A Magnetic Resonance Imaging Technique for Quantitative Mapping of Moisture and Fat in a Cheese Block[1]

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ABSTRACT

Separate magnetic resonance images of water and fat of oil-in-water emulsions and cheese blocks were obtained using the chemical shift selective suppression technique. With this technique, the proton signals emitted from water can be readily separated from those emitted from fat in the same sample through a single experiment using magnetic resonance imaging. Relaxation compensation was made to improve the quality of suppression. The experiment using oil-in-water emulsions demonstrated an excellent linear relationship between the intensity of the signal and the concentrations of water or fat. Images of a cheese slice showing only water or fat revealed that moisture distribution was less uniform than was fat distribution. Moisture contents determined by the magnetic resonance imaging technique were very close to those obtained by the oven-drying method.

(**Key words**: cheese, chemical shift selective, magnetic resonance imaging, moisture and fat distribution)

Abbreviation key: **CHESS** = chemical shift selective, **MRI** = magnetic resonance imaging, **RF** = radio frequency, **S/N** = ratio of signal to noise.

INTRODUCTION

Large cheese blocks that are placed in paperboard forms after pressing are commonly aged at a low temperature (5°C) to allow the development of desirable flavor, body, and texture (14) and to restrict the growth of contaminants (8). However, rapid cooling creates uneven distribution of moisture in large cheese blocks to create variable composition and pH values within the blocks, which may result in texture and flavor defects (9). Uneven distribution of moisture is thought to be a result of its redistribution of within the blocks that is driven by temperature gradients established across the blocks during curing at low temperature. That is, when warm cheese blocks are placed in a cold environment, the temperature of the outer regions of the blocks drops rapidly, but the temperature of the central regions decreases more slowly. Temperature gradients are therefore formed between the central and outer regions and drive moisture from high to low temperature regions. It is important to be able to visualize how moisture redistributes in cheese blocks during cooling to improve current cooling processes or to develop new ones.

The patterns of uneven cheese texture can be observed visually on a cut surface of a block withdrawn immediately after cooling. Quantitative chemical analysis, in combination with multiple samplings at designated spatial locations of a cheese block, have been employed to provide information on the distribution of fat and moisture contents within a cheese block (9). This technique, although valid, is destructive, time-consuming, and does not allow noninvasive observation on a continuous basis. Conventional light and electron microscopy involve extensive preparations that severely damage the integrity of the samples and interrupt the processes that the samples are undergoing. A nondestructive, noninvasive technique for mapping or imaging moisture and fat contents would provide real-time and real-location information on the distribution of moisture and fat in cheese blocks while the cheese blocks are being cured or cooled or aged.

Magnetic resonance imaging (**MRI**) may be a technique that satisfies those requisites. Currently, most applications of MRI are in clinical situations to acquire images of organs or tissues for diagnostic purposes. In recent years, application of MRI to food science and technology has received increasing attention as food scientists and technologists realize the

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unique nondestructive and noninvasive features and power of the technique. In principle, MRI is based on the magnetic behavior of certain nuclei (such as protons ^1H) in a sample placed in an external magnetic field while being subjected to radio frequency (**RF**) pulses. The ^1H magnetic resonance parameters that are usually used to produce image contrast include proton density, spin-lattice relaxation time (T_1), spin-spin relaxation time (T_2), and self-diffusivity. In MRI experiments, a sample is observed without any prechemical or physical fixation or sectioning, indicating the unique nondestructive and noninvasive nature of the technique. In addition, MRI can acquire an image of a plane at any location and direction of a sample; thus, a sample can be virtually sectioned in all possible directions without the limitation to number of sections. When a process (e.g., cooling, drying, or steeping) is coupled with the MRI instrument, real-time, nondestructive, and noninterrupted testing and monitoring of the process become possible.

It is difficult to obtain magnetic resonance images of only moisture or fat because moisture protons and fat protons simultaneously contribute to the magnetic resonance signal. In clinical MRI, for body regions that are rich in fat, a strong fat signal may interfere with the imaging of nearby structures and obscure the definition of body organs and tissue. Therefore, medical researchers have been trying to separate fat and water signals (2, 4, 6, 7, 10, 11, 12, 13, 16). However, in these medical studies, emphases were placed mainly on tissue discrimination and contrast; few focused on quantifying water and fat and examining their distribution in tissue. Limited work has been done to measure moisture and fat contents in foods using MRI. Winkler et al. (20) used T_1 weighted and T_2 weighted spin echo imaging sequence to measure water and lipid in an emulsified water-oil system. Heil et al. (5) measured moisture and fat in French-style dressings, and Farkas et al. (2) measured the oil-water interface in potatoes during frying using similar methods However, only a partial separation of the water and fat proton signal was achieved in these images, which could lead to misinterpretation of the results. These methods also have problems associated with reduced signal to noise ratio (**S/N**) and poor image quality.

