Simultaneous and Nondestructive Measurement of Transient Moisture Profiles and Structural Changes in Corn Kernels During Steeping Using Microscopic Nuclear Magnetic Resonance Imaging

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ABSTRACT

Magnetic resonance imaging (MRI) of corn kernels during steeping was used to determine the internal three-dimensional transient moisture profiles. The following variables were tested: kernels with and without pericarp damage, kernels dried at high and low temperatures, kernels with high and low initial moisture contents, steepwater with and without lactic acid, and high and low steeping temperatures. The path of moisture flow into the kernel was found to follow the traditional path (into the tip cap, through the cross and tube cells, and into the endosperm), but a second major pathway through the germ and into the interior of the endosperm was confirmed. The results illustrate how MRI can be used to better understand mass transfer phenomena during steeping, including rates and paths of moisture transfer, factors influencing the moisture distribution, and major barriers to moisture transfer into the corn kernel. The results also indicate that MRI can provide insight concerning stress crack development and concomitant structural changes.

Cereal Chem. 69(6):600–606

Annual U.S. corn production is more than double that of any other cereal crop, averaging over seven billion bushels. Approximately 15% is processed by conventional wet-milling technology to produce starch and starch-based products such as high-fructose corn syrup and ethanol. In conventional wet milling, the corn is initially steeped in a solution of lactic acid and sulfurous acid for 24–40 hr. This steeping is a complex process with a variety of chemical and biochemical reactions and physical changes that result in a weakening of the endosperm protein matrix. One of the physical changes that occurs during steeping is the absorption of water, which helps to soften the kernel as well as acting as a carrier for the sulfurous acid. The rate of diffusion and the diffusional pathways affect the efficacy of the steeping operation, since disruption of the protein matrix cannot occur without the presence of the sulfurous acid being carried by the water.

Fan et al (1965) studied the diffusion of pure water and of sulfurous acid into corn kernels and found that sulfurous acid had a higher diffusion rate than water alone. Cox et al (1944) found that 8 hr is required for the SO_2 solution steep to completely penetrate the whole corn kernel. Wolf et al (1952) suggested that water is taken up through the tip cap, since the outer layer of the pericarp is cutinized. As water enters the tip cap, capillary forces move the water rapidly through the cross and tube cells of the pericarp to the top of the kernel. The water then slowly diffuses across the seed coat and the aleurone layer into the corn germ and endosperm (Krochta et al 1980).

The presence of lactic acid in steepwater may help to increase starch yields by creating holes or pits in the endosperm cell walls. C. F. Earp, C. M. McDonough, and L. W. Rooney, in a 1985 report to the Corn Refiners Association (*unpublished*), reported that the pits appeared faster in corn steeped with sulfurous acid and lactic acid than in corn steeped with sulfurous acid alone. Eckhoff and Tso (1991) showed that with the addition of lactic acid into the steeping process, both the yield and the quality of the starch produced improved. However, lactic acid has no significant effect on the rate of water absorption (Roushdi et al 1981).

Higher steeping temperatures might help open or expand the pathways for water penetration. Fan et al (1965) reported that an acceleration in absorption rate occurs with increasing temperature. Steeping temperatures in the range of 49–53°C have been found to result in optimum starch recovery, best quality starch, maximum germ separation, maximum amount of lactic acid production, and the optimum rate of steepwater evaporation (Watson et al 1955).

The heterogeneity of composition and irregularity in shape of grain kernels makes analysis of the diffusion problem complicated (Grosh and Milner 1959). Diffusion of water is not uniform through the various corn components. Vitreous endosperm takes longer to hydrate than floury endosperm. The heterogeneity of water absorption processes apparently results in degradation of the starch and subsequent lower starch viscosity (Eckhoff and Tso 1991). Little research has been done on the pattern of moisture movement inside a corn kernel during water absorption and on the effects of the corn kernel components on the resistance to moisture movement.

Fan et al (1962) indicated that the mechanism of water diffusion into cereal kernels originally proposed for wheat may also apply to other grains such as corn. That is, the volume increase of the grain accompanying absorption of water may be due to the continuous formation of cracks inside the kernels and the flow of water into the cracks during the sorption process (Grosh and Milner 1959). Specific sorption mechanisms for corn and various corn components have not been investigated.

The initial moisture content of corn has a significant effect on the moisture absorption rate (Wagoner 1948, Guritno et al 1989). Damage to kernels, stress cracks, and different drying methods may also affect the water absorption rate. However, very little information is available with which to understand these effects.

