JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

NMR Signal Analysis To Characterize Solid, Aqueous, and Lipid Phases in Baked Cakes

F. LE GRAND, M. CAMBERT, AND F. MARIETTE*

CEMAGREF, Food Process Engineering Research Unit (IRM-Food), 17 Avenue de Cucillé, 35044 Rennes cedex, France

Proton mobility was studied in molecular fractions of some model systems and of cake using a ^{1}H nuclear magnetic resonance (NMR) relaxation technique. For cake, five spin–spin relaxation times (T_2) were obtained from transverse relaxation curves: T_2 (1) $\approx$ 20 μs, T_2 (2) $\approx$ 0.2 ms, T_2 (3) $\approx$ 3 ms, T_2 (4) $\approx$ 50 ms, and T_2 (2) $\approx$ 165 ms. The faster component was attributed to the solid phase, components 2 and 3 were associated with the aqueous phase, and the two slowest components were linked to the lipid phase. After cooking, the crust contained more fat but less water than the center part of the cake. The amount of gelatinized starch was lower in the crust, and water was more mobile due to less interaction with macromolecules. This preliminary study revealed different effects of storage on the center and crust.

KEYWORDS: Cakes; phases; crust; storage; NMR; relaxometry; spin–spin relaxation; T_2

INTRODUCTION

Because consumers are becoming more and more demanding regarding the preservation of foodstuffs, it is necessary to understand molecule mobility within food and chemical reactions during storage. Preservation of baked products such as breads, cakes, and biscuits is of particular interest. The aging of these systems, all composed of flour (gelatinized starch and gluten) with limited quantities of water, is called staling. Previous studies on the staling process of bread have emphasized the importance of drying (as a result of water migration) and starch recrystallization or "retrogradation" (*1, 2*).

Nuclear magnetic resonance relaxation analysis, also called NMR relaxometry, is a powerful tool for studying molecular dynamics and structural modifications in food products (*3*). Spin–spin relaxation time (T_2) is particularly sensitive to molecular mobility (*4–6*). Many studies involving NMR relaxometry have investigated water and the retrogradation of starch in simple matrices [starch−water solutions (*7–9*), starch−sugar−water solutions (*10, 11*), gluten−starch−water solutions (*12, 13*), and bread (*1, 14*)]. In these studies, the T_2 value in the solid state (tens of microseconds, detected with a single-pulse experiment) differs by several orders of magnitude from those in the liquid state (>1 ms, detected through a CPMG sequence) (*8, 15*).

In starch−water gels, Farhat et al. found one main component with a T_2 distribution between 5 and 40 ms and the beginnings of a low T_2 distribution centered around 0.2 ms for the liquid part of the system (*15*). In bread, three spin–spin relaxation time constants were identified from the one-pulse and the CPMG pulse decays at about 10 μs, 0.2 ms, and 2.5 ms (*1*). In white bread containing milk fat, Roudaut et al. found three components in T_2 relaxation curves (0.2, 30, and 100 ms), whereas relaxation of polymers could not be detected because of a dead time of 20 μs (*16*). The faster decaying component was ascribed to water protons, whereas the other two were associated with the protons of the lipids contained in the product.

Spin–spin relaxation parameters are affected by the retrogradation process and by the reordering of the gelatinized starch (*2*). The recrystallization of starch is accompanied by a decrease in the T_2 of the solid-like component and an increase in the corresponding contribution to the total signal (*8*). Several factors are known to affect starch retrogradation, including the starch source, the amylase to amylopectin ratio, heating and storage temperatures, and addition of sugars, polyols, and food emulsifiers (*10–12, 17*). The initial (fresh) and the equilibrium (retrograded) T_2 values increase with the water content of the sample (*7*).

Few studies have described the mobility of molecules in complex systems containing fat. Assifaoui et al. (*18*) recently studied biscuit batter using NMR relaxometry. The distribution of less mobile protons [T_2 (s) $\approx$ 11 μs] was attributed to the solid-like components (starch, proteins). Three populations were found in the liquid part of the system [T_2 (1) = 1.9 ms, T_2 (2) = 12.4 ms, and T_2 (3) = 104.7 ms]. T_2 (1) and T_2 (2) were attributed to water protons in association with gluten, starch, and sucrose. The third population corresponded to palm oil protons. This system was similar to that used in our study, but the matrix was not cooked.

The NMR relaxation signal from a complex product such as cake is hard to interpret because of its multiexponential behavior, and attributing the relaxation components to each phase (liquid, solid, fat, water, ...) is difficult. Moreover, baked cakes are not homogeneous samples because stronger cooking occurs at the

* Author to whom correspondence should be addressed (e-mail francois.mariette@cemagref.fr).

10.1021/jf071735r CCC: $37.00
Published on Web 11/29/2007

Table 1. Proportions (Expressed in Percent Wet Basis) of Various Ingredients Used in the Preparation of Standard Cake Batter ("Madeleine")[a]

water	6.0	sorbitol	3.0
rapeseed oil	18.0	eggs	18.0
wheat flour	29.7	baking powder	0.4
sucrose	22.2	salt	0.5
glucose syrup	2.1		

[a] The vanilla flavor in the original recipe (0.1%) was omitted from our preparation.

periphery (crust). The aim of this study was to attribute components of the NMR signal to specific molecular fractions, and certain model systems and a standard recipe for cake were analyzed: the spin–spin relaxation times and the signal intensities were studied quantitatively. In addition, the NMR relaxation signals were compared between the crust and the central part of the cakes and between fresh and stored samples. We emphasize that these findings represent a preliminary study of the effects of storage.

