model by Kontogiorgos,[39] which extends an earlier one by Belton.[40] According to Kontogiorgos,[39] gluten strands are organized in parallel "sheets" with 5 nm spacings between. These spaces are filled with confined water molecules. The gluten sheets are surrounded by bulk water, which evidently has a higher mobility.

On the basis of this model, we suggest population A to contain rigid CH protons of the gluten strands in the sheets not in contact with water. Possibly, population B (and C) contained CH protons of gluten in the sheets, which are in little contact with water (e.g., CH protons in contact with confined water between the sheets). Population D with intermediate mobility then possibly contains, besides some mobile CH protons of gluten in the sheets in contact with the bulk water fraction, the –NH, –OH, and/or –SH protons of gluten, which can exchange[9] with confined water present between the sheets. The protons of the bulk water itself would then be present in the most mobile third CPMG population (population E). Because the T_2 of this population is still much smaller than that of free water, the bulk water surrounding the sheets also exchanges with the exchanging gluten protons on the outside of the sheets.

Unlike what is the case for other proteins, such as egg white proteins[41] and β-lactoglobulin,[42] which undergo a sol–gel transition during heating with an increase in solid-like component in NMR spectra, heat treatment did not markedly change the proton distribution of the gluten–H_2O sample in this study (Figure 4a,b). Although the structure and rheological properties of gluten changed during heating due to the formation of additional disulfide cross-links and polymerization of gliadin and glutenin,[43,44] these changes did not induce significant changes in the NMR profiles. As a result of heating, the large CPMG population E with exchanging protons of bulk water surrounding the gluten sheets became less intense and more heterogeneous. Also, an additional small population appeared at high T_2 in the CPMG spectrum (population F). These changes can be the result of the expulsion of water from the gluten network due to gluten polymerization, whereby the protons of the expelled water fraction have a higher mobility

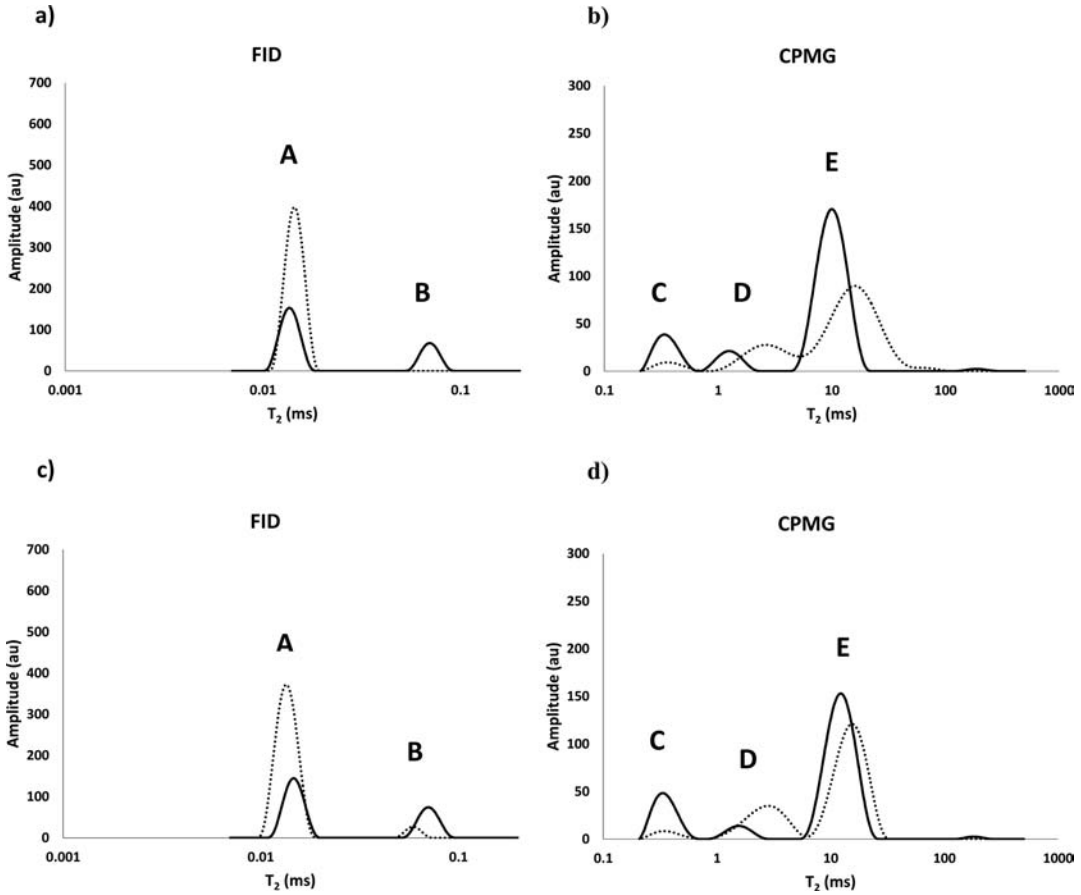

Figure 5. Free induction decay (FID) (a) and Carr–Purcell–Meiboom–Gill (CPMG) (b) proton distributions of unheated (···) and heated (——) flour–water model systems (water content = 47%) and FID (c) and CPMG (d) proton distributions of unheated (···) and heated (——) starch–gluten–water model systems (water content = 47%, starch content = 45%, and gluten content = 8%) obtained by the inverse Laplace transformation of the FID and CPMG pulse sequence. Amplitudes are given in arbitrary units (au). The different proton populations are indicated with capital letters in order of their increasing mobility.