Our objective was to develop MRI techniques based on the chemical shift characteristics of water and fat. It is known that nuclei such as protons and carbons in different chemical environments resonate at different frequencies when placed in a magnetic field (3). The resonance frequencies of these nuclei depend on the chemical shifts of the nuclei concerned, which are determined by the chemical environments such as electron density and the type of bonding. Protons in water have a range of resonance frequencies or chemical shifts that are distinguishable from those of protons in fat. An imaging pulse sequence, which sets the timing for RF pulses and gradients, takes advantage of the chemical shift characteristics of water and fat and makes possible selective suppression of the proton signals emitted from one compound and the acquisition of proton signals emitted only from the other compound. This chemical shift selective (**CHESS**) suppression technique therefore enables the construction of images of water only or fat only through a single imaging experiment without postprocessing. An attempt also was made to improve the suppression quality.

MATERIALS AND METHODS

Materials

Oil-in-water emulsions. Oil-in-water emulsions were prepared to serve as homogeneous samples for the purpose of development and testing of the MRI techniques. The emulsions were freshly made before the MRI experiments by mixing known amounts of vegetable oil (Crisco oil; Proctor & Gamble, Cincinnati, OH) with 3 m*M* $CuSO_4$ in 20-mm diameter glass tubes. The spin-lattice relaxation time of emulsified vegetable oil was similar to that of Cheddar cheese. Percentages of oil by volume were 0, 20, 30, 40, 50, 60, 70, and 100%. Three percent of Tween 40 (polyoxyethylene sorbitan monooleate) by volume of the oil was added to improve the stability of the mixture. The mixture was homogeneously mixed using an Osterizer blender (Sunbeam-Oster Household Products, Schaumburg, IL). Separation of the mixture into fat and water layers was not noticed over 1 wk. The emulsion was placed vertically in a holder, and a slice of 3-mm thickness through the middle of the samples was imaged.

Cheese block. A small commercial Cheddar cheese block (14 cm × 8 cm × 3 cm) was used to obtain images of moisture and fat contents. The cheese block was sealed in a plastic bag and placed horizontally in a holder within the RF coil.

Instrument and Pulse Sequence

A 4.7 T SISCO imaging spectrometer with a 330-mm diameter bore superconducting magnet (SISCO, Fremont, CA) and a GE volume bird cage RF coil were used for spectrum analysis and imaging. The FID (free induction decay) data were Fourier transformed into image data using STIMULATE 4.0

(The Center for Magnetic Resonance Research, University of Minnesota, St. Paul). Images were displayed and processed in NIH 1.54 (National Institutes of Health, Bethesda, MD). In the CHESS pulse sequence, a frequency selective 90° RF pulse is applied first with carrier frequency set on the unwanted spin resonance, which flips the unwanted spin magnetization into the transverse plane; subsequently, a spoiling gradient dephases the transverse magnetization. After presaturation of the spin system, no net magnetization of unwanted component is retained, but the desired component remains entirely unaffected in the form of z magnetization. A conventional spin-echo imaging sequence is then applied to obtain the desired proton image. Figure 1 shows an ^1H spectrum of cheese at 4.7 T. The left peak represents protons in water molecules, and the right peak represents protons in fat molecules. These two peaks have a chemical shift difference of about 3.5 ppm, which is about 700 Hz in the 4.7-T spectrometer. Without suppression of one of the peaks, the acquired magnetic resonance image represents the total signal intensity of the sample, and the water signal cannot be separated from the fat signal.

Relaxation Compensation and Suppression Quality

Incomplete suppression may come from imperfect RF pulses (shape and duration), inaccurate calibration of 90° and 180° RF pulses, and magnetization-relaxation phases in the manipulation and sampling process. It is very important to examine the suppression quality of water or fat that shows the fraction of water or fat being suppressed in spectra and images. The quality of suppression cannot be easily visualized from the images because the moisture signal cannot be distinguished from the fat signal. However, on the spectrum, moisture and fat signals can be easily distinguished because of different chemical shifts shown on the spectrum. Based on this ability, a quantitative spectrum was obtained using the CHESS sequence. The number of phase-encoding steps was set to 1, phase-encoding increment to 0, readout gradient to 0, and slice number to 1. The other sequence parameters remained unchanged. Fourier transform analysis was performed on the acquired signal to generate a spectrum of the signal obtained by nuclear magnetic resonance after saturation.