MATERIALS AND METHODS

Preparation of Samples. The cake recipe was based on that for a "madeleine", a shell-shaped sponge cake. All ingredients (wheat flour, eggs, sucrose, glucose syrup, rapeseed oil, sorbitol, baking powder, and salt) were from commercial suppliers, stored and used at ambient temperature, except for the eggs (4 °C). Wheat flour contained 12.2% wet basis (wb) of water; eggs, 77%; glucose syrup, 13.4%; sucrose, 0.1%; sorbitol, 0.8%; and baking powder, 14.4%.

The cake batter was prepared by first mixing sucrose, glucose syrup, sorbitol, salt, eggs, and water. Then flour and baking powder were introduced into the preparation and mixed. Oil was introduced and mixed. About 28 g of batter was placed into each shell-shaped tin. Cakes were baked in a 220 °C oven for 7.5 min. Each cooked madeleine had a final mass of about 25 g. Ingredient quantities for this recipe are listed in **Table 1** corresponding to about 40 madeleines. From each batch of 40 madeleines, 6 madeleines were characterized by NMR, and 6 more madeleines were used to determine the water content and the water activity. To investigate the behavior of the product during storage, three bags of six madeleines from the same batch were sealed and stored at room temperature for 1, 7, and 15 days, the corresponding results being denoted "D1", "D7", and "D15", respectively.

Model systems were also analyzed in the form of pure flour, pure rapeseed oil, a sugar−water solution, and a flour−water−sugar solution. "Sugar" included sucrose, glucose syrup, and sorbitol. The latter two solutions were prepared with exactly the same proportions of ingredients as in the standard recipe. To obtain gelatinized starch in the model solutions, the corresponding NMR tubes were subjected to a heat treatment of 5 min in an oven at 133 °C, and the final temperature inside the tube was 131 °C. This value was close to the temperature measured inside the cakes during cooking, 120 °C.

Chemical Determinations of Water Content and Fat Content. The central region and the crust were carefully manually separated with a kitchen knife just before measurements and then separately ground with a mixer and analyzed.

Water content was estimated by measuring the difference in weight after drying in an oven at 103 °C overnight. The standard error, calculated on three samples, did not exceed 0.2%.

Fat content was estimated by acid hydrolysis of samples (boiling 4 M HCl for an hour), filtration, and Soxhlet recuperation with petroleum ether.

NMR Measurements. NMR measurements were performed on samples from the center and the crust, after careful manual separation with a kitchen knife. Stamping out for NMR tube preparation was performed with a pastry cutter. Tubes were filled to about 10 mm in height in order to place samples in a homogeneous region of the NMR magnet, then weighed and hermetically closed. A Teflon rod was introduced into each tube before closing to avoid vertical expansion of the sponge cake. Each sample was prepared in triplicate, and the final results were expressed as a mean value with associated standard error ($\pm\sigma$).

The NMR measurements were carried out with a low-field NMR spectrometer (Minispec PC120, Brüker SA, Wissembourg, France), operating at 20 MHz for ^{1}H (0.47 T). The sample coil was 10 mm in diameter. A temperature control device regulated the NMR tube temperature at 24 ± 0.15 °C, and all measurements were performed at this temperature.

The spin–spin relaxation (T_2) was measured from the free induction decay (FID) and Carr−Purcell−Meiboom−Gill (CPMG) pulse curves, simultaneously recorded. The sampling rate for the acquisition of the FID was one point per 0.4 μs, and the delay between the 90° and 180° pulses of the CPMG sequence was 0.1 ms.

Data Analysis. Different relaxing components (numbered 1, 2, etc.) in the relaxation decay curve may be encountered if protons belong to different molecules (e.g., protein or water) or if they are involved in different physical states (e.g., solid or liquid).

Determination of the relaxation time and its relative population is known to be sensitive to the fitting method selected. To exclude misadjustment, we compared the results with two different methods: the discrete method Marquardt (*19*) and the continuous maximum entropy method (MEM) (*20*). This strategy was applied on CPMG (MEM was not applied to the FID sequence due to the small number of points to be fitted). Because the results given by both methods were consistent, only the results from the Marquardt method are presented here, owing to their greater accuracy.

The reconstructed spin–spin relaxation curves from FID and CPMG were fitted to Gaussian (FID) and exponential (CPMG) decay using eq 1

$$S(t) = S(k)\exp^{-\left(\frac{t}{T(k)}\right)^2} + \sum_{k'=1}^{n} S(k')\exp^{-\left(\frac{t}{T(k')}\right)} + \text{cst} \qquad (1)$$

where $S(t)$ is the intensity of the total relaxation signal, t is the time of the relaxation process, T_2 is the spin–spin relaxation time of component k (or k'), and S is the associated signal. k and k' refer to any of the relaxing components of the FID and CPMG, respectively.

