JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Molecular Changes in Soy and Wheat Breads during Storage as Probed by Nuclear Magnetic Resonance (NMR)

ALESSIA LODI,† STEFANO TIZIANI,§ AND YAEL VODOVOTZ*,†

Department of Food Science and Technology, Parker Food Science and Technology Building, The Ohio State University, 2015 Fyffe Road, Columbus, Ohio 43210, and CR UK Institute for Cancer Studies, Henry Wellcome Building for Biomolecular NMR Spectroscopy (HWB-NMR), University of Birmingham, Vincent Drive Edgbaston, Birmingham B15 2TT, United Kingdom

Addition of raw ground almond has been shown to improve loaf quality (e.g., loaf specific volume) of soy bread. To better understand the effects of almond addition to soy bread and to follow these through storage, nuclear magnetic resonance spectroscopy relaxation times and cross-relaxation experiments were performed. Spin−spin relaxation times of water protons were similar for the two soy breads, and therefore changes of water interactions with the other components of the soy breads (with and without almond) were not considered to be major contributors to the differences in loaf quality observed between these breads. Additionally, T_2 values of water protons were found to have a similar decreasing trend during storage, especially up to day 3, for all of the products studied. On the other hand, during storage, lipid proton relaxation times exhibited only small changes in wheat and regular soy bread, whereas the soy−almond bread showed a major decrease of lipid proton mobility in particular after day 3 and up to day 10. These findings may indicate that, after a few days of storage, the lipid fraction contributes to better plasticization of the soy bread with almond, which can affect acceptability and storage stability of the final product. Thus, the higher amount of lipids introduced in the almond-enriched soy bread is likely to be responsible for the improved loaf quality and may significantly affect shelf stability of the soy-containing product.

KEYWORDS: Relaxation times; cross-relaxation; bread; lipid; water

INTRODUCTION

Soybeans have a unique nutritional profile that incorporates essential proteins, isoflavones, and other phytochemicals such as saponins, lignans, and phytic acids (*1*, *2*). When these components are consumed together, they function synergistically to reduce the risk of disease (*1*, *2*). However, soy inclusion in the U.S. diet remains relatively low. A strategy involving the incorporation of soy into products commonly consumed in a Western diet represents a viable alternative for increasing soy consumption in such populations. Attempts to adding soy ingredients to bread significantly affect loaf quality, due to increased water absorption (*3*, *4*) and decreased loaf specific volume (*5*−*7*).

Recently, a soy bread of highly acceptable quality has been developed in our laboratory, requiring an adjustment in formulation, and resulting in higher moisture content than traditional wheat bread in the final baked product (*8*). A variant of this soy bread, containing 5% (w/w of dry ingredients) of ground raw almond (referred to henceforth as soy−almond bread), was also formulated with the objective of altering the isoflavone profile of the original product (*9*). The β-glucosidase, naturally present in almonds, hydrolyzes the glycosides, thereby increasing the concentration of the aglycones, which may lead to greater and faster adsorption of isoflavones (*10*) and potentially greater biological efficacy of these phytochemicals.

Various factors need to be considered to assess the impact of soy and almond addition on the quality and stability of baked bread. The higher moisture content, coupled with dilution of gluten with soy protein (*11*, *12*) and a decrease in starch content, is likely to cause changes in water states and distribution in fresh and stored soy bread as compared to wheat bread. These factors have been shown previously to affect staling of baked goods (*13*).

Aside from altering the isoflavone profile, almond addition may affect loaf quality of fresh and stored breads. Almond increases the lipid content of the soy bread by about 2.5% (w/w dry ingredients), because >50% of almond kernels' weight is composed of lipids (distributed as follows among the different forms: saturated, 3.9%; monounsaturated, 32.2%; polyunsaturated, 12.2%). The addition of lipids (in the form of glycolipids or lecithin) and lipid-related surfactants in soy bread has been shown to play an important role in product quality, leading to improved loaf volume (*5*−*7*, *14*, *15*). One hypothesis formulated to explain this behavior deals with the role of lipids in glutenin

* Author to whom correspondence should be addressed [telephone (614) 247-7696; fax (614) 292-0218; e-mail vodovotz.1@osu.edu].
† The Ohio State University.
§ University of Birmingham.

10.1021/jf070636a CCC: $37.00
Published on Web 06/19/2007

and gliadin interactions. The addition of soy proteins to wheat flour was thought to disrupt direct interactions between glutenin and gliadin, whereas upon addition of polar lipids to the wheat−soy system, lipids and soy protein could interact concurrently with glutenin and gliadin to form a stronger network (*16*, *17*).

Therefore, an in-depth molecular characterization was needed to understand the role of water and lipids in the soy−almond bread as compared to the soy bread.

NMR is one of the most powerful techniques to follow dynamic processes and elucidate molecular and conformational structure of both small compounds and macromolecules in food products (*18*). Various magnetic resonance techniques have been applied to study bread and its individual ingredients. For example, solid-state NMR has been used to study hydration of wheat components (*19*), high-resolution magic angle spinning (HR-MAS) techniques were used to characterize flours of various origin (*20*), and magnetic resonance imaging (MRI) experiments were performed to investigate mobility and distribution of water in baked goods (*21*). However, for water and polymer mobility, relaxation times and cross-relaxation techniques are valuable and easily executable experiments able to provide information on water and solid dynamics.

Water mobility was investigated in bread and starch gel systems by means of spin−spin and/or spin−lattice relaxation times (*22*−*27*). Relaxation times, which are time constants of the magnetization vector evolution to equilibrium, correlate to rotational diffusion and are therefore considered to be a good indication of the molecular mobility of compounds, larger values of relaxation times indicating higher mobility. However, it should be pointed out that the interpretation of the water relaxation times results is complicated by the effects of chemical exchange and cross-relaxation with macromolecules such as proteins and carbohydrates and molecular diffusion (*28*). To the best of our knowledge, it appears that investigations of lipid relaxation times, in the baked goods field, have been performed only on glassy breads (*29*) and crackers (*30*). In glassy breads the low moisture content most likely resulted in a water proton signal with very short relaxation times and therefore was most likely not detected. For the crackers samples, at 3.5% moisture content, it was determined that the relaxation times calculated were due to lipid protons (*30*). The authors designed the experiments, by increasing the moisture content of the samples, such that they could determine the moisture level that allows acquisition of both water and lipid proton relaxation times using the CPMG sequence (usually $T_2 > 500$ μs) (*30*). In this study, spin−spin relaxation times of water and lipid in the fresh and stored soy breads were used to probe the effects of the almond addition on the interactions among ingredients and their changes during storage.