Techniques that have been used for steeping research have been limited to destructive, invasive measurements that are not taken in real time. Advancements in research tools and the importance of steeping to the wet-milling industry suggest that state-of-the-art research techniques should be utilized to study steeping.

Magnetic resonance imaging (MRI) is a technique for producing images of heterogeneous systems based on the nuclear magnetic resonance (NMR) properties of a bulk fraction distributed within the sample. In most food materials, the largest fraction is likely to be the water, although, in some imaging pulse sequences, lipids may need to be taken into account due to the chemical shift effect. Frequently used NMR properties include the proton spin density (ρ), spin-lattice relaxation time (T_1), spin-spin relaxation time (T_2), and the diffusion coefficient. ρ directly reflects the apparent water content.

The study of water in foods, in fact, was a very early application of NMR. However, the use of MRI to study foods is still in its infancy. MRI technology has great potential as a tool for the study of foods because it is noninvasive, nondestructive, accurate, and has high resolution.

Corn steeping involves a solid-liquid diffusion system, in which the corn kernel has a relatively lower proton concentration while

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the steepwater generates a large signal, which causes dynamic signal range problems (Wehrli et al 1983). Moreover, corn kernels have a very short T_2 of about 6 msec. Therefore, it is difficult to obtain a clear image of the corn kernel with a conventional spin-echo technique. Ruan and Litchfield (1992) developed a method using short interpulse sequence delay times with the gradient refocused echo technique, or fast low-angle shot (FLASH), which uses readout gradient reversals for magnetization refocusing instead of adding the 180° rephasing pulse, as in the Hahn spin-echo technique. Therefore the time between the excitation radio frequency (rf) pulse and the echo formation depends mainly on the gradient rising and falling rate and can be greatly reduced so that the T_2 decay and the diffusion signal loss can be decreased. Because of the short interpulse sequences delay time, the signal from the steepwater surrounding the corn kernel is well suppressed due to the fact that it requires longer signal recovering time. Hence, both the short T_2 and the dynamic signal range problems are solved. Therefore, this MRI technique can be used to study the steeping of the corn kernel.

Steeping technology has existed in its present form for over 100 years. To improve the steeping process, it is important to obtain some fundamental information, such as how steepwater moves through the kernel during the steeping. The objectives of this research were to develop an MRI-based quantitative method for nondestructive and noninvasive study of corn kernels during steeping, and more specifically to: 1) measure transient moisture profiles inside corn kernels and inside different corn components during steeping processes and 2) investigate the effects of steeping temperature, drying temperature, lactic acid content of the steepwater, initial kernel moisture content, and pericarp quality on water absorption during steeping processes.

TABLE I
Corn Steeping Conditions

Experiment	Temperature, °C Steeping	Temperature, °C Drying[a]	Lactic Acid (%)	Initial Moisture Content (% wb)	Percarp Quality[b]
1	53	L	0.55	13	G
2	53	L	0.55	13	D
3	53	H	0.55	13	G
4	53	L	0	13	G
5	25	L	0	13	G
6	53	L	0.55	26	G

[a] L = dried at low temperature (25°C), H = dried at high temperature (100°C).
[b] G = pericarp good, D = pericarp damaged.

MATERIALS AND METHODS

Steeping Experiments

Five variables (steeping temperature, drying temperature, lactic acid content of the steepwater, initial kernel moisture content, and pericarp quality) were tested (Table I). The steeping temperature was maintained by circulating the steepwater through a water bath with controlled temperature. During data acquisition (15 min per image), water circulation was stopped to reduce the sample vibration. The tubing for steepwater circulation was well insulated, so the steepwater temperature decreased less than 5°C during the data acquisition period. To test the effect of high initial moisture content, some corn kernels were steeped in water for 3 hr, then sealed in a bottle to equilibrate for 24 hr. To test the effect of pericarp quality, a section of pericarp 5 mm long and 1 mm deep was removed from a corn kernel on the side opposite the germ.

All samples were steeped in a tube with an 11-mm inside diameter, open at both ends. Corn kernels were held in place in the tube by a small rubber ring. Yellow dent corn kernels, variety FR27 × FR32, were used for the experiments, and all of the corn kernels used were similar in size and shape.