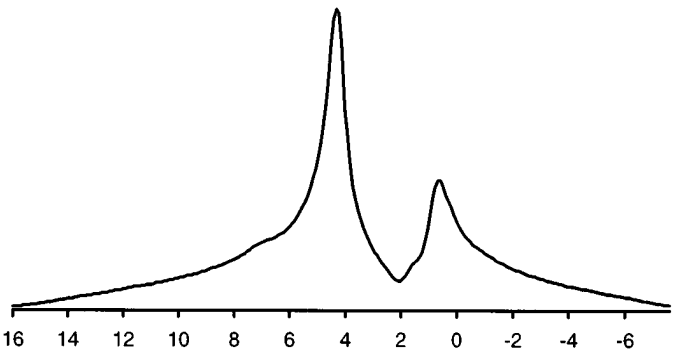

Figure 1. Proton spectrum of a Cheddar cheese block in 4.7 T SISCO spectrometer (SISCO, Fremont, CA).

The saturation pulse was Gaussian in shape with a width of 2500 μs. Repetition time was 800 ms, and echo time was 25 ms; 256 data points were acquired. A slice of 3-mm thickness through the middle of the cheese block was imaged.

Usually a 90° saturation pulse is used in CHESS sequence to flip the unwanted component from longitudinal magnetization to a transverse plane so that this component can be dephased by the subsequent spoil gradients. There is a time interval between the 90° saturation pulse and the center of the spoil gradient in which magnetization relaxes from the transverse plane to the longitudinal direction; therefore, a saturation pulse that is slightly greater than 90° is preferred for complete suppression.

Imaging and Quantification of Water and Fat

The repetition time, echo time, RF pulse shape and width, and slice thickness and orientation used for imaging experiments were the same as those used in the spectrum experiment. The data matrix was 128 × 128. For the oil-in-water emulsions, the field of view was 12 cm in the readout direction and 5 cm in the phase-encoding direction. For the cheese blocks, the field of view was 9 cm in the readout direction and 5 cm in the phase encoding direction.

After the MRI experiment, the cheese block was cut into 5 × 7 uniform rectangular portions (Figure 2). The portions along the middle lines in both readout and phase-encoding directions (the shaded area) were oven-dried at 100°C for 16 h to measure the moisture contents. The calibration of moisture content for the moisture image was done by measuring mean image intensity and mean moisture content of the cheese portions.

RESULTS AND DISCUSSION

Signal Suppression

Suppression of unwanted signals through the CHESS sequence is illustrated in Figure 3. When fat or water signals were suppressed, the peak represent-

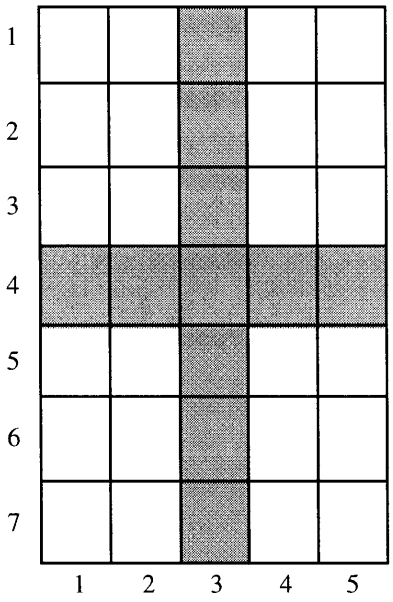

Figure 2. Schematic diagram showing the locations of portions that were used for moisture content determination using the oven-drying method. The vertical direction is the read-out direction, and the horizontal direction is the phase-encoding direction.

ing the unsuppressed signals appears to be unaffected (Figure 3). However, perfect suppression was not achieved, as indicated by the residue of the suppressed fat (25%) and water signals (21%). Here, the fat (water) residue is defined as the ratio of the residual fat (water) peak area after suppression to before suppression. The residue may be caused by imperfect RF pulse design and magnetic field inhomogeneity. This imperfection of suppression can be reduced by optimizing the experimental parameters and using relaxation compensation. Figure 4 shows that the residue of the suppressed signals was greatly reduced (9% for fat and 10% for water) with relaxation compensation.