To study the component intensities in various systems quantitatively, certain concepts used are introduced below. The contribution $S(i)$ (in volts) of a component i to the total NMR signal can also be expressed as mass intensity (MI_i) (in volts per gram)

$$\mathrm{MI}_i = \frac{S(i)}{m_i} \qquad (2)$$

where m_i is the mass of the component i. MI of one component (e.g., water) is also taken to be constant in all recipes, all other things being equal.

Because $S(i)$ is directly dependent on the receiver gain, all MI expressed in this paper have been converted to a constant receiver gain (75 dB).

Conversely, when the MI of rapeseed oil in cakes was known, the signal intensity from the lipid phase S_{oil} allowed calculation of the oil content m_{oil} in the sample using

$$m_{\mathrm{oil}} = \frac{S_{\mathrm{oil}}}{\mathrm{MI}_{\mathrm{oil}}} \qquad (3)$$

NMR measurement thus allowed determination of fat content in a completely different way from the previously described chemical method. Moreover, fat content can be determined without any preparation or even drying of the sample. Low-field NMR measurement is a well-known method for fat content determination (*21, 22*).

RESULTS AND DISCUSSION

Model Systems. *Rapeseed Oil.* Fitting of a continuous distribution from MEM described the spin–spin relaxation of pure rapeseed oil according to two wide distributions between 10 and 130 ms and between 150 and 300 ms (data not shown).

Table 2. Spin–Spin Relaxation Time T_2 (in Milliseconds) and Corresponding Peak Intensity I (in Percent of Total Signal) for Model Systems[a]

sample	I (1)		T_2 (1)		I (2)		T_2 (2)		I (3)		T_2 (3)		I (4)		T_2 (4)	
pure oil	52.6	*0.5*	**51.3**	*0.5*	47.4	*0.5*	**179.9**	*1.6*								
sugar−water solution	20.6	*0.3*	**38.7**	*0.7*	79.4	*0.3*	**294.3**	*2.6*								
pure flour	100		**0.016**	*0.000*												
flour−water solution, raw	27.5	*0.3*	**0.022**	*0.000*	25.5	*0.2*	**3.0**	*0.03*	47.1	*0.1*	**23.3**	*0.09*				
flour−water solution, cooked	25.5	*0.7*	**0.026**	*0.001*	23.2	*0.1*	**0.3**	*0.01*	51.4	*0.8*	**8.0**	*0.05*				
flour−water−sugar solution, raw	23.9	*0.4*	**0.018**	*0.000*	12.7	*0.2*	**1.0**	*0.03*	15.7	*0.6*	**11.2**	*0.54*	47.6	*1.1*	**38.3**	*0.32*
flour−water−sugar solution, cooked	32.3	*2.0*	**0.022**	*0.001*	33.0	*0.4*	**0.4**	*0.09*	34.7	*2.4*	**4.1**	*0.69*				

[a] Standard deviations are indicated in italics.

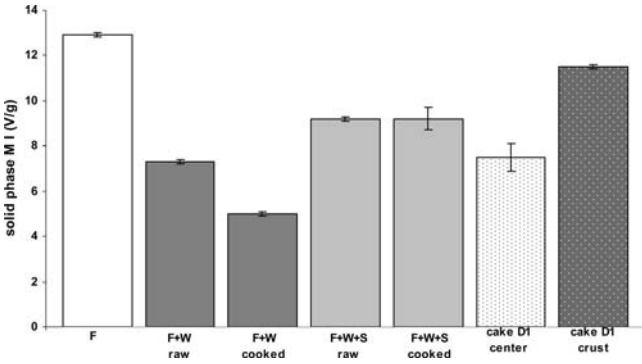

Figure 1. Mass intensities (MI) (expressed in V/g for a receiver gain of 75 dB) of the solid phase: F, pure wheat flour; F+W, flour−water solution; F+W+S, flour−water−sugar (sucrose, glucose syrup, sorbitol) solution. Solutions were prepared with exactly the same proportions of ingredients as in the cake recipe.

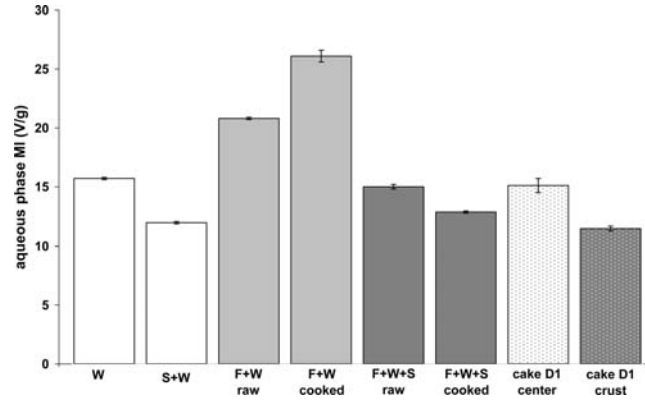

Figure 2. Mass intensities (MI) (expressed in V/g for a receiver gain of 75 dB) of the aqueous phase: W, pure water; S+W, sugar−water solution (sucrose, glucose syrup, sorbitol); F+W, flour−water solution; F+W+S, flour−water−sugar solution. Solutions were prepared with exactly the same proportions of ingredients as in the cake recipe.