(Figure 4b). That heat treatment has only a limited impact on the mobility of gluten protons has also been suggested by Ablett et al.[16] on the basis of 60 MHz ^{1}H NMR studies of gluten exchanged with D_2O.

Flour–H_2O. For the unheated flour–water mixture, one FID population (population A, Figure 5a) and three CPMG populations (populations C, D, and E, Figure 5b) were detected. As for the unheated starch and gluten model systems, two predominant populations were distinguished containing either rigid protons not in contact with water (population A) or mobile protons in strong interaction with water (population E). Panels c and d of Figure 5 show the FID and CPMG profiles of both unheated and heated starch–gluten–H_2O model systems. Because these profiles were very similar to the ones of the flour–H_2O model system (Figure 5a,b), we interpreted the NMR profiles of the flour model system on the basis of the profiles of starch and gluten model systems. Population A then contained rigid CH protons of starch (amylopectin crystals and densely packed amorphous starch not in contact with water) and rigid CH protons of gluten strands in the gluten sheets not in contact with water. The large CPMG population (population E) was assigned to mobile protons of water in exchange with hydroxyl protons of starch on the granule surface and to water protons surrounding the sheets in exchange with gluten protons. Population C consisted of CH protons of amorphous starch and CH protons of gluten in the sheets in little contact with confined water. Population D then contained hydroxyl protons of intragranular water and starch, but also some CH protons of gluten and exchanging protons of confined water and gluten. In contrast to an earlier study[18] that assigned two FID populations in wheat flour dough to solid protons from gluten and starch, we believe that only the first FID population (population A, Figure 5a) contains rigid protons from starch and gluten, whereas the second FID (population B, Figure 5a) and first CPMG (population C, Figure 5a) populations contained CH protons of amorphous starch and gluten in contact with water molecules. Because we studied both starch and gluten model systems (in water and D_2O), we were able to make a profound interpretation of the flour–water NMR spectra.

Heating and cooling changed the NMR profile of the flour–water mixture (Figure 5a,b). The decrease in area of population A and the increase in area of populations B and C were attributed to starch gelatinization and gelation (cfr. supra). Population A contained mainly CH protons of immobile crystallized amylose and/or amorphous starch and rigid CH protons of gluten, whereas populations B and C consisted of CH protons of amorphous starch and gluten in the sheets in contact with confined water. Population D was attributed to some CH protons of gluten and exchanging protons of confined water, starch, and gluten. The largest CPMG population (population E) consisted of mobile exchanging

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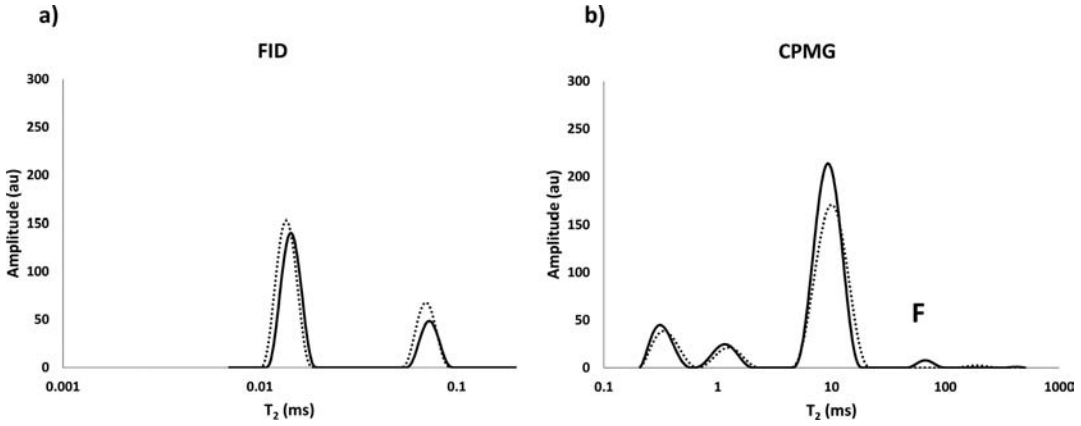

Figure 6. Distribution of transverse relaxation times of fresh bread crumb (——) and a heated flour–water model system obtained by the inverse Laplace transformation of the free induction decay (FID) and Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence. Amplitudes are given in arbitrary units (au).