Imaging and Quantification of Water and Fat in Homogeneous Fat and Water Phantoms

Figure 5 shows a series of water-suppressed (A) and fat-suppressed (B) magnetic resonance images of the oil-in-water emulsion phantoms. Greater brightness indicates a stronger signal. The signal intensities vary as the water and fat contents vary. When signals that were emitted from water were suppressed (Figure 5A), the signal intensity increased as oil concentration increased. When signals emitted from oil were suppressed (Figure 5B), the signal intensity increased as water content increased. When fat or water was absent from the mixture, little signal can be seen in the water-suppressed or fat-suppressed images, indicating that reliable selective signal suppression was achieved with the optimized CHESS technique. An excellent linear correlation between the MRI signal intensity and water and fat contents was found as indicated by the following linear regression equations for oil and water contents:

$$I = 0.9887C_o + 2.6416 \quad [1]$$
$$r^2 = 0.9764$$

$$I = 0.8593C_w + 19.544 \quad [2]$$
$$r^2 = 0.97961$$

where I = image intensity, C_o and C_w = oil content and water content, respectively. This relationship suggests that the quantitative mapping of water and fat in materials containing them is possible with the CHESS technique. The very small signal noticed in the water-suppressed image of the oil-free phantom

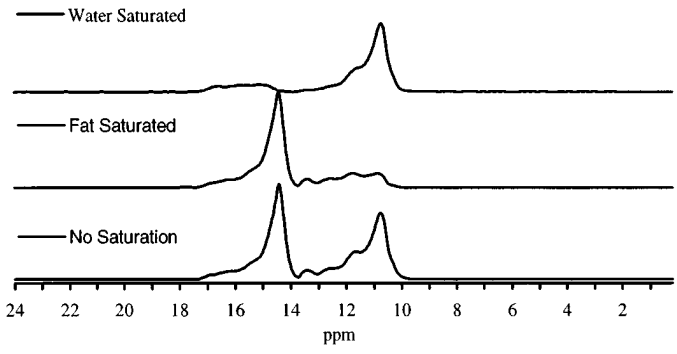

Figure 3. Spectra of a selected slice of the cheese block before and after saturation without parameter optimization.

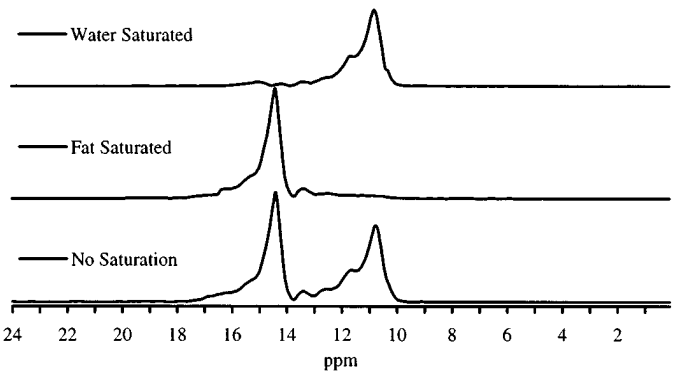

Figure 4. Spectra of a selected slice in the cheese block before and after saturation with parameter optimization.

TABLE 1. Measurement of images of moisture and fat with edge exclusion.

	Image	
	Moisture	Fat
Mean	113.2	122.7
SD	14.6	14.83
Minimum	66	49
Maximum	188	165

and in the oil-suppressed image of water-free phantom may be attributed to the imperfect RF pulses and the unsaturated proton component in oil.

Imaging of Water and Fat in the Cheese Block

The distribution of water and fat in a Cheddar cheese block is demonstrated in the image of water (Figure 6A) and the image of fat (Figure 7A) that were acquired using the CHESS technique. Even though the signal suppression was applied during the MRI experiments, the S/N was still about 50, which was certainly sufficient to construct good images. The images and surface plots of moisture (Figure 6) and fat contents (Figure 7) show the variation in the distribution of moisture and fat that existed throughout the cheese block. The moisture distribution was slightly more variable than was the fat distribution. This observation can be further supported by the statistical analyses in Table 1. Areas of high moisture concentration can be found throughout the cheese (Figure 6A). The moisture image had a low mean value, and fat had a high mean value; the standard deviation was about the same for both moisture and fat images. The higher the ratio of standard deviation to mean value was, the lower was the uniformity of the image.

TABLE 2. Moisture contents of seven locations in the read-out direction in the cheese slice: a comparison between oven-drying and magnetic resonance imaging (MRI) methods.