Cross-relaxation NMR spectroscopy uses the saturation transfer method and involves perturbing a spin system (represented by the solid component of the sample) and observing changes occurring in another spin system (the liquid component) (*31*). Investigations on changes in starch gels and bread (*32*−*34*) showed that cross-relaxation is suitable to depict changes in rigidity of food macromolecules. A radio frequency pulse is used to irradiate the sample at a variable offset frequency from the observed peak resulting in partial saturation of the immobile protons of the sample. In systems having a significant magnetization transfer rate, saturation transfer phenomena, from the immobile spin system to the observed protons, cause a decrease of the acquired spin system signal intensity. Interaction between the two pools takes place primarily through dipolar coupling (*33*). In bread systems, protons on macromolecules forming bread structure (immobile protons), such as gluten and starch, are partially saturated via radio frequency irradiation of the sample, at an offset frequency from the signal of water protons. The saturation transfer phenomenon from the immobile macromolecules to the water protons causes a decrease of the water signal intensity, which leads to a minimum intensity in correspondence to the null frequency offset.

Z-spectra of carbohydrate systems, obtained from cross-relaxation data by plotting the normalized magnetization intensity versus the frequency offset, were deconvoluted to sums of Lorentzian and/or Gaussian components to better describe changes during storage of components of different rigidity present in the sample (*33*, *34*). For example, starch gel systems analyzed during storage using cross-relaxation spectroscopy were found to have Z-spectra approximated by a single Lorentzian component for the freshly gelatinized system and, upon storage, required the use of combinations of Lorentzian and Gaussian curves to describe the more mobile (narrow component) and highly restricted (broader component) conformations, respectively (*34*). Previous work in our laboratory found that two Lorentzian curves provide the best fit for starch gels (waxy and wheat), gluten, and bread dough, whereas coupled Lorentzian and Gaussian curves best fitted bread Z-spectra (*32*).

These various techniques, in combination, can be used to characterize the molecular changes in soy, soy−almond, and wheat breads during storage and relate these to product quality.

MATERIALS AND METHODS

Sample Preparation. Wheat, soy, and soy−almond bread samples were freshly baked for each set of experiments. The wheat bread samples were prepared in a bread machine (model BBCC-V20, Zojirushi America Corp., Commerce, CA) using the wheat bread program, to reduce variability (preheat time, 40 min; kneading time, 30 min; three rising periods with a stir between them; and baking time, 40 min). The obtained baked loaves were ∼455 g (1 lb). The soy breads could not be baked in this machine due to insufficient mixing of the ingredients and consequent poor quality of the baked product. Therefore, the soy breads were made and baked at The Ohio State University Food Industry Center following a patent-pending process (*8*, *9*). The soy and soy−almond bread ingredients were mixed (at maximum speed in a Hobart mixer) until uniform dough was obtained. The dough was hand-kneaded and proofed at 48 °C and humidity >90% (CM2000 combination module, InterMetro Industries Corp, Wilkes-Barre, PA) for 45 min and baked (jet air oven, model JA14, Doyon, Liniere, QC, Canada) at 160 °C for 50 min. The proofing temperature was previously optimized (*35*, *36*) to obtain maximum conversion of isoflavones to the aglycone form. The soy−almond sample had the same formulation as the regular soy bread with the addition of 5% w/w (dry ingredients) of raw almond (Wild Oats Markets, Inc., Boulder, CO) to the dough mixture. The raw almonds were ground to a fine powder immediately before dough preparation. Bread formulas of the three products are presented in **Table 1**. After baking, loaves were allowed to cool at room temperature for 4 h. One set of experiments was run immediately after finishing sample preparation and constituted the "day 0" data point of the storage study. Further experiments were run at days 1, 2, 3, 6, and 10 of storage. Samples were stored at 4 °C between analyses and allowed to equilibrate at room temperature (∼20 °C) for 2 h prior to analysis. The refrigerated storage conditions were chosen as these have been shown to accelerate staling processes (*37*). The same sample was kept and analyzed at different time points rather than sample a stored loaf (whole) at the different storage times to prevent effects of moisture migration (which is likely to take place in baked products) on water behavior.

Moisture Content. Moisture content of the products (percent, wet basis) after baking was measured by weight difference before and after drying of the samples in a vacuum oven according to AACC method 44-40 (*38*).

Table 1. Formulations and Crude Composition of the Wheat, Regular Soy, and Soy–Almond Breads (Footnotes Include the Manufacturers of Ingredients)

ingredient	wheat bread formulation (%)	soy bread formulation (%)	soy–almond bread formulation (%)
water	37.7	45.35	44.14
soy milk powder[a]		6.61	6.43
soy flour[b]		19.92	19.39
wheat flour[c]	54.3	17.52	17.05
gluten[d]		2.30	2.24
dough conditioner[e]		0.20	0.19
sugar	4.0	4.50	4.38
yeast[f]	0.9	1.00	0.97
salt	1.0	0.90	0.88
shortening[g]	2.1	1.70	1.66
almond powder[h]			2.67

ingredient	wheat bread crude composition (%)	soy bread crude composition (%)	soy–almond bread crude composition (%)
soy protein		27.57	26.15
non-soy protein	16.06	8.61	9.29
sugar	1.14	10.82	10.52
carbohydrate	81.66	48.81	47.36
fat	1.14	4.20	6.68

[a] Soy Milk Powder, Devansoy Farms, Carrol, IA. [b] Baker's Soy Flour, ADM Protein Specialties Division, Decatur, IL. [c] Bakers High Gluten Enriched Bromated Wheat Flour Bleached, General Mills Operations, Inc., Minneapolis, MI. [d] Vital Wheat Gluten with Vitamin C, Hodgson Mill, Inc., Teutopolis, IL. [e] Dough Conditioner, Caravan Products Co., Totowa, NJ. [f] Red Star Instant Active Dry Yeast, Universal Foods Corp., Milwaukee, WI. [g] Crisco All-Vegetable Shortening, 50% less saturated fat than butter, Procter & Gamble, Cincinnati, OH. [h] Raw Almond, Wild Oats Markets, Inc., Boulder, CO.

Specific Loaf Volume. Loaf volume of fresh samples was measured using the rapeseed displacement method (AACC method 10-05) (*38*). Specific loaf volume was calculated from the ratio of loaf volume and weight.

NMR Sample Preparation. NMR tubes (5 mm; Wilmad-Labglass, Buena, NJ) were filled to about 4 cm with the central part of the crumb of each bread sample. The tubes were sealed to minimize moisture loss during storage.

Experimental Condition. All of the NMR experiments were performed on a Bruker DMX 300 MHz (Bruker Biospin, Rhinstetten, Germany) spectrometer, at room temperature.

Relaxation Time Acquisition. Spin−spin relaxation time was calculated from the data acquired using the CPMG (*39*, *40*) pulse sequence. A 90−180 pulse spacing, τ, of 0.5 ms and a 5 s relaxation delay were used, and eight acquisitions were averaged.

Data Analysis. Fourier transform of the obtained time domain data led to spectra containing three very broad and partially superimposed peaks (**Figure 1**). To fully quantify the contribution of each component to the signal, the Padè−Laplace method (discussed under Results) was used to separate the signals. Each of these components was Fourier transformed to obtain the corresponding peak.

