Nuclear Magnetic Resonance Imaging and Spectroscopic Studies of Wheat Flake Biscuits During Baking

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ABSTRACT

Wheat flake biscuits of known moisture and lipid contents were studied by nuclear magnetic resonance (NMR) imaging (MRI) to determine whether the moisture content of these low moisture samples could be measured by this technique. The water and lipid components of the observed NMR image signal were assigned from a ^{1}H relaxation study. A serial imaging study of the baking of a wheat flake biscuit mapped the spatial and temporal changes in moisture content across the biscuit during baking and resting.

Nuclear magnetic resonance (NMR) is a well-established spectroscopic technique in food research that allows the chemical composition, microdynamics, and morphology of optically opaque and heterogeneous samples, such as food, to be probed. NMR relaxation measurements are routinely used to quantitatively measure moisture and lipid contents and the solid-to-liquid ratio of foods (Chapman et al 1959, Conway and Earle 1963, Miller and Kaslov 1963, Pohle and Gregory 1968, Shanbhag et al 1971, Moisio et al 1972). The development of NMR imaging (MRI) has further extended the application of NMR in food research. There have been a number of MRI studies of cereal products: the circulation and diffusion of water within wheat grains has been measured (Eccles et al 1988, Jenner et al 1988); the ingress of water into kernels of corn and maize during steeping has been investigated (Ruan and Litchfield 1992, Ruan et al 1992); the drying of an ear of corn and maize kernels has been studied (Song and Litchfield 1990, Song et al 1992); and recently, a baking study of American-style biscuits has been reported (Heil et al 1993). An image is a map of the spatial distribution of mobile protons (typically those of water and lipids) in the sample and can be obtained completely noninvasively from large, intact samples. The variation in image intensity, referred to as image contrast, depends on a number of sample-dependent variables such as the proton density, their longitudinal (T_1) and transverse (T_2) relaxation times, and locally induced variations of magnetic susceptibility. A number of instrument-dependent factors are also important, such as the type of pulse sequence used and the associated time delays (Callaghan 1991). At present, considerable effort is being invested to produce imaging protocols that give quantitative data (Attard et al 1991, Callaghan 1991, Doran et al 1992) where the signal in the image is sensitive to a single parameter.

Magnetic field gradients are used in the imaging experiment to encode the spatial coordinates of the protons. Typically it takes several milliseconds to apply these gradients and during this period the NMR signal from the sample can dephase. Consequently, it is far easier to image high water content samples with long water ^{1}H T_2 relaxation times (>50 msec), such as plant tissue, than low moisture content samples with short water ^{1}H T_2 relaxation times (<10 msec), such as biscuits. The main objective of this study was to acquire images of wheat-flake biscuits and investigate the correlation between the signal intensity in the spin echo images and the moisture content of the biscuit. There are a number of reasons why measuring moisture content in biscuits is not easy. First, the low water content of these samples, and the fact that the water protons have short T_2 relaxation times, lowers the intrinsic NMR sensitivity of the experiment. Second, the protons on the lipids in liquid domains will also contribute to the image signal. Third, the proton signal in the image is a function of the temperature of the biscuit. Fourth, such cereal-based products are often highly porous, and even if image intensity is proportional to the volumetric water content, there may be little correlation between image intensity and gravimetric moisture content. Despite these problems, wheat flake breakfast cereal biscuits with moisture contents that ranged from 5 to 20% were studied to investigate the possibility of mapping the distribution of moisture content in biscuits by this technique. A serial imaging study of the baking of a biscuit that mapped the spatial and temporal changes in moisture content across the biscuit during baking and resting was also reported.