This broad distribution of relaxation times was in accordance with the wide distribution of T_2 found by Assifaoui et al. for pure palm oil [between 30 and 300 ms (*18*)]. Indeed, oil is a complex mixture of triglycerides. The relaxation time of a triglyceride is modulated by both the length of the carbon chain and the number of unsaturations (*23*). In the case of a triglyceride mixture, the relaxation signal reflects the mixture's complexity, so that discrete adjustment of the signal is an oversimplification (*24*). Nevertheless, optimum adjustment of the relaxation curve of pure rapeseed oil by the discrete method was achieved by a biexponential model, with $T_2 = 51.3$ and 179.9 ms at 24 °C (**Table 2**).

Sugar−Water Solution. Adjustment of the relaxation signal of the sugar−water solution was performed by a biexponential model: T_2 (1) = 38.7 ms and T_2 (2) = 294.3 ms (**Table 2**). This biexponential model was in agreement with previous results obtained on sucrose−water systems (*25*), where two components were attributed, one for nonexchangeable protons of sucrose and one for water protons in exchange with hydroxyl groups of sucrose. Moreover, because of their long T_2, some nonexchangeable protons were included in the second component. Therefore, simple assignment of the two components to water and sugar protons, respectively, was also not representative, and both T_2 (1) and T_2 (2) were partially attributed to protons from sugar.

Flour−Water Solution. Flour is mostly constituted of starch and proteins. The macromolecules of this system have very low mobility, so their T_2 is very short [on the order of 10–20 μs (*1*)]. The T_2 of pure flour is 16 μs (**Table 2**) and shows a Gaussian behavior in the relaxation curve. Its mass intensity is 12.9 ± 0.1 V/g (**Figure 1**).

Adjustment of the relaxation signal of the flour−water solutions (raw and cooked) was optimized with a Gaussian biexponential model (**Table 2**). T_2 (1) $\approx$ 26 μs was attributed to the macromolecules of the system (flour). T_2 (2) and T_2 (3) described the aqueous phase, decreasing between the raw and cooked systems and, hence, emphasizing the growing interactions between water and starch due to starch gelatinization induced by heat treatment (*26*).

When water was added to flour, some macromolecules from the flour interacted with water and their mobility was increased, so that a lower intensity of the relaxation signal came from the solid phase for the same flour mass. Taking into account the flour mass, the mass intensity of the solid phase clearly decreased to 7.3 ± 0.1 V/g (**Figure 1**). Starch was (at least partly) gelatinized after heat treatment of this solution and contributed to the aqueous phase signal. Thus, still taking into account the flour mass, the mass intensity of the solid phase decreased further to 5.0 ± 0.1 V/g (**Figure 1**).

The same phenomenon induced the opposite effect on the mass intensity of the aqueous phase: starting from 15.7 ± 0.1 V/g in pure water, it increased to 20.8 ± 0.1 V/g when interacting with proteins and to 26.1 ± 0.5 V/g with gelatinization of the starch (**Figure 2**).

Flour−Water−Sugar Solution. Adjustment of the relaxation signal of the cooked flour−water−sugar solution was optimized with a Gaussian biexponential model: T_2 (1) = 22 μs, T_2 (2) = 0.4 ms, and T_2 (3) = 4.1 ms (**Table 2**).

As mentioned above, macromolecules have very low mobility, and thus their T_2 values are very short [on the order of 10–20 μs (*1*)]. Moreover, the corresponding population of protons shows Gaussian behavior in the relaxation curve (*27*). Also, T_2 (1) was attributed to all the solid parts of the sample (starch, insoluble proteins). The mass intensity deduced for the solid phase of the raw model solution (9.2 ± 0.1 V/g) was slightly below that of flour 12.9 ± 0.1 V/g (**Figure 1**). As discussed earlier for the flour−water system, this discrepancy was explained by the decrease in the contribution to the total signal from flour in the presence of water, because some macromol-

Table 3. Comparison of Spin–Spin Relaxation Time T_2 (in Milliseconds) and Corresponding Peak Intensity I (in Percent of Total Signal) between the Center and the Crust of Fresh (D1) and Stored (D7, D15) Cakes[a]

sample	I (1)		T_2 (1)		I (2)		T_2 (2)		I (3)		T_2 (3)		I (4)		T_2 (4)		I (5)		T_2 (5)	
center, D1	16.8	*1.3*	**0.020**	*0.0003*	12.3	*0.4*	**0.11**	*0.01*	41.5	*2.5*	**2.6**	*0.4*	13.8	*0.4*	**47.4**	*0.6*	15.7	*0.6*	**153**	*2.6*
crust, D1	26.9	*0.3*	**0.018**	*0.0003*	16.6	*1.6*	**0.24**	*0.05*	22.0	*2.2*	**2.2**	*0.1*	15.4	*0.7*	**49.8**	*2.6*	19.0	*0.2*	**159**	*4.3*
center, D7	22.5	*0.1*	**0.019**	*0.0002*	16.9	*0.2*	**0.20**	*0.01*	30.4	*0.2*	**1.8**	*0.0*	15.3	*0.0*	**52.9**	*0.6*	14.9	*0.2*	**160**	*0.7*
crust, D7	25.6	*0.4*	**0.018**	*0.0003*	18.5	*0.2*	**0.47**	*0.03*	22.2	*0.4*	**3.4**	*0.2*	15.2	*0.1*	**47.0**	*0.7*	18.5	*0.4*	**156**	*2.3*
center, D15	22.7	*0.3*	**0.019**	*0.0003*	15.6	*0.1*	**0.20**	*0.02*	31.4	*0.3*	**1.8**	*0.0*	15.5	*0.1*	**52.5**	*0.3*	14.8	*0.0*	**163**	*0.3*
crust, D15	26.0	*0.4*	**0.018**	*0.0002*	18.0	*0.4*	**0.52**	*0.04*	22.6	*0.7*	**3.7**	*0.2*	15.3	*0.2*	**45.6**	*0.8*	18.0	*0.1*	**154**	*2.0*