The obtained peak intensity data versus $2n\tau$ (n being the echo number) were fit to exponential equations, resulting in the values of T_2.

Cross-Relaxation Spectroscopy. A magnetization transfer experiment, cross-relaxation spectroscopy (*31*, *41*, *42*), was performed on all of the bread samples. In these experiments, saturation transfer techniques were used to investigate magnetization transfer processes between mobile water protons and immobile macromolecules (*43*).

A Gaussian-shaped pulse lasting 3 s was used for partial saturation of immobile protons. The frequency offset of the saturating pulse was swept from −50 to 50 kHz (as referred to the water peak) in 29 experiments (increments of 5 kHz between 50 and 5 kHz and increments of 1 kHz between −5 and 5 kHz). The intensity of the longitudinal magnetization was obtained by acquiring the transverse component after application of a 90° pulse. Z-spectra were constructed by plotting the normalized intensity of the longitudinal magnetization of the water spins, M_z^A/M_0^A, versus the frequency offset used for the saturating pulse (M_z^A and M_0^A are the intensities of the water peak in the presence and absence of the saturating pulse, respectively). Combinations of Lorentzian and/or Gaussian functions were used to fit the Z-spectra data (*32*, *34*). The combination of lineshapes giving the best-fitting result (highest R^2) was chosen for each sample.

All of the data processing was performed using MatLab 6.5 (The MathWorks, Inc., Natick, MA). The code for the Padè−Laplace method was written in-house for the appropriate analysis. A Levenberg−Marquardt algorithm was used for all of the fitting procedures.

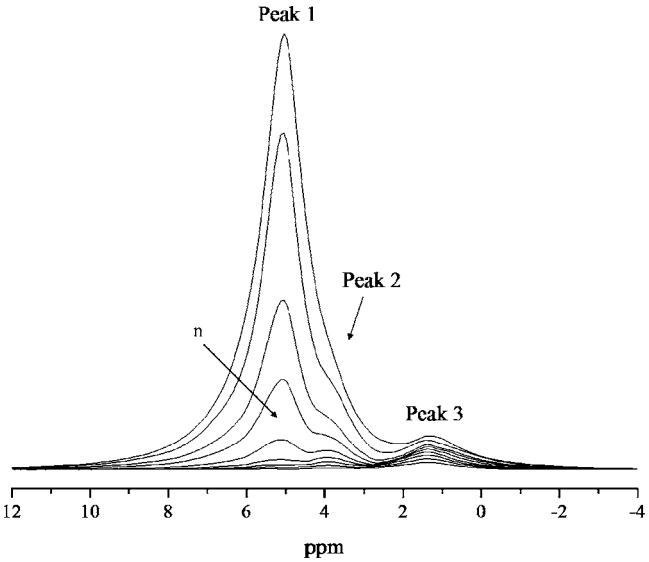

Figure 1. ^{1}H spectra (obtained using CPMG) used in the calculation of the spin–spin relaxation time of soy bread at day 0 of storage (nine spectra at different echo numbers, n, are shown).

Table 2. Moisture Content and Specific Loaf Volume of Wheat, Soy, and Soy–Almond Bread Samples

sample	moisture content (%, wet basis)	specific loaf volume (cm^3/g)
wheat bread	39.3 ± 0.5	4.69 ± 0.05
soy bread	44.8 ± 0.3	2.13 ± 0.06
soy–almond bread	42.8 ± 0.5	2.33 ± 0.05

RESULTS AND DISCUSSION

Moisture content and specific loaf volume of the fresh bread samples are presented in **Table 2**. The wheat bread was lower in moisture content (39.3%) as compared to the soy breads (∼43−45%) due to formulation differences. Loaf volume was much greater for the wheat bread as compared to the soy formulations. However, the soy−almond bread had a slightly (∼9%) greater loaf volume as compared to the regular soy bread.

Due to the significant differences in formulation and preparation between the wheat and soy samples, no direct comparisons are possible between the properties of these samples. However, the wheat bread NMR and loaf attribute results have been included as a reference to a more traditional and well-known bread sample.

Relaxation Times. Fourier transformation of the FIDs (half-echoes) acquired using the CPMG pulse sequence led to the spectra reported in **Figure 1** (only nine spectra of the soy bread samples at day 0 of storage are included for presentation clarity).

Spectra of similar shape were found also for the soy bread samples at different storage times and for all of the wheat and soy−almond bread samples. The presence of three broad peaks can be clearly recognized in **Figure 1**, in particular as the number of echoes, *n*, increases. To evaluate the decay of each single peak, without the influence of the others, the Padè−Laplace method was applied to the acquired time domain decays (half-echoes) to obtain the data of each component (*44*, *45*). Separation of the signals due to the major components present in the system allows unequivocal assignment of decay rates to the corresponding protons.

NMR spectroscopy time domain data can be represented as the sum of an appropriate number, *M*, of exponentially decaying sinusoids, each characterized by specific amplitude and decay constants (*46*), as in eq 1

$$f(t_i) = \sum_{m=1}^{M} A_m \exp(\mu_m t_i) + e(t_i) \quad t_i = 0, \Delta t, 2\Delta t, ..., (N-1)\Delta t \quad (1)$$

where A_m, μ_m, and $e(t_i)$ denote amplitude, decay constants, and noise, respectively, and Δt is the sampling time interval. Each decay is due to a spin system present in the sample under investigation.

One of the great advantages of the Padè−Laplace method is that it is not necessary to input the number of components to be considered, as required with standard fitting procedures (*44*, *45*). As a consequence, when one of the components has completely decayed, it stops appearing as an outcome of the Padè−Laplace calculations. Each exponential decay, obtained from the above treatment, was then separately Fourier transformed, resulting in separate peaks, and pure absorption spectra (*47*) were considered in the calculation of relaxation times.

The decays of the intensity of all three peaks (around 1, 4, and 4.5 ppm) were used to calculate T_2 of each component. R^2 of the exponential fittings of T_2 were ≥0.9931, respectively.

In **Figure 1**, the major peak (peak 1) around 4.5 ppm represents a water proton signal (*48*), peak 2, around 4 ppm (only seen as a shoulder of the water peak in the experiments with small $2n\tau$ values) likely was due to proteins (*19*) and/or starch (*49*) protons, and the lipid signal (*50*) was evident around 1 ppm (peak 3).

In previous studies, sums of a number of exponentials, resulting from multiple components underlying the same signal decay, have been required, in some cases, to obtain appropriate fitting of T_2 data (*22*, *29*). Many authors have also used continuous methods for the analysis of relaxation decays, showing the existence of multiple components for T_2 distributions (*23*, *25*−*27*, *51*). For the calculation of T_2 of our samples, either a single- or a double-exponential decay was found to be suitable to fit the data, for each peak. The exponential fitting was performed using standard nonlinear least-squares procedures instead of the Padè−Laplace method because the data were initially acquired using $2n\tau$ values that are not linearly increasing.