Data Acquisition

A Spectroscopy Imaging System (SISCO, Fremont, CA) instrument (200 MHz, 4.7 T, 330-mm bore diameter) and a

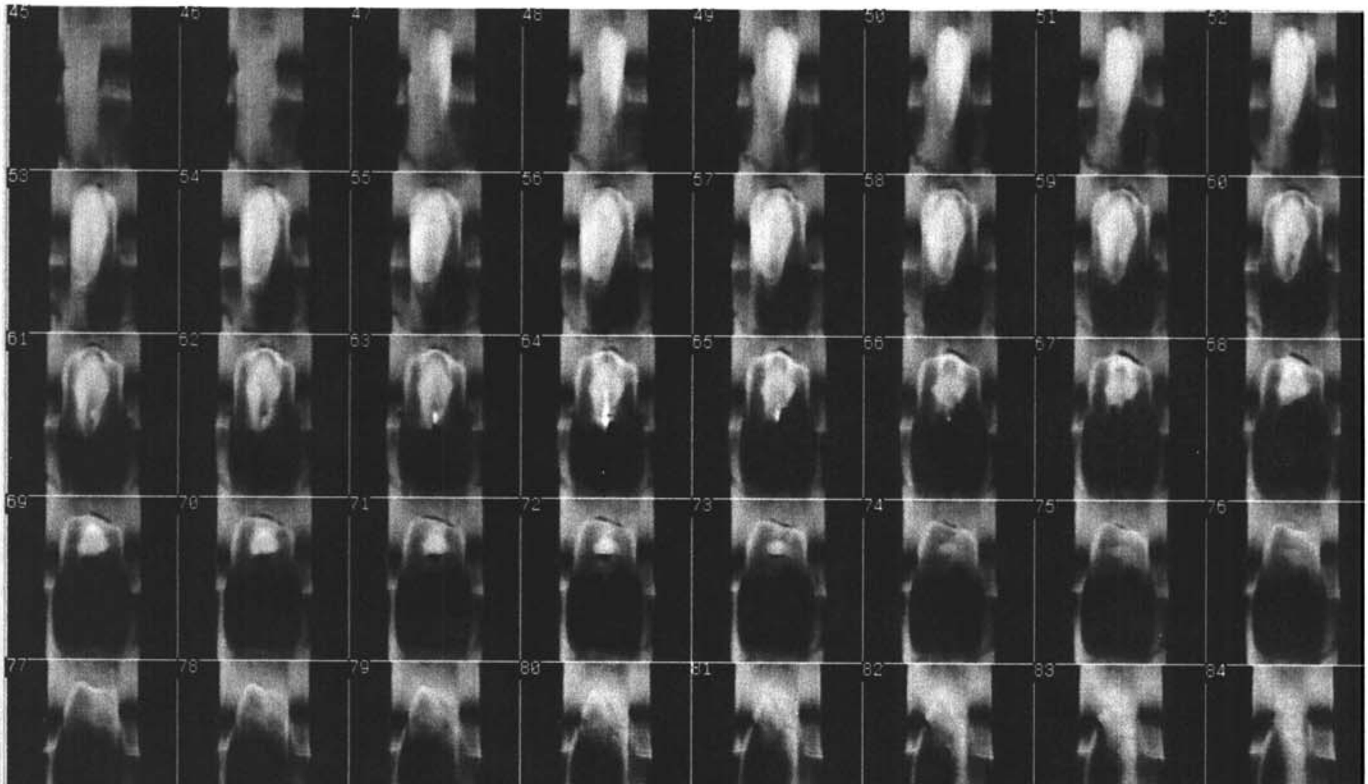

Fig. 1. Forty two-dimensional slices (128 × 128) showing the three-dimensional magnetic resonance data (128 × 128 × 128) from front to back of a corn kernel during steeping (front view). Steeping conditions: temperature, 53°C; lactic acid content, 0.55%; steeping time, 4.5 hr.

microscopic imaging probe (Doty Scientific, Inc., Columbia, SC) were used for the MRI data acquisition. The rf coil region of the probe was 17 mm (longitudinal direction), and the clear bore of the probe was 12 mm. The probe permits acquisition of high-resolution imaging data because of its small rf receiver coils, high and linear magnetic field gradients, and fast pulse/signal response time.