Location	Oven-drying	MRI	Difference
	(%)		
1	39.80	42.26	–2.45
2	39.03	43.00	–3.97
3	39.67	39.62	0.05
4	39.73	40.67	–0.94
5	39.23	38.85	0.38
6	39.24	36.77	2.47
7	39.48	36.49	2.99

Comparison of Moisture Contents Between Oven-Drying and Magnetic Resonance Imaging

The moisture contents that were determined using an oven-drying method and the MRI method at 7 locations in the readout direction and 5 locations in the phase-encoding direction of the cheese block (Figure 2) are compared in Tables 2 and 3. The moisture contents that were determined using the oven-drying method can be used as a reference to evaluate the performance of the imaging techniques. Table 2 illustrates that the result from the MRI techniques deviated from those obtained with the oven-drying method. The result from oven-drying, with a mean content of 39.8% and standard deviation of 0.3%, demonstrates a more uniform distribution than was indicated by the MRI result. The slight departure of the MRI result from the oven-drying result may be caused by magnetic field inhomogeneity. Table 3 shows that, in the phase-encoding direction, the imaging result is very close to the oven-drying result; mean moisture content was 39.9%, and standard deviation was 0.5%, indicating that moisture contents in

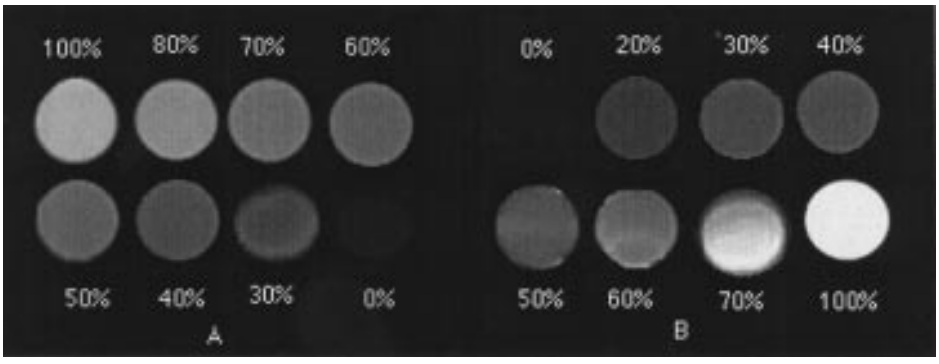

Figure 5. Magnetic resonance images of oil-in-water phantoms: A, water-suppressed images (the percentages indicate the oil contents of the mixtures); B, oil-suppressed images (the percentages indicate the water contents of the mixtures).

this direction were very uniform. The MRI result in the phase-encoding direction matches the oven-drying result better than that in the read-out direction. In the read-out direction, the large difference in moisture contents between the two methods was found at the both ends, which was probably because both ends of the cheese block were not within the uniform region of the RF field.

CONCLUSIONS

The unwanted proton signals in oil-in-water emulsion phantoms and cheese could be reliably suppressed using the adapted CHESS method. This signal suppression enables the acquisition of magnetic resonance images of water or of fat through a single experiment, and no postprocessing of data is neces-