Previous studies performed on starch, gluten, and bread systems reported only relaxation times of water as a way to investigate changes in water mobility in systems of different compositions or during storage (*22*, *23*, *25*−*27*, *51*, *52*). However, most of these papers do not detail whether additional signals, not attributable to water protons but possibly interfering with their signal decay, were observed. Exceptions are the studies by Roudaut et al. (*29*) and Desbois and Lebotlan (*30*), which probed the mobility of lipids in glassy bread and crackers, respectively. In the present study, changes in water and lipid spin−spin relaxation time were investigated as these may reflect the microscopic basis of the different loaf quality attributes of soy and soy−almond breads.

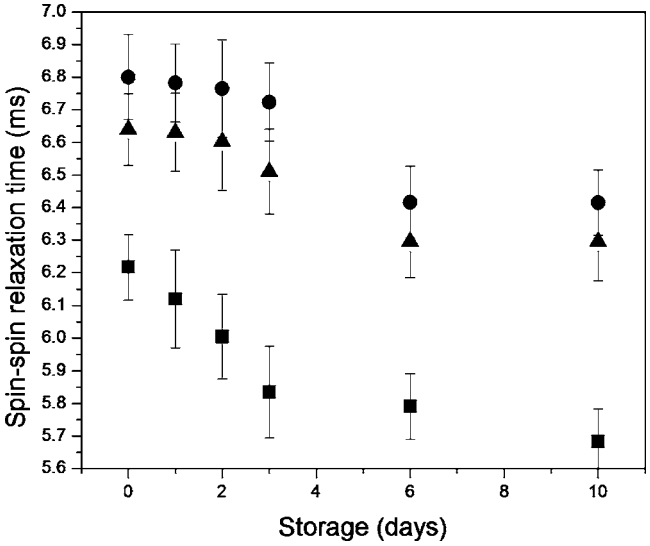

Figure 2. Spin–spin relaxation times of water protons (peak 1 in **Figure 1**) during storage: (■) wheat bread; (●) soy bread; (▲) soy–almond bread.

Changes of relaxation times during storage for the water peak of all the products are shown in **Figure 2**. Spin−spin relaxation times, reported in **Figure 2**, were obtained by fitting the decay of the water peak intensity with a single exponential.

T_2, calculated from the water proton signal decay, resulted in only one component [very short components may have been missed due to the use of CPMG pulse sequence (*30*)]. For all breads, the T_2 decreased during storage, more markedly during the first 3 days of the study. The soy-containing breads resulted in higher values of T_2 [and this can be partly due to higher moisture content, as previously observed in starch−water systems (*53*)] and, during storage, exhibited smaller changes than wheat bread.

Spin−spin relaxation times of the lipid proton component were obtained from the exponential fit of peak 3 data (in **Figure 1**) and are reported in **Figure 3**. For the lipid peak of all the bread samples, magnetization intensity changes at different $2n\tau$ values were best fit by the sum of two components for the calculation of T_2 data.

The T_2 of the longest component of the soy−almond sample was the highest (∼100 ms), as compared to the other breads (∼75 ms), and decreased to ∼70 ms (comparable to T_2 values at the end of the storage period of wheat and regular soy breads), with the greatest decrease occurring after day 3 of storage. For regular soy and wheat breads, the longest T_2 components of lipid protons were very similar throughout the considered storage period (between ∼75 ms, in fresh samples, and ∼65 ms, in breads stored for 10 days). The shortest T_2 components, due to a small spin population for all of the products, were found to be around 4.5 ms for regular soy and wheat formulations and remained almost unchanged during the study. For the soy−almond bread the shortest T_2 component was found to be slightly shorter (between 2 and 3 ms) than that of the other products.

Relaxation times for the third component (peak 2 in **Figure 1**) were found to be ∼20 ms and did not depict changes during storage for any of the considered products and, thus, are not further discussed.

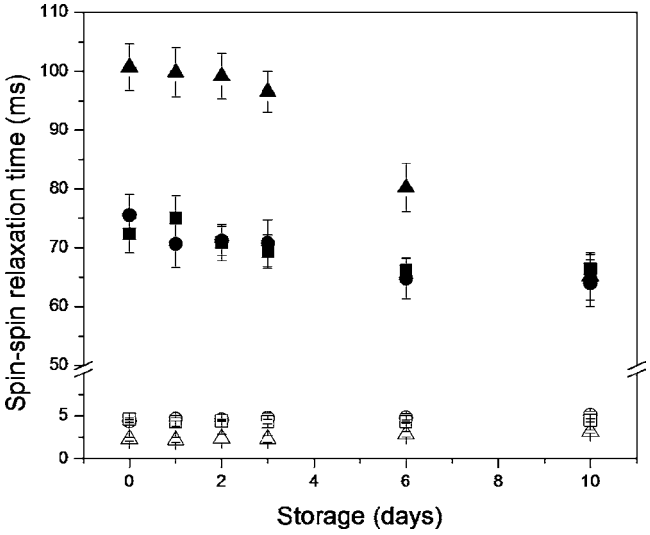

Figure 3. Spin–spin relaxation times of lipid protons (peak 3 in **Figure 1**) during storage: (■) wheat bread; (●) soy bread; (▲) soy–almond-bread; the solid symbols represent the longest and the open ones the shortest T_2 component.

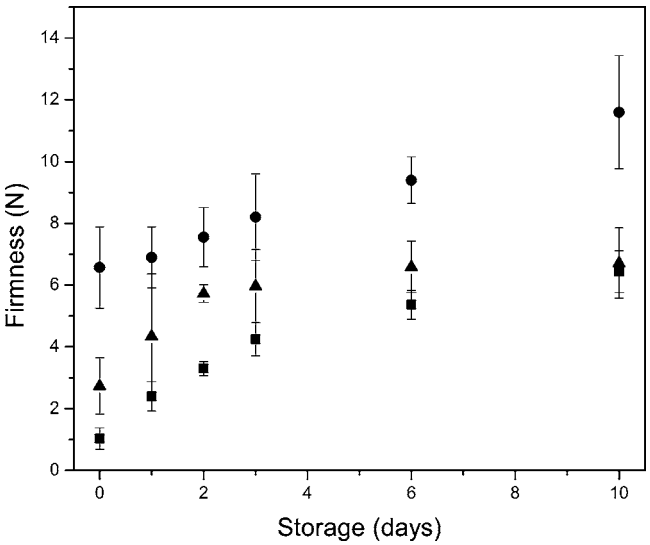

Figure 4. Changes of firmness [maximum compression load (N), as measured using Instron] of wheat (■), soy (●), and soy–almond (▲) breads during 10 days of storage.