The improved three-dimensional (3D) FLASH (gradient refocused echo) sequence was used for the MRI data acquisition. Although a two-dimensional (2D) imaging sequence takes less time to obtain an image, it requires the use of long-slice selection pulses, which makes a short echo delay time (TE) impossible. As a result, 2D imaging sequences were not suitable for samples with short T_2, such as corn kernels. On the other hand, a 3D image is necessary to fully understand the water movement inside the corn kernel during steeping. The field of view for the 3D FLASH sequence was set to 1.3 cm in each direction. The linear magnetic field gradients used were about 0.6 mT/cm, or 6 G/cm. The TE was set to 1 msec. Seven sets of image data as a function of steeping time were acquired for each sample. Each image took about 15 min to acquire.

Data Processing and Moisture Profile Calibration

The data acquired from the imaging system were processed and reconstructed with image processing software (Potter 1990). NIHImage software (Rasband 1990) was used for processing the region of interest (ROI) of the original image and obtaining the one-dimensional (1D) moisture profiles. NCSAImage (National Center for Supercomputing Application 1990) was used for producing the 2D moisture profiles and the moisture contour images. The signal intensity (S) of each voxel using the 3D FLASH (gradient refocused echo) sequence is (Ruan and Litchfield 1992):

$$S = \rho \frac{(1 - e^{-\mathrm{TR}/T_1})\sin\theta}{1 - e^{-\mathrm{TR}/T_1}\cos\theta} e^{-\mathrm{TE}/T_2^*} \qquad (1)$$

where TR is the repetition delay time between successive pulse sequences, TE is the time between the excitation rf pulse and echo formation pulse, θ is the small flip angle, ρ is the proton spin density, T_1 is the spin-lattice relaxation time, and T_2^* is the apparent spin-spin relaxation time. T_1 values were about 0.45 and 0.35 sec, and T_2^* values were 0.017 and 0.006 sec for the germ and endosperm, respectively, of the variety of corn tested, and they changed little during the steeping tests. Image acquisition time should be short enough so that large dynamic changes do not occur during acquisition, but it should be long enough to obtain an acceptable signal-to-noise ratio for good image contrast. A 15-min acquisition time was selected, which gave a TR of 0.1 sec and, in turn, an optimum θ of 40° (equation 1).

From equation 1, the signal intensity from this pulse sequence is a function of three factors: the proton density, the T_1 factor $[1 - \exp(-\mathrm{TR}/T_1)]\sin\theta/[1 - \exp(-\mathrm{TR}/T_1)\cos\theta]$, and the T_2^* factor $\exp(-\mathrm{TE}/T_2^*)$. Therefore, with a TE setting of 1 msec, the combination of T_1, and T_2^* factors was found to be 0.3115 for germ and 0.3199 for endosperm. The difference was 0.0084, or less than 2.7%. Although T_1 and T_2^* might increase during steeping, they increase simultaneously, which tends to cancel any differences. Hence, the signal intensity was considered a linear function of proton density and moisture content, with a nonlinearity of less than 3%. The average intensity value of a corn kernel was compared to the average moisture content of the corn kernel to calibrate the moisture content profiles. This calibration was verified by slicing three different kernels and comparing the average moisture content with the moisture content obtained from the average intensity value of slices at the same location. The moisture contents of the three slices from the same location of the three different corn kernels were 36.7, 35.7, and 35.4% (wb). The moisture content obtained from the average signal intensity of the slice was 37.5%. This error was less than 4.5%, and some of the error might have come from other factors, such as different kernel shapes and slicing errors. Therefore, this technique can be used as an approximate method to determine the moisture content profiles. For more accurate moisture values, exact proton density mapping must be done, as discussed previously (Ruan and Litchfield 1992).

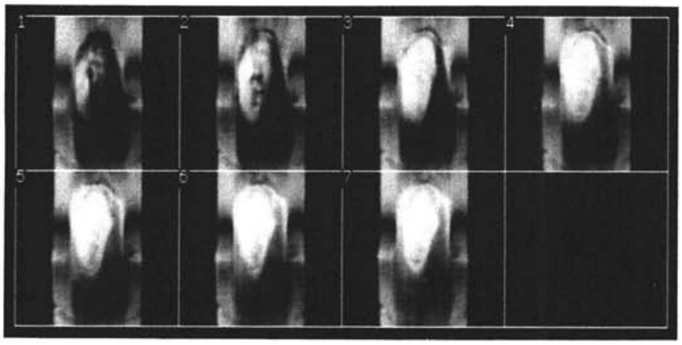

Fig. 2. Two-dimensional magnetic resonance images of a corn kernel (slice no. 59 from Fig. 1) during steeping. Images 1–7 are for steeping times of 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 hr, respectively.