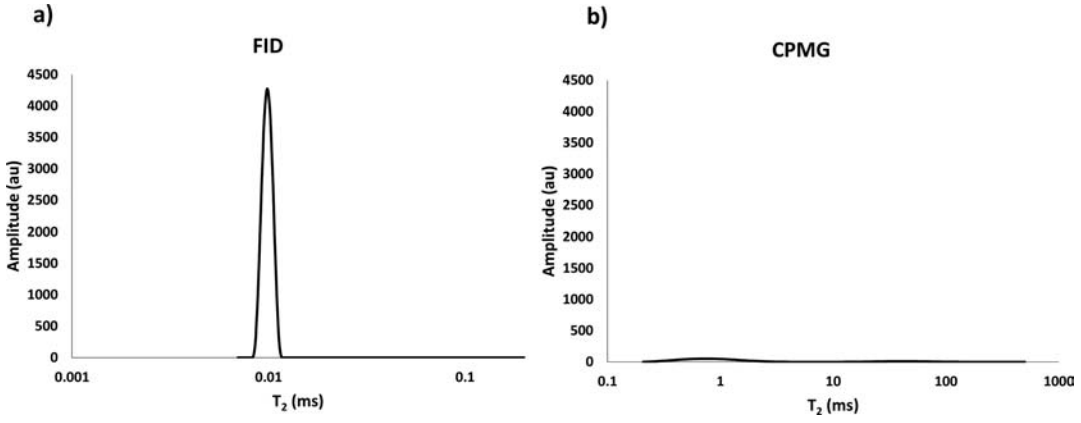

Figure 7. Distribution of transverse relaxation times of fresh bread crust obtained by the inverse Laplace transformation of the free induction decay (FID) and Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence. Amplitudes are given in arbitrary units (au).

protons of water, starch, and gluten present in the formed gel network. The reduced mobility of this population was also observed for the starch model system and attributed to starch gelatinization and gelation. However, the reduction in mobility was not as large as for the starch–water mixture. This was explained by the presence of gluten in flour, because population E did not have a reduced mobility in the heated gluten–water sample (Figure 4b).

The minor levels of nonstarch polysaccharides and lipids in flour seem to have little if any impact on the NMR profiles, given the similarities of the profiles of the flour model system and those of the starch–gluten model system (Figure 5c,d). Also, the addition of salt and sugar in concentrations typically used in breadmaking did not influence the proton distributions to a large extent (results not shown).

Bread. The NMR profiles of *fresh bread crumb* were very comparable to those of the heated flour–water model system (Figure 6). Therefore, population assignment was done in a similar way. With the CPMG pulse sequence of fresh bread, a small additional proton population was detected at high T_2 (population F). This population probably consisted of protons from shortening used to grease the mixing bowls, sheeter, and baking tins, as lipid protons typically appear at higher T_2 values.[19] Native flour lipids[45] were probably also present in this population.

For *fresh bread crust*, one very large FID population was detected, which represented rigid protons. Due to the very low moisture content of the crust, almost all protons were in a rigid environment and therefore present in the population with lowest mobility. However, two very small CPMG populations (compared to the FID population) were also detected. They probably represented mobile exchanging protons (Figure 7). These protons arose from the small amount of water present in the crust, which exchanged with protons of crust constituents.

Changes in proton distributions that occur in starch–, gluten–, and flour–water model systems during heat treatment were investigated. The effect of starch gelatinization was clearly visible in the starch– and flour–water model systems by a decrease in area of the population with rigid protons of starch (crystals and densely packed amorphous starch) and an increase in area of the populations with protons of amorphous starch in contact with water. By studying the changes in proton distribution in waxy and high-amylose maize starch–water model systems, a distinction between protons from amylose and amylopectin was made. Heat treatment did not affect the NMR profiles of the gluten–water model system to a large extent, although the gluten rheological properties had changed. The existence of different proton populations in gluten was related to conceptual models of gluten structure. On the basis of the model systems, an in-depth interpretation of the proton distributions of fresh bread crumb and crust was performed. To

conclude, the developed LR ^{1}H NMR technique holds promise for studying changes during the storage of bread (e.g., water migration from crumb to crust and amylopectin crystallization with concomitant water migration on molecular scale) and the influence of antifirming additives on proton distributions.

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Notes

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ABBREVIATIONS USED

^{1}H NMR, proton nuclear magnetic resonance; FID, free induction decay; CPMG, Carr−Purcell−Meiboom−Gill; LR, low resolution; T_1, spin−lattice relaxation time; T_2, spin−spin relaxation time; D_2O, dideuterium oxide; DSC, differential scanning calorimetry.; au, arbitrary units.

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