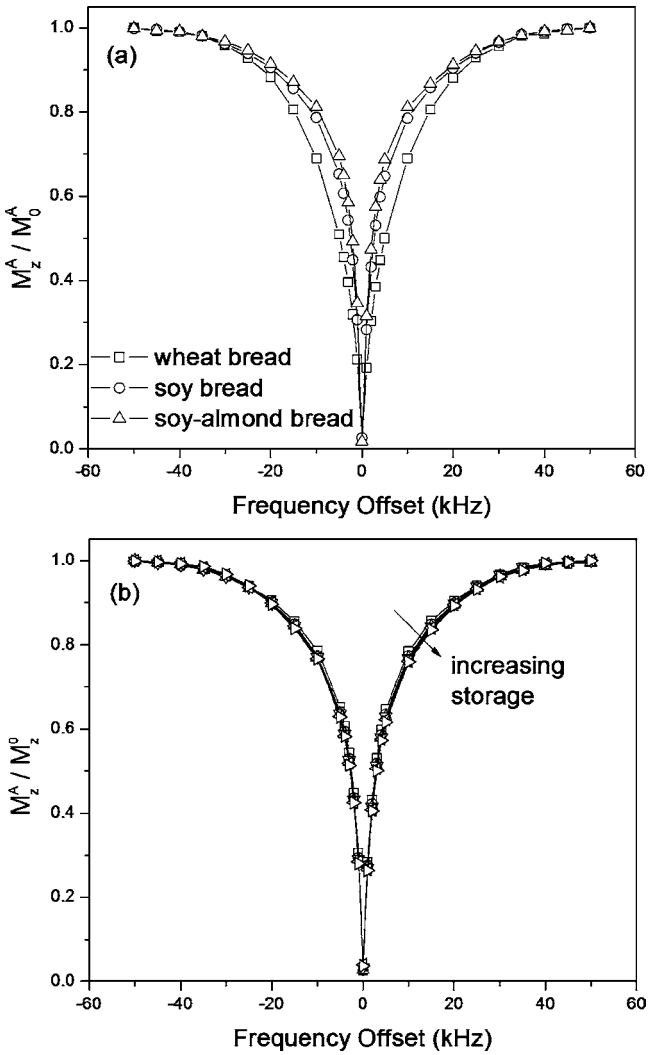

Figure 5. (**a**) Z-spectra (normalized longitudinal magnetization intensity, M_z^A/M_0^A, versus frequency offset) of wheat, soy, and soy–almond breads at day 0 of storage; (**b**) Z-spectra of soy bread during 10 days of storage. Error bars were not included in the plots for better visualization (maximum standard deviation value < 0.011).

Changes in relaxation times of water in various systems of protein and/or carbohydrates in mixture with water have been related to different states of water binding (*22*−*27*). However, it is well recognized that the value of relaxation times can be affected not only by rotational diffusion (which can be correlated to mobility) of water molecules but also by chemical exchange and cross-relaxation processes between water and macromolecules (proteins or carbohydrates) and translational diffusion of water molecules (*28*), which complicate the interpretation of the observed variations in complex systems (such as foods). Relaxation times in the two soy bread samples under analysis, which have very similar compositions, were not found to be significantly different and decreased slightly during storage. Further investigations (e.g., using deuterium or ^{17}O-labeled samples) may have provided a rationale for these trends; however, the main objective of this study was to investigate changes in interactions among ingredients that induce changes in loaf attributes in the products with and without almond. The outcomes of the relaxation times indicate that water is not likely to be responsible for the improved loaf quality of almond-enriched soy bread; therefore, further investigations were not warranted.

Changes of lipid proton mobility during storage were found to provide better differentiation between the soy breads with and without almonds. Although lipid proton relaxation times are expected to be different between the two soy breads (due to the addition of almond to one formulation, which causes different lipid profile composition and about 2.5% higher lipid content in almond-enriched bread), trends of relaxation times during storage in these products were investigated because these may elucidate changes in other macroscopic loaf quality attributes (e.g., firmness, **Figure 4**), observed using other techniques [e.g., Instron, dynamic mechanic analysis, (*54*)]. T_2 components (in particular the longest component) depicted very different values of lipid spin−spin relaxation time values and trends during storage between the soy bread and its almond-containing variant. The presence of shortening in both the regular soy and the soy−almond bread and the addition of almond to the latter can be hypothesized to affect the amount of lipids bound by the matrix of the two fresh soy products, as well as the bulk free lipids. The longest T_2 component for lipid protons, related to the more mobile "free" lipids, exhibits much