MATERIALS AND METHODS

NMR Imaging

The NMR imaging experiments were performed on an Oxford Research Systems Biospec I spectrometer, operating at 84.7 MHz for protons, connected to an Oxford Instruments 31-cm horizontal bore, 2 Tesla superconducting magnet. The samples were studied in a 6-cm i.d. split ring resonator radio frequency probe (Hall et al 1985). Linear magnetic gradients of 8 kHz/cm were generated using 20-cm dia. home-built gradient coils (Carpenter et al 1989). The images were acquired with a spin echo imaging pulse sequence (Edelstein et al 1980). However, because of the thinness of the biscuit, no slice selection pulse was used. An echo time (TE) of 3.5 msec and a recycle time (TR) of 6 sec was used. The 90° pulse length was 50 μsec, the NMR signal was sampled every 8 μsec, and 128 complex points were acquired. The sequence was repeated using 128 phase encode steps without signal averaging. The field of view was 11 cm in both image directions. All experiments were performed at ambient temperature (295 K).

NMR Relaxation Study

The NMR relaxation experiments were performed on a Bruker MSL 300 NMR spectrometer operating at a frequency of 300 MHz for protons. An RT of 10 sec was used throughout the study, which was performed in a 5-mm NMR probe operating at a temperature of 298 K. The free induction decay (FID) signal was recorded following a single 90° pulse (2 μsec). Longitudinal relaxation times (T_1) were determined using a 180°–τ–90° inversion recovery pulse sequence. Transverse relaxation times (T_2) were determined using the Carr Purcell Meiboom Gill (CPMG) pulse sequence. In both cases, the experimental data were analyzed using the SIMFIT program supplied by Bruker, which calculates the T_1 or T_2 relaxation time constants by fitting the data to exponential components.

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Sample Preparation

The wheat biscuits were fabricated by compressing flakes of wheat in 10- × 5.5- × 2-cm ovoid shapes. Biscuits were prepared with moisture contents that ranged between 5 and 20% by baking the biscuits in an oven for different time periods, and with lipid contents of ~2.5%. The water and lipid contents (%, w/w) of the samples studied were determined by gravimetric oven drying and acid hydrolysis analysis, respectively.

RESULTS AND DISCUSSION

The moisture and fat analyses of the seven samples are shown in Table I. Representative images of those with moisture contents of 20.6, 18.1, 13.5, and 5.8% are displayed in Figure 1, which shows that image intensity drops as a function of decreasing moisture content. The T_2 relaxation time of the water in these low moisture content samples was very short (Table I). Hence, to minimize loss of signal during the sequence, a TE of 3.5 msec was used, which was the shortest TE possible using this hardware and experimental procedure. As a result, the image contrast was significantly T_2-weighted. A long TR was used to allow the water protons to return to thermal equilibrium. Consequently, there was no T_1 weighting in the images. Each biscuit was separately imaged three times, and the average pixel intensity of the image of each biscuit was measured and plotted against the gravimetrically determined moisture content of that biscuit (Fig. 2). These biscuits were fabricated by compressing flakes of wheat and were relatively uniform. The image intensity was determined from a large area of the biscuit image. For these samples, the porosity of the biscuits can be assumed to be similar. The biscuits with moisture contents of <15% all have similar image intensity (Fig. 2). From this we infer that the water protons make a negligible contribution to these images; rather the image signal arises from the liquid lipid in the biscuit. However, the water protons do contribute to the image signal in the samples when the moisture content in the biscuit is >15% (Fig. 2), because the signal increases as a function of increasing moisture content, although not linearly.

To understand this system further, a ^{1}H relaxation study at 300 MHz was undertaken (Table I). It was found that the longitudinal (T_1) relaxation profiles of the biscuits were single exponential, and the longitudinal relaxation rate ($1/T_1$) is proportional to the moisture content of the biscuit (Fig. 3A). The transverse (T_2) relaxation behavior of the wheat biscuit was multicomponent. Two components were clearly observed in the FID of the biscuit, with the major component decaying very rapidly, corresponding to a Gaussian lineshape with a T_2 relaxation time on the order of microseconds. This signal was assigned to the protons of immobile starch and solid fat. This rapidly decaying NMR signal is not observed in the imaging experiment because the transverse magnetization from those components completely dephases before the spin echo image signal is sampled. The second component of the FID has a much longer transverse relaxation time, on the order of milliseconds. This component arises from water and liquid lipid molecules and is the one that contributes to the imaging experiment. A CPMG sequence was used to characterize the T_2 relaxation behavior of these protons, and in each case, the T_2 relaxation profiles of the biscuits could be resolved into two exponentially relaxing components (Table I). The intensity of the fast relaxing CPMG component increases and its relaxation time lengthens as moisture content of the biscuit increases, whereas the intensity of the slower relaxing CPMG component is invariant to changes in moisture content and has a constant value of ~45 msec. The transverse relaxation rate ($1/T_2$) of the rapidly decaying component is proportional to the moisture content of the biscuit (Fig. 3B). Thus, the rapidly decaying CPMG component arises from water protons, and the more slowly decaying CPMG component was assigned to the protons of the mobile lipid molecules.