[a] Standard deviations are indicated in italics.

ecules were more mobile and also contributed to the aqueous phase signal. The expected value was much lower (about 7.3 V/g, as for flour mixed with water). We also concluded that sugar, which was found in the liquid phase, interacted with water molecules and made them less available for interaction with proteins and starch, thus explaining the reduced relaxation times compared to the flour−water system.

Furthermore, the mass intensities of the solid phase before and after cooking were 9.2 ± 0.1 and 9.2 ± 0.5 V/g, respectively (**Figure 1**). The expected value after cooking was much lower, that is, about 5.0 V/g, as for the flour−water solution after heat treatment, when starch gelatinization was observed. The absence of significant variation in mass intensity after heat treatment suggested a low gelatinization rate. This hypothesis is consistent with the relatively high proportion of sugar in the mixture, which is known to limit starch gelatinization. Sugar interacted with water molecules and made them less available for starch, thus reducing the amount of gelatinized starch (*10*).

In the cooked system, the two slower components T_2 (2) and T_2 (3) also reflected the aqueous phase relaxation. This biexponential behavior was consistent with that of the sugar−water solution. The strong decrease in T_2 values compared to the previous sugar−water solution was attributed to interactions with macromolecules. The mass intensity of the aqueous phase in this solution model (12.9 ± 0.1 V/g) was close to the value obtained with the sugar−water solution (12.0 ± 0.1 V/g) (**Figure 2**).

Mass Intensities versus Proton Densities. The mass intensities and the proton densities of molecules are strongly correlated. Proton densities of carbohydrates, water, and triglycerides are of the order of 0.06, 0.11, and 0.13, respectively. Mass intensities obtained here were of the order of 10, 15, and 18 V/g, for solid phase, aqueous phase, and lipid phase, respectively. Mass intensities obtained were also in agreement with the number of protons by molecule.

Assignment of Relaxation Components in Relation to Composition of Cakes. The spin–spin relaxation curve was fitted by one Gaussian function (FID) and four exponential functions (CPMG). Five relaxation peaks were also identified [T_2 (1) = 20 μs, T_2 (2) = 0.11 ms, T_2 (3) = 2.6 ms, T_2 (4) = 47.4 ms, and T_2 (5) = 153 ms for the center of sample at D1 (**Table 3**)]. Center and crust contained 15.4 and 11.2% (±0.2%) wb water, respectively.

Lipid phase. Because of the few interactions between the lipid phase and aqueous phase when oil is introduced into food products, we know that these T_2 did not differ much (*18*). Our results were consistent with the study of lipids in bread by Roudaut et al., that is, two wide distributions between 10 and 50 ms and between 50 and 300 ms (*16*). The slight discrepancies between T_2 values of pure rapeseed oil (51 and 180 ms) and rapeseed oil in baked cakes (50 and 157 ms) were mainly attributed to heat treatment and molecular organization during cake baking. Assifaoui et al. also reported such variations in T_2 values after heating (25−55 °C) and cooling of palm oil (*18*). Because of the wide distribution of relaxation times in fat, relaxation decay curve fitting by the discrete method revealed variations in T_2 for which two explanations could be proposed: (i) modification of the behavior of lipids or, more likely, (ii) variation in the distribution of T_2 resulting from the curve fitting of the discrete method itself. T_2 (4) and T_2 (5) were therefore attributed to the lipid phase. Moreover, the MI values calculated for the lipid phase on the basis of populations (4 + 5) were 16.2 ± 0.4 V/g for the cake center and 16.8 ± 0.2 V/g for the crust, which were consistent with the mass intensity of pure rapeseed oil (17.1 ± 0.1 V/g). Fat content was calculated from the NMR for a few cake samples and compared to the results of the chemical analysis (**Figure 3**). NMR slightly underestimated (−1.4%) the lipid content compared to the Soxhlet chemical extraction. This NMR determination of fat content was similar to that reported for fish muscle (*21*). This method has proved to be accurate, solvent-free, and not time-consuming compared to chemical extraction. We emphasize that there is no universal method for fat determination in food and that the officially proposed methods depend on fat proportion and composition. Fat content results can be very different according to the method or solvent used (*28*, *29*). In chemical extraction, the use of a preliminary phase of hydrolysis results in the extraction of complex and polar lipids such as phospholipids and short-chain fatty acids (*29*). Moreover, the risk of extracting unwanted sugars or proteins increases with the polarity of the solvent used. The NMR technique allows measurement of all the lipids contained in fat in an amorphous state, with the exception of certain components such as phospholipids with very short T_2, too close to the behavior of the solid protons.

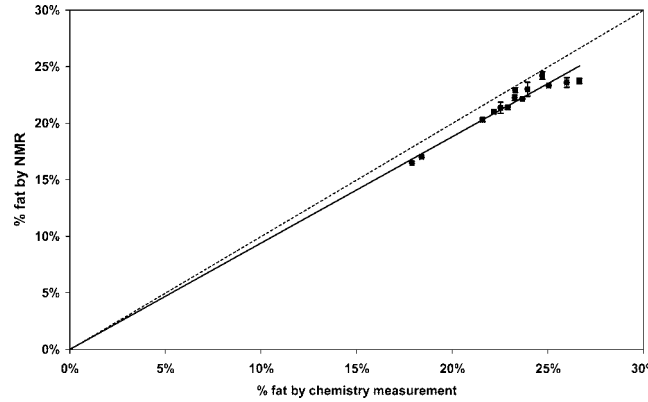

Figure 3. Comparison of results of fat content (expressed as percent wet basis) determination by chemical method (acid hydrolysis and petroleum ether recuperation) and by NMR.

NMR determination of fat content in fresh cakes (D1) was 22.8 and 25.9% wb for the center and crust, respectively (22.5 and 24.7% wb by chemical determination). Allowing for variations in water content, we could distinguish a difference in fat content between the center and the crust (27.0 ± 0.6 and

29.2 ± 0.4% dry basis, respectively). Indeed, the crust looked more "oily" than the center of the cakes.

The T_2 of water observed at 294 ms for sugar−water solution could not be linked to the T_2 (5) of cakes because this component was strongly reduced after the addition of flour in the flour−water−starch system (T_2 = 11 ms) and after cooking (T_2 = 4 ms).

Solid Phase. The first component of the relaxation curve with T_2 (1) ≈ 20 μs was attributed to the solid part of the sample (starch, insoluble proteins). This T_2 value was in fact close to the first component of the flour−water−sugar solution and also showed Gaussian behavior. The mass intensities calculated for the solid phase on the basis of flour mass were 7.5 ± 0.6 V/g for the center and 11.5 ± 0.1 V/g for the crust of the cakes (**Figure 1**). In view of the previous deductions of mass intensities of the model solutions, we concluded that starch was very little gelatinized in the crust (MI = 12.9 V/g for pure flour), but partly gelatinized in the center. The slightly lower T_2 (1) value in the crust indicated lower mobility of starch and proteins. Thus, our results suggested that the crust had a characteristic, more rigid structure than the center.

Aqueous Phase. The two populations T_2 (2) and T_2 (3) presented values similar to those of the aqueous phase in the model solutions. They were also attributed to the aqueous phase, containing water and mobile molecules (proteins, sugar, and gelatinized starch). The MI values calculated for this aqueous phase on the basis of sugar and water masses were 15.1 ± 0.6 V/g for the center and 11.5 ± 0.2 V/g for the crust of the cakes (**Figure 2**). As discussed above, very little starch was gelatinized in the crust or contributed to the aqueous phase relaxation signal. The mass intensity of the aqueous phase in the crust was lower than that of the cooked flour−water−sugar solution (12.9 ± 0.1 V/g). In contrast, starch was partially gelatinized in the center, and the mass intensity was greater than that of this model solution.

The T_2 (2) and T_2 (3) values were very short for the liquid phase, especially the T_2 (2) of about 0.2 ms. The T_2 values were thought to be shortened by the increase in viscosity of the aqueous phase through the presence of sucrose, as reported elsewhere (*2*). These findings were consistent with the assignment of population 2 to nonexchangeable protons. The corresponding precision was less than for other components (on the order of 10−20%) because of the fitting process for the NMR relaxation curve, composed of FID and CPMG decays. In fact, the FID was sampled on 0.07 ms due to field inhomogeneity. The first CPMG point was acquired at 0.2 ms. The NMR signal between 0.07 and 0.2 ms was thus not sampled in our experiments, and fitting of the relaxation curve around 0.2 ms was less precise.

T_2 (3) was lower in the crust than in the center at D1, due to lower water content and hence lower mobility of the aqueous protons.

Populations 2 and 3 constituted a single phase, so that a simple chemical classification was not representative of the fractions present in the sample, and these two components were both considered. The proportion of the aqueous signal [calculated by I (2) + I (3) in **Table 3**] decreased from 54 to 38% for the center and crust, respectively (fresh samples, D1). Water loss during cooking explained this lower intensity of the aqueous phase in the crust (15.4 and 11.2% wb of water for the center and crust at D1, respectively).

Effects of Storage. *Aqueous Phase.* After 15 days of storage, the water contents of the center and crust were 13.3 and 12.7%, respectively. The initial gradient (15.4–11.2% wb) of water

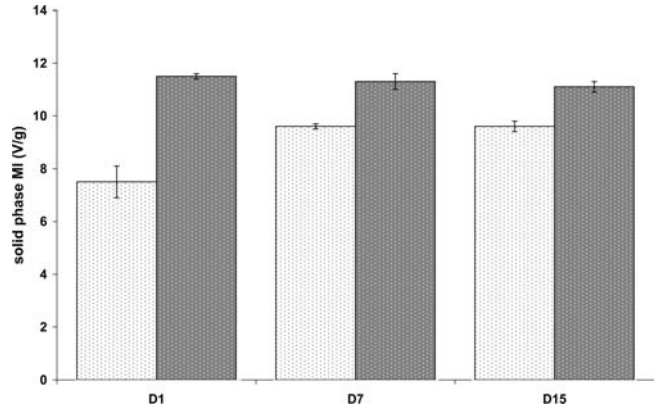

Figure 4. Mass intensities (MI) (expressed in V/g for a receiver gain of 75 dB) of the solid phase at D1, D7, and D15, in center (white) and crust (gray).

concentration between the center and crust almost disappeared. The variations in I (2) + I (3) were consistent with water content measurements (decrease for center, increase for crust). The system tended toward balance of the water concentrations (between center and crust, between crust and sealed air).

After storage, T_2 (2) and T_2 (3) were not identical between the center and crust, despite their similar water contents. T_2 values of the aqueous phase were expected to be identical for the same water content, when the mobility of protons is identical. The discrepancy between the center and the crust at D15 underscored a difference in molecular structure. Our hypothesis is that the water content in the crust was very low during cooking (immediate evaporation), so that water molecules were much less available for starch gelatinization than in the center. Therefore, the starch was less mobile in the crust than in the center after cooking. However, the T_2 of the water was affected by the relaxation of the polymer due to proton exchange between water and starch (*7*). Fewer interactions between water and starch in the crust resulted in a more mobile aqueous phase.

Moreover, drying of the center during storage resulted in a decrease in T_2 value [decrease of T_2 (3)]. In the crust, the water content increased during storage, aqueous protons became more mobile, and the T_2 of the aqueous phase increased.

Lipid Phase. The initial oil concentration gradient (27.0 ± 0.6 and 29.2 ± 0.4% db) between the center and the crust was very similar after 15 days of storage (26.4 ± 0.2 and 28.5 ± 0.1% db). Both T_2 (4) and T_2 (5) slightly increased in the center of cakes during storage, whereas they slightly decreased in the crust (**Table 3**).

Solid Phase. In the center of the cakes, the solid phase had a slightly lower T_2 after storage (**Table 3**) and provided a greater contribution to the total signal (**Figure 4**). Indeed, the NMR parameters were affected by the retrogradation of starch, as reported extensively elsewhere (*7–10*). These results were indicative of the decrease in polymer mobility during the recrystallization process.

In the crust, T_2 (1) parameters were not significantly changed after storage (**Table 3**; **Figure 4**). As discussed above, the crust initially had a more rigid structure than the center, with a lower water content, which could explain the greater stability. Farhat et al. reported that lower water content delayed the retrogradation process while decreasing the original (fresh) and final (retrograded) T_2 values (*7*).

Conclusion. The main aim of this study, that is, assignment of spin–spin relaxation components to specific molecular fractions, was reached despite the compositional and structural complexity of cakes. The spin–spin relaxation times provided

information regarding molecular dynamics. The relaxation signal was not only influenced by water content but also by changes in the molecular structure resulting from cooking. Crystallization, dissolution, and gelatinization of ingredients could be detected through the calculation of mass intensities. The results showed that the center and the crust did not have the same structure or the same behavior during aging and that relaxometry was a relevant tool for studying the behavior of the solid, aqueous, and lipid phases.

We demonstrated that a careful analysis of both relative intensities (or MI) and T_2 relaxation times improves the interpretation of the NMR results and expands the capabilities of the NMR for quantitative analysis. Indeed, because MI values are related to proton density values, this approach can be developed for use with any NMR system assuming a standard calibration method.

Future studies are planned to investigate the effects of initial water content and long-term storage (one to several months) on various cake fractions. These results should make possible greater understanding of the mechanisms involved in the aging of cake.

ACKNOWLEDGMENT

We thank Mr. El Gersiffi from "Pâtisseries gourmandes" and Mrs. Huchet from "ADRIA Développement" for technical and scientific assistance.

LITERATURE CITED

(1) 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* (2), 178–183.

(2) Farhat, I. A.; Blanshard, J. M. V.; Mitchell, J. R. Time domain ^{1}H NMR: its relevance to the processing and storage of starch systems. In *Advance in Magnetic Resonance in Food Science*; Belton, P. S., Hills, B. P., Webb, G. A., Eds.; Royal Society of Chemistry: London, U.K., 1999; pp 280–288.

(3) Ruan, R. R.; Chen, P. L. *Water in Foods and Biological Materials. A Nuclear Magnetic Resonance Approach*; Technomic Publishing: Lancaster, PA, 1998; p298.

(4) 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.

(5) Bertram, H. C.; Purslow, P. P.; Andersen, H. J. Relationship between meat structure, water mobility, and distribution: a low-field nuclear magnetic resonance study. *J. Agric. Food Chem.* **2002**, *50*, 824–829.

(6) Duval, F.; Cambert, M.; Mariette, F. NMR study of tomato pericarp tissue by spin-spin relaxation and water self-diffusion. *Appl. Magn. Reson.* **2005**, *28* (1–2), 29–40.

(7) Farhat, I. A.; Blanshard, J. M. V.; Mitchell, J. R. The retrogradation of waxy maize starch extrudates: effects of storage temperature and water content. *Biopolymers* **2000**, *53* (5), 411–422.

(8) Teo, C. H.; Seow, C. C. A pulsed NMR method for the study of starch retrogradation. *Starch/Staerke* **1992**, *44* (8), 288–292.

(9) Lin, Y. S.; Yeh, A. I.; Lii, C. Y. Correlation between starch retrogradation and water mobility as determined by differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR). *Cereal Chem.* **2001**, *78* (6), 647–653.

(10) Le Botlan, D.; Desbois, P. Starch retrogradation study in presence of sucrose by low-resolution nuclear magnetic resonance. *Cereal Chem.* **1995**, *72* (2), 191–193.

(11) Farhat, I. A.; Blanshard, J. M. V.; Descamps, M.; Mitchell, J. R. Effect of sugars on retrogradation of waxy maize starch−sugar extrudates. *Cereal Chem.* **2000**, *77* (2), 202–208.

(12) 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* (4), 371–379.

(13) Ottenhof, M. A.; Farhat, I. A. The effect of gluten on the retrogradation of wheat starch. *J. Cereal Sci.* **2004**, *40* (3), 269–274.

(14) Wang, X.; Choi, S. G.; Kerr, W. L. Water dynamics in white bread and starch gels as affected by water and gluten content. *Lebensm.-Wiss. -Technol. (Food Sci. Technol.)* **2004**, *37* (3), 377–384.

(15) Farhat, I. A.; Ottenhof, M. A.; Marie, V.; De Bezenac, E. ^{1}H NMR relaxation study of amylopectin retrogradation. In *Magnetic Resonance in Food Science*; Belton, P. S., Gil, A. M., Webb, G. A., Rutledge, D., Eds.; Royal Society of Chemistry: London, U.K., 2003; pp 172–180.

(16) Roudaut, G.; vanDusschoten, D.; VanAs, H.; Hemminga, M. A.; Lemeste, M. Mobility of lipids in low moisture bread as studied by NMR. *J. Cereal Sci.* **1998**, *28*, 147–155.

(17) Miura, M.; Nishimura, A.; Katsuta, K. Influence of adition of polyols and food emulsifiers on the retrogradation rate of starch. *Food Struct.* **1992**, *11* (3), 225–236.

(18) Assifaoui, A.; Champion, D.; Chiotelli, E.; Verel, A. Characterization of water mobility in biscuit dough using a low-field ^{1}H NMR technique. *Carbohydr. Polym.* **2006**, *64* (2), 197–204.

(19) Marquardt, D. W. An algorithm for least squares estimations of nonlinear parameters. *J. Soc. Ind. Appl. Math.* **1963**, *11*, 431.

(20) Mariette, F.; Guillement, J. P.; Tellier, C.; Marchal, P. Continuous relaxation time distribution decomposition by MEM. In *Signal Treatment and Signal Analysis in NMR*; Elsevier: Paris, France, 1996; pp218–234.

(21) Toussaint, C. A.; Medale, F.; Davenel, A.; Fauconneau, B.; Haffray, P.; Akoka, S. Determination of the lipid content in fish muscle by a self-calibrated NMR relaxometry method: comparison with classical chemical extraction methods. *J. Sci. Food Agric.* **2002**, *82* (2), 173–178.

(22) Sorland, G. H.; Larsen, P. M.; Lundby, F.; Rudi, A. P.; Guiheneuf, T. Determination of total fat and moisture content in meat using low field NMR. *Meat Sci.* **2004**, *66* (3), 543–550.

(23) Le Botlan, D.; Helie, I. Assessment of the intermediate phase in milk fat by low-resolution nuclear magnetic resonance. *Anal. Chim. Acta* **1995**, *311* (2), 217–223.

(24) Mariette, F. NMR relaxation of dairy products. In *Handbook of Modern Magnetic Resonance*; Webb, A., Ed.; 2005;Vol. sous presse.

(25) Lucas, T.; Mariette, F.; Dominiawsyk, S.; Le Ray, D. Water, ice and sucrose behaviour in frozen sucrose−protein solutions as studied by ^{1}H NMR. *Food Chem.* **2004**, *84* (1), 77–89.

(26) Tananuwong, K.; Reid, D. S. DSC and NMR relaxation studies of starch−water interactions during gelatinization. *Carbohydr. Polym.* **2004**, *58* (3), 345–358.

(27) Blanshard, J. M. V.; Derbyshire, W.; MacNaughtan, W.; Ablett, S.; Martin, D.; Izzard, M. J. ^{1}H relaxation of hydrated carbohydrate systems. In *Advances in Magnetic Resonance in Food Science*; Belton, P. S., Hills, B. P., Webb, G. A., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1999; p 293.

(28) *Approved Methods of the AACC*; American Association of Cereal Chemists: St. Paul, MN, 1995;30-10 to 30-40.

(29) Wolff, J. P. Analyse et dosage des lipides. In *Techniques d'Aalyse et de Cntrôle dans les Industries Agro-alimentaires*; Multon, J. P., Ed.; Lavoisier: Paris, France, 1991; Vol. 4, pp 157–199.

Received for review June 13, 2007. Revised manuscript received October 4, 2007. Accepted October 19, 2007. This study was financed by Région Bretagne and the Ministry of Agriculture and Fishing.

JF071735R