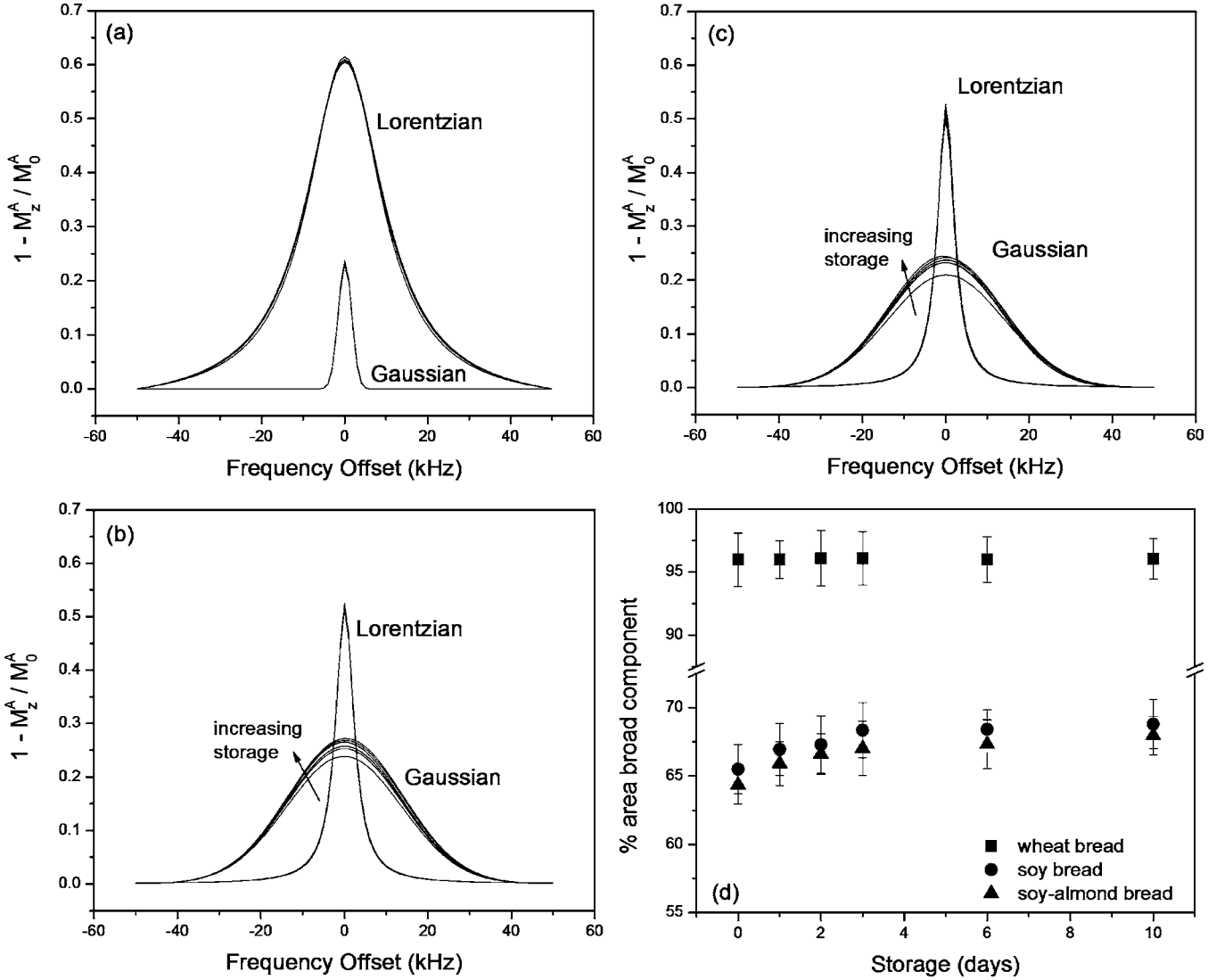

Figure 6. Lorentzian and Gaussian curves resulting from deconvolution of Z-spectra (normalized longitudinal magnetization intensity, M_z^A/M_0^A, versus frequency offset) during 10 days of storage of (**a**) wheat, (**b**) soy, and (**c**) soy–almond breads; (**d**) percent of total area contributed by the broad component obtained from deconvolution of the Z-spectra during storage.

longer initial values for the almond-containing product, indicating that the bulk fat is more mobile in soy−almond bread. The higher mobility of the bulk fraction of lipids in fresh soy−almond bread may additionally contribute to enhanced loaf quality by improving loaf volume (interaction with proteins) (*55*) and the increased plasticizing effect that contributes to reduced firmness, as shown in **Figure 4**.

During storage, the mobility of the lipid proton fraction exhibited a relevant decrease only in the almond-containing soy bread (after day 3 of storage). The final T_2 value was comparable to T_2 values of the other bread samples. It is worth noting that these changes mainly occurred after the relaxation times of water had reached an almost steady value. This fact may indicate that the larger portion of lipids in soy−almond bread prolongs the plasticizing effect of water (by acting on the bread matrix at a later stage) possibly contributing to better retention of freshness (e.g., slower increase in firmness as shown in **Figure 4**). The shortest component of lipid proton T_2, related to more "bound" lipid fraction interacting with the other component of the bread matrix, did not change during storage.

Cross-Relaxation Spectroscopy. Z-spectra of the fresh (day 0 of storage) wheat, regular soy, and soy−almond bread samples are shown in **Figure 5a**. Wheat bread was found to have the broadest line shape as compared to the soy-containing samples. There were little differences between the lineshapes of the two soy breads. The broadness of Z-spectra peaks is considered to be directly proportional to the rigidity of the system under analysis (*34*); therefore, broader lineshapes of the Z-spectra imply higher rigidity of the matrix.

During 10 days of storage only minor changes (a slight decrease of normalized magnetization intensity at longer storage times) were observed in the Z-spectra lineshapes for the wheat and both of the soy bread samples (e.g., soy bread in **Figure 5b**), agreeing with previous findings (*32*). Therefore, a more detailed characterization (by curve deconvolution) was needed to elucidate any changes taking place during storage.

Wu and Eads applied cross-relaxation techniques to the study of retrogradation of starch gels and reported that, although a simple Lorentzian can successfully fit Z-spectra of fresh starch gels, the evolution of the system during storage required the use of sums of Lorentzian and Gaussian curves to properly fit the results of cross-relaxation experiments (*34*). For the complicated bread systems under analysis, deconvolution of the curves required the use of sums of Lorentzian and/or Gaussian curves. The best fits (R^2 values were ≥ 0.9992) were always found using the sum of one Lorentzian and one Gaussian curve.

Examples of curves resulting from deconvolution of Z-spectra are reported in **Figure 6a−c**. Wheat bread lineshapes were best fit with a broad Lorentzian and a narrow Gaussian peak function (**Figure 6a**). However, lineshapes of both the soy bread products were deconvoluted to a broad Gaussian and a narrow Lorentzian (**Figure 6b**,**c**). The deconvolution results (**Figure 6a−c**) reflected a complex solid consisting of one solid-like proton fraction in highly restricted conformation (broader curve) and another that is relatively more mobile (narrower curve) (*32*−*34*). The area under the curve of the broader and narrower components is believed to directly correlate with the amount of the solid proton fraction in each of these conformations (*34*). The width at half-height of each component is considered to be directly proportional to the solid proton fraction rigidity (*34*).

Only very minor changes during storage can be observed for the narrow components alone of deconvoluted spectra for all samples in **Figure 6** [the width at half-height of the narrow component of the deconvoluted curves for the fresh samples was found to be around 4 kHz for the wheat bread and 5 kHz for the soy-based products and remained constant during storage (data not shown)]. Similar results have been previously seen for white bread (*32*). The width at half-height of the broad component was 21 kHz for fresh wheat bread and 32 and 33 kHz for regular soy and soy−almond breads, respectively (data not shown). During storage, a slight increase was observed only for the wheat sample, whereas the soy-based samples maintained almost constant width.

Changes in the relative amounts of the two solid fractions could be evaluated on the basis of variations in peak area. However, a more suitable comparison of the changes occurring in these fractions can be obtained from the percent of the area of the broad (or narrow) component fraction over the total area. Changes during storage of percent of the broad component area under the Z-spectra [$A_{broad} \times 100/(A_{narrow} + A_{broad})$] are reported in **Figure 6d**. The highly restricted solid fraction in wheat bread was much greater (>95% of total area) than that in the soy-based products (<68% of total area) and remained almost constant during the storage period, as found previously (*32*). Regular soy and soy−almond bread showed a slight increase in the percent of the area due to the broad component (from about 63 to 67%) up to day 3 of storage and only minor increases after day 3 and up to day 10. These results indicated an increase in the amount of the highly restricted solid fraction during storage of the soy-based products (however, the rigidity of this fraction did not change). It is worth noting that these changes occurred early during storage as did the decrease of water proton relaxation times (**Figure 2**). The increase in the amount of the highly restricted solid fraction may thus be due to a less efficient plasticizing effect of water on the bread matrix.

From these observations it is also possible to infer that the rigidity of the highly restricted solid fraction in the wheat bread sample increased slightly during storage, whereas the distribution of solid between the two fractions of different mobilities did not change (**Figure 6d**). These observations resulted in general agreement with those made by Wu and Eads (*34*) on a retrograding waxy starch gel and may indicate that amylopectin retrogradation and other microscopic changes in the state of gluten, taking place in wheat bread during storage, underlie these effects.

In summary, ^{1}H spin−spin relaxation times (T_2) of water and lipid protons were monitored during storage of wheat, regular soy, and soy−almond bread samples. Cross-relaxation spectroscopy was also performed to analyze changes in rigidity of the solid matrix of breads. T_2 of water was calculated using a single-exponential decay. The values and profile of T_2 during storage in both of the soy-containing products were not significantly different. Changes in water dynamics and interaction with the other ingredients during storage in these products can thus be hypothesized to be very similar and do not contribute to the differences in loaf quality (especially loaf volume) observed.

Spin−spin relaxation times of lipid protons in wheat and regular soy bread remained almost constant during storage. On the contrary, changes were evident in the soy−almond bread, in particular after day 3 of the storage time studied, which coincided with water proton relaxation times reaching a steady state. Thus, plasticization of the soy−almond bread matrix is first accomplished by water (up to day 3) and then by the lipid fraction. Similarly, Z-spectra, derived from cross-relaxation data, indicated that regular soy bread had slightly higher rigidity (broader peaks) than soy−almond bread. Such plasticization has been shown to delay firming in breads enriched with increased shortening content (*55*).

In conclusion, this study indicated that the higher amount of lipids (about 2.5%), introduced in the soy−almond product by the addition of 5% (w/w, dry ingredients) almond powder might reinforce the plasticizing effect of water, as well as improve loaf quality (loaf specific volume in particular). The added lipid fraction was able to better interact with proteins as well as complement the plasticizing role of water through interactions with the hydrophobic components of the bread matrix. Further work is underway to correlate these findings with other physicochemical properties related to bread staling.

LITERATURE CITED

(1) Erdman, J. W., Jr. AHA Science Advisory: Soy protein and cardiovascular disease: a statement for healthcare professionals from the Nutrition Committee of the AHA. *Circulation* **2000**, *102*, 2555−2559.

(2) Potter, S. M. Soy protein and cardiovascular disease: the impact of bioactive components in soy. *Nutr. Rev.* **1998**, *56*, 231−235.

(3) Ofelt, C. W.; Smith, A. K.; Derges, R. E. Baking behavior and oxidation requirements of soy flour. I. Commercial full-fat soy flours. *Cereal Chem.* **1954**, *31*, 15−22.

(4) Ofelt, C. W.; Smith, A. K.; Mills, J. M. Baking behavior and oxidation requirements of soy flour. II. Commercial defatted soy flours. *Cereal Chem.* **1954**, *31*, 23−28.

(5) Mizrahi, S.; Zimmermann, G.; Berk, Z.; Cogan, U. The use of isolated soybean proteins in bread. *Cereal Chem.* **1967**, *44*, 193−203.

(6) Pomeranz, Y.; Shogren, M. D.; Finney, K. F. Improving breadmaking properties with glycolipids. I. Improving soy products with sucroesters. *Cereal Chem.* **1969**, *46*, 503−511.

(7) Pomeranz, Y.; Shogren, M. D.; Finney, K. F. Improving breadmaking properties with glycolipids. II. Improving various protein-enriched products. *Cereal Chem.* **1969**, *46*, 512−518.

(8) Vodovotz, Y.; Ballard, C. Formula and process for making soy-based bakery products. Patent Appl., Docket 10-267845, 2002.

(9) Vodovotz, Y.; Zhang, Y. C.; Schwartz, S. J. Methods for enhancing soy-containing foods and foods made thereby. Patent Appl., Docket 22727. 2004.

(10) Izumi, T.; Piskula, M. K.; Osawa, S.; Obata, A.; Tobe, K.; Saito, M.; Kataoka, S.; Kubota, Y.; Kikuchi, M. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr.* **2000**, *130*, 1695−1699.

(11) Chen, S. S.; Rasper, V. F. Functionality of soy proteins in wheat-flour soy isolate doughs. 3. Protein and lipid-binding during dough mixing. *Can. Inst. Food Sci. Technol. J.* **1982**, *15*, 302−306.

(12) Fleming, S. E.; Sosulski, F. W. Dough conditioners in wheat-soy-gluten breads. *Can. Inst. Food Sci. Technol. J.* **1974**, *7*, 51−55.

(13) Maga, J. A. Bread staling. *CRC Crit. Rev. Food Technol.* **1975**, *5*, 443−492.
(14) Finney, K. F.; Shogren, M. D. Surfactants supplement each other, make foreign proteins compatible in bread making. *Baker's Dig.* **1971**, *45*, 40−41.
(15) Tsen, C. C.; Hoover, W. J.; Phillips, D. High-protein breads. Use of sodium 2-stearoyl lactylate and calcium 2-stearoyl lactylate in their production. *Baker's Dig.* **1971**, *45*, 20−23, 26, 74.
(16) Aidoo, E. S.; Tsen, C. C. Influence of surfactants or soy proteins on the extractability, gel filtration, and disc electrophoretic patterns of wheat proteins. *Cereal Sci. Today* **1973**, *18*, 302.
(17) Aidoo, E. S.; Tsen, C. C. Surfactant−protein interactions in model systems. *Cereal Sci. Today* **1973**, *18*, 301.
(18) Engelsen, S. B.; Belton, P. S.; Jakobsen, H. J. *Magnetic Resonance in Food Science. The Multivariate Challenge*; The Royal Society of Chemistry: Cambridge, U.K., 2005.
(19) Calucci, L.; Forte, C.; Galleschi, L.; Geppi, M.; Ghiringhelli, S. ^{13}C and ^{1}H solid state NMR investigation of hydration effects on gluten dynamics. *Int. J. Biol. Macromol.* **2003**, *32*, 179−189.
(20) Brescia, M. A.; Sgaramella, A.; Ghelli, S.; Sacco, A. ^{1}H HR-MAS NMR and isotopic investigation of bread and flour samples produced in southern Italy. *J. Sci. Food Agric.* **2003**, *83*, 1463−1468.
(21) Ruan, R.; Almaer, S.; Huang, V. T.; Perkins, P.; Chen, P.; Fulcher, R. G. Relationship between firming and water mobility in starch-based food systems during storage. *Cereal Chem.* **1996**, *73*, 328−332.
(22) Chen, P. L.; Long, Z.; Ruan, R.; Labuza, T. P. Nuclear magnetic resonance studies of water mobility in bread during storage. *Food Sci. Technol.−Lebensm.-Wiss. Technol.* **1997**, *30*, 178−183.
(23) Le Botlan, D.; Rugraff, Y.; Martin, C.; Colonna, P. Quantitative determination of bound water in wheat starch by time domain NMR spectroscopy. *Carbohydr. Res.* **1998**, *308*, 29−36.
(24) Roudaut, G.; Maglione, M.; Van Dusschoten, D.; Le Meste, M. Molecular mobility in glassy bread: a multispectroscopy approach. *Cereal Chem.* **1999**, *76*, 70−77.
(25) Ruan, R. R.; Wang, X. A.; Chen, P. L.; Fulcher, R. G.; Pesheck, P.; Chakrabarti, S. Study of water in dough using nuclear magnetic resonance. *Cereal Chem.* **1999**, *76*, 231−235.
(26) Wang, X.; Choi, S. G.; Kerr, W. L. Effect of gluten content on recrystallisation kinetics and water mobility in wheat starch gels. *J. Sci. Food Agric.* **2004**, *84*, 371−379.
(27) Wang, X.; Choi, S. G.; Kerr, W. L. Water dynamics in white bread and starch gels as affected by water and gluten content. *Food Sci. Technol.−Lebensm.-Wiss. Technol.* **2004**, *37*, 377−384.
(28) Hills, B. P.; Takacs, S. F.; Belton, P. S. A new interpretation of proton NMR relaxation time measurements of water in food. *Food Chem.* **1990**, *37*, 95−111.
(29) Roudaut, G.; van Dusschoten, D.; Van As, H.; Hemminga, M. A.; Le Meste, M. Mobility of lipids in low moisture bread as studied by NMR. *J. Cereal Sci.* **1998**, *28*, 147−155.
(30) Desbois, P.; Lebotlan, D. Proton low-field NMR measurements on crackers. *J. Food Sci.* **1994**, *59*, 1088−1090.
(31) Grad, J.; Bryant, R. G. Nuclear magnetic cross-relaxation spectroscopy. *J. Magn. Reson.* **1990**, *90*, 1−8.
(32) Vodovotz, Y.; Vittadini, E.; Sachleben, J. R. Use of ^{1}H cross-relaxation nuclear magnetic resonance spectroscopy to probe the changes in bread and its components during aging. *Carbohydr. Res.* **2002**, *337*, 147−153.
(33) Wu, J. Y.; Bryant, R. G.; Eads, T. M. Detection of solidlike components in starch using cross-relaxation and Fourier transform wide-line ^{1}H NMR methods. *J. Agric. Food Chem.* **1992**, *40*, 449−455.
(34) Wu, J. Y.; Eads, T. M. Evolution of polymer mobility during ageing of gelatinized waxy maize starch: a magnetization transfer ^{1}H NMR study. *Carbohydr. Polym.* **1993**, *20*, 51−60.
(35) Riedl, K. M.; Zhang, Y. C.; Schwartz, S. J.; Vodovotz, Y. Optimizing dough proofing conditions to enhance isoflavone aglycones in soy bread. *J. Agric. Food Chem.* **2005**, *53*, 8253−8258.
(36) Zhang, Y. C.; Lee, J. H.; Vodovotz, Y.; Schwartz, S. J. Changes in distribution of isoflavones and β-glucosidase activity during soy bread proofing and baking. *Cereal Chem.* **2004**, *81*, 741−745.
(37) Farhat, I. A.; Blanshard, J. M. V. Modeling the kinetics of starch retrogradation. In *Bread Staling*; Chinachoti, P., Vodovotz, Y., Eds.; CRC Press: Boca Raton, FL, 2000; 163 pp.
(38) AACC. *Approved Methods of the American Association of Cereal Chemists*, 10th ed; American Association of Cereal Chemists: St. Paul, MN, 2000.
(39) Carr, H. Y.; Purcell, E. M. Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Phys. Rev.* **1954**, *94*, 630−638.
(40) Meiboom, S.; Gill, D. Modified spin-echo method for measuring nuclear relaxation times. *Rev. Sci. Instrum.* **1958**, *29*, 688−691.
(41) Forsen, S.; Hoffman, R. A. Study of moderately rapid exchange reactions by means of nuclear magnetic double resonance. *J. Chem. Phys.* **1963**, *39*, 2892−2901.
(42) Wu, X. Z. Lineshape of magnetization transfer via cross relaxation. *J. Magn. Reson.* **1991**, *94*, 186−190.
(43) Balaban, R. S.; Ceckler, T. L. Magnetization transfer contrast in magnetic resonance imaging. *Magn. Reson. Q.* **1992**, *8*, 116−137.
(44) Aubard, J.; Levoir, P.; Denis, A.; Claverie, P. Direct analysis of chemical relaxation signals by a method based on the combination of Laplace transform and Pade approximants. *Comput. Chem.* **1987**, *11*, 163−178.
(45) Yeramian, E.; Claverie, P. Analysis of multiexponential functions without a hypothesis as to the number of components. *Nature* **1987**, *326*, 169−174.
(46) Hoch, J. C.; Stern, A. S. *NMR Data Processing*; Wiley-Liss: New York, 1996.
(47) Bi, Z. Q.; Bruner, A. P.; Li, J.; Scott, K. N.; Liu, Z. S.; Stopka, C. B.; Kim, H. W.; Wilson, D. C. Spectral fitting of NMR spectra using an alternating optimization method with a priori knowledge. *J. Magn. Reson.* **1999**, *140*, 108−119.
(48) Calucci, L.; Forte, C. 1H magnetization transfer in hydrated gluten and flour: effects of wheat aging. *Biomacromolecules* **2004**, *5*, 1824−1831.
(49) McIntyre, D. D.; Ho, C.; Vogel, H. J. One-dimensional nuclear-magnetic-resonance studies of starch and starch products. *Starch/Staerke* **1990**, *42*, 260−267.
(50) Mitchell, D. G. *MRI Principles*; Saunders: Philadelphia, PA, 1999.
(51) Choi, S. G.; Kerr, W. L. Effect of hydroxypropylation on retrogradation and water dynamics in wheat starch gels using ^{1}H NMR. *Cereal Chem.* **2003**, *80*, 290−296.
(52) Wynne-Jones, S.; Blanshard, J. M. V. Hydration studies of wheat-starch, amylopectin, amylose gels and bread by proton magnetic-resonance. *Carbohydr. Polym.* **1986**, *6*, 289−306.
(53) Cornillon, P.; Salim, L. C. Characterization of water mobility and distribution in low- and intermediate-moisture food systems. *Magn. Reson. Imaging* **2000**, *18*, 335−341.
(54) Lodi, A. Physico-chemical and molecular characterization of soy bread containing almond. Ph.D. Thesis, The Ohio State University, Columbus, OH, 2006.
(55) Rogers, D. E.; Zeleznak, K. J.; Lai, C. S.; Hoseney, R. C. Effect of native lipids, shortening, and bread moisture on bread firming. *Cereal Chem.* **1988**, *65*, 398−401.

Received for review March 5, 2007. Revised manuscript received May 12, 2007. Accepted May 16, 2007.

JF070636A