The relaxation results support the imaging results. Although the relaxation study was performed at a higher magnetic field strength than the imaging study, the T_2 relaxation time of water protons in such low moisture content carbohydrate systems is insensitive to field strength. Thus, it is possible to use the relaxation data to interpret these T_2 weighted images. The intensity of signal M in a spin echo image is dependent on several parameters, but principally on the density of protons (ρ) in the region, their longitudinal (T_1) and transverse (T_2) relaxation times, the TE, and the TR in the pulse sequence, such that:

$$M = k \times \rho \times \exp(-\mathrm{TE}/T_2) \times [1 - \exp(-\mathrm{TR}/T_1)] \quad (1)$$

where k is a constant, assuming that TR>>TE, and mass transport of the nuclear spins during the echo period is negligible. In the imaging experiments used in this study, the TR was at least five times longer than the ^{1}H longitudinal relaxation time of the water in the sample; thus the last term in Eq. 1, which includes T_1, can be neglected as it equates to unity. Hence, three variables determine the intensity of the image signal originating from the water in the biscuit: the concentration of the water, the transverse relaxation time of its proton resonances, and the TE. The T_2 relaxation times given in Table I confirm that the transverse magnetization arising from the water in the biscuits with moisture content of <15% will have completely dephased during the 3.5

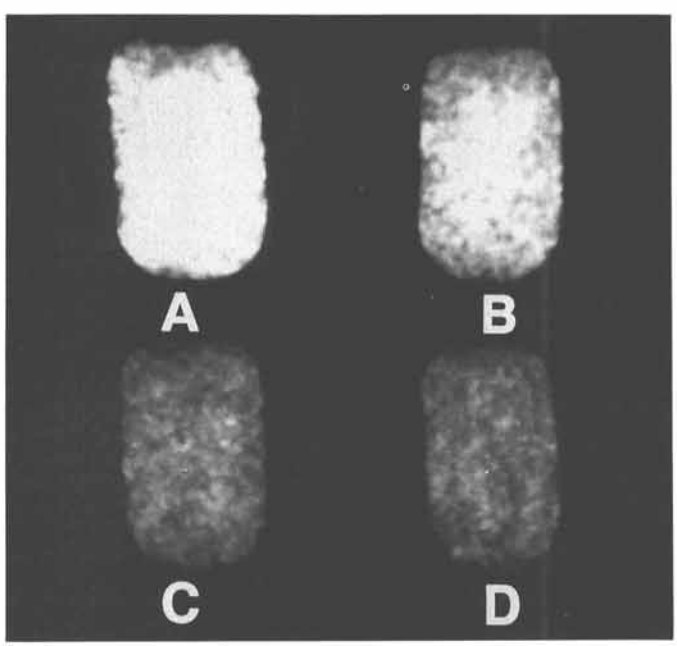

Fig. 1. 128 × 128 spin echo images of wheat biscuits with moisture contents (%, w/w) of 20.6, 18.1, 13.5, and 5.8 (A–D, respectively).

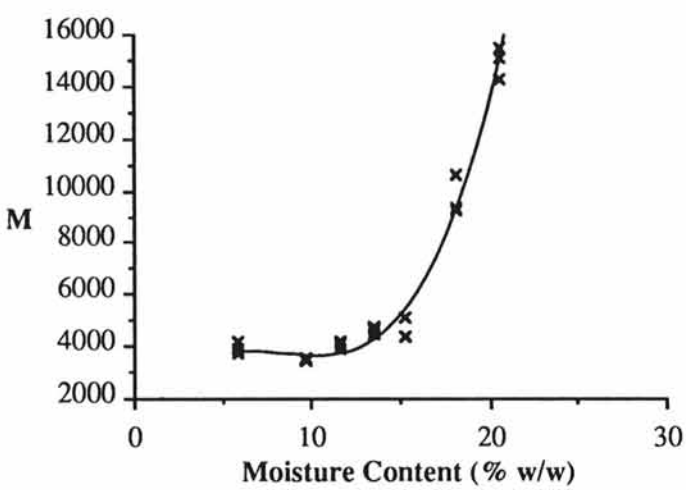

Fig. 2. Graph of average signal intensity (M) from the image (see Eq. 1) against the gravimetric moisture content of the wheat biscuit.

msec TE; hence, no water signal will be detected. Only the liquid lipids with their T_2 relaxation times on the order of 40 msec will be detected. Nevertheless, as the graph in Figure 2 demonstrates, for biscuits with moisture content >15%, the water protons do contribute to the image signal. The T_2 relaxation time of water in the biscuit with 20% moisture content is ~1.7 msec. Although this is shorter than the 3.5 msec TE, and thus most of its transverse magnetization will dephase during the imaging echo period, there will be a finite amount of magnetization remaining that contributes to the image.

The origins of the observed signal in the NMR image have thus been established. Under the experimental conditions used in this study, where the image TE is 3.5 msec, water protons only contribute to the NMR images of wheat flake biscuits when the moisture content is >15%. Although the relationship between water content and image contrast is not linear, this relationship nevertheless provides a suitable calibration curve from which moisture contents can be estimated from the NMR image.

A serial imaging study of the baking of a wheat flake biscuit was then undertaken. The biscuit was baked in a portable oven at 473 K for 16 min. The wheat biscuit was imaged on three different occasions: before baking, 5 min after being removed from the oven, and after 2 hr. The respective images are displayed in Figure 4. Again, the image intensity drops as the moisture content of the biscuit is reduced during baking. Each biscuit was studied at room temperature (295 K), the biscuit was relatively thin with a large surface area, and as a result, it cools quickly back to room temperature. From the calibration curve shown in Figure 2, the moisture distribution across the biscuit can be estimated from the intensity of the signal in the different pixels in the image. The intensity of signal in the image of the unbaked biscuit is very strong (Fig. 4A), and there is a small moisture gradient across the biscuit. The central region of the biscuit has a moisture content of 21–21.5%, whilst the outer region has a moisture content of 19–21%. Baking reduces the moisture content of the biscuit significantly, and as a result, the image intensity drops. However, the image of the biscuit 5 min after baking (Fig. 4B) demonstrates that the biscuit still has a region with high moisture content of between 19 and 20% near the center of the sample; the actual center of the biscuit has a slightly lower moisture content of 16.8–17.2%; whilst the moisture content in the outer region is <15%. During the 2-hr resting period, the high water region in the center of the biscuit disappears (Fig. 4C). The whole biscuit now has a moisture content of <15%, and the water protons are no longer detected in this image.

This study demonstrates that it is possible to spatially map moisture content in biscuits with low moisture contents of >15%. It would be necessary to implement an imaging sequence with a TE of <1 msec to detect the water proton signal from biscuits with moisture contents of <10%. Strong eddy currents produced in the magnet as the magnetic field gradients are switched on and off meant that it was not possible to implement such short TE with the hardware used in this study. However, shorter TE could be achieved by either reducing the diameter of the gradient coils or using shielded gradients.

TABLE I
Moisture and Lipid Content, T_1 and T_2 Relaxation Times of Water and Liquid Lipid Protons in Wheat Biscuits Measured at 300 MHz

Moisture Content (%, w/w)	Lipid Content (%, w/w)	T_1 (sec)	T_2 Water (msec)	T_2 Liquid Lipid (msec)
20.6	2.5	1.38	1.7	46
18.1	2.3	1.26	1.4	46
15.3	2.2	0.9	1.0	46
13.5	2.3	0.5	0.8	46
11.6	2.3	0.37	0.5	43
9.7	2.5	0.34	0.3	43
5.8	2.4	0.24	0.3	45

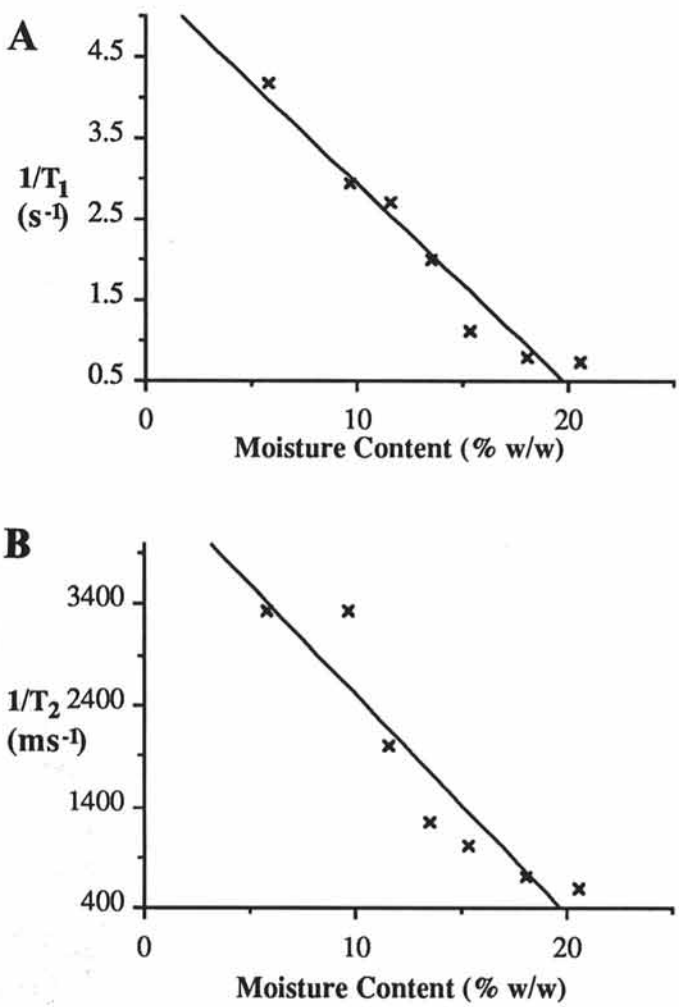

Fig. 3. A, ^{1}H Longitudinal relaxation rates ($1/T_1$) of the water in the wheat biscuit against the moisture content. **B**, ^{1}H Transverse relaxation rate ($1/T_2$) of the water in the wheat biscuit against the moisture content.

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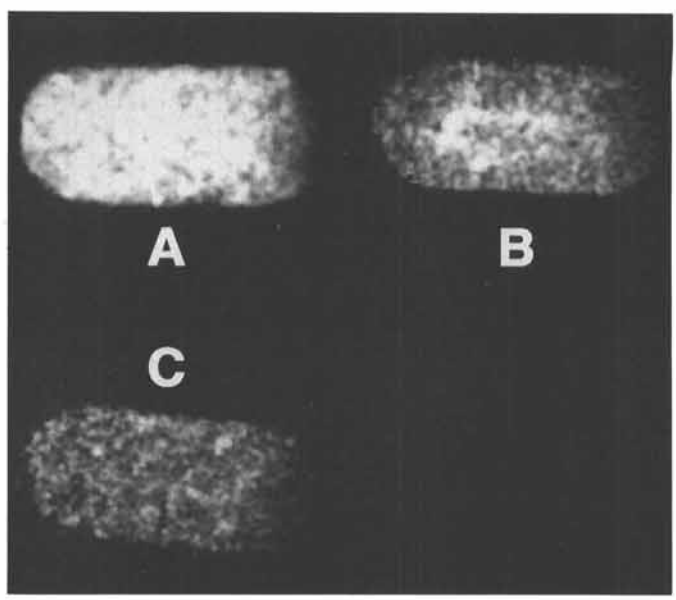

Fig. 4. 128 × 128 spin echo images of wheat biscuits before baking, 5 min after baking, and 2 hr after baking (A–C, respectively).

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