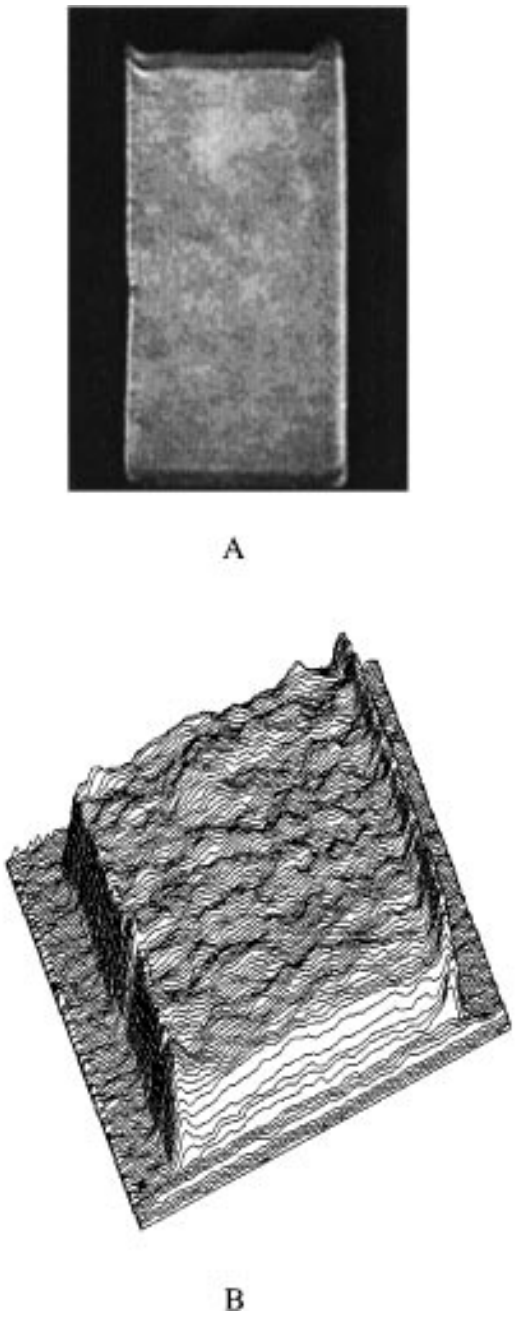

Figure 6. Moisture image (A) and corresponding surface plot (B) of the block of Cheddar cheese.

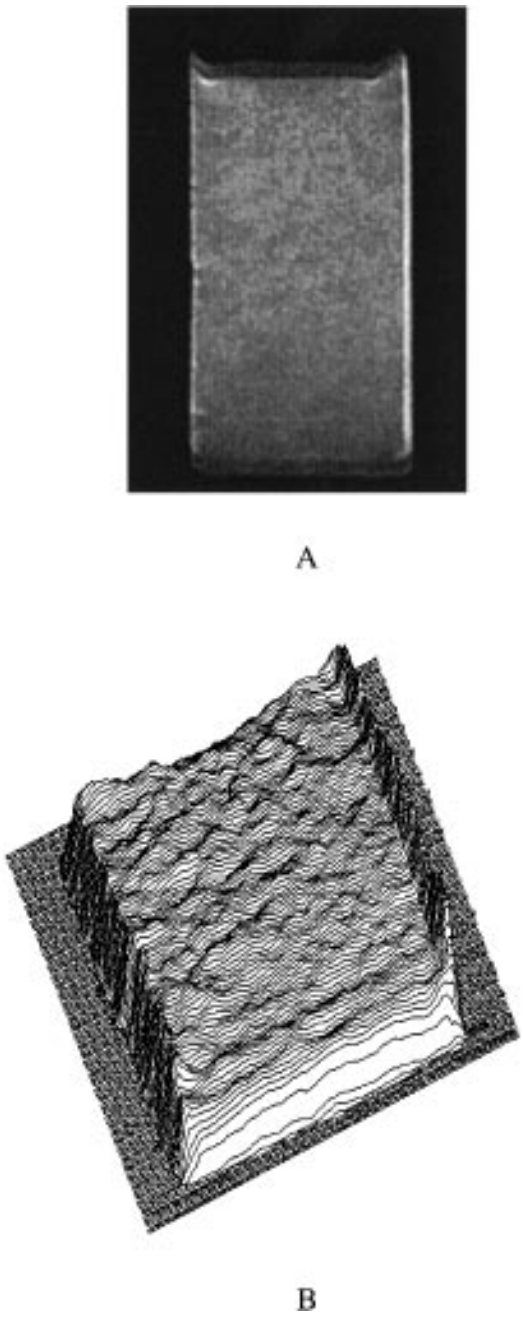

Figure 7. Fat image (A) and corresponding surface plot (B) of the block of Cheddar cheese.

TABLE 3. Moisture contents of five locations in the phase-encoding direction in the cheese slice: a comparison between oven-drying and magnetic resonance imaging (MRI) methods.

Location	Oven-drying	MRI	Difference
	(%)		
1	40.25	39.31	0.94
2	39.04	40.00	–0.96
3	39.70	40.25	–0.55
4	40.12	40.77	–0.65
5	40.17	39.22	0.95

sary. Suppression quality in the CHESS sequence can be examined directly by viewing the proton spectrum after suppression. The fat residue after fat saturation was less than 9% of the original fat peak, and the water peak residue after saturation was less than 10% of the original water peak. The experiment using water-in-oil emulsions demonstrated an excellent linear correlation between the MRI signal intensity and the concentrations of water or fat, indicating that the adapted CHESS technique can be used for quantitative analysis of water and fat distribution in food materials that are rich in water and fat. This result was supported by the success in obtaining isolated water and fat images of cheese blocks in this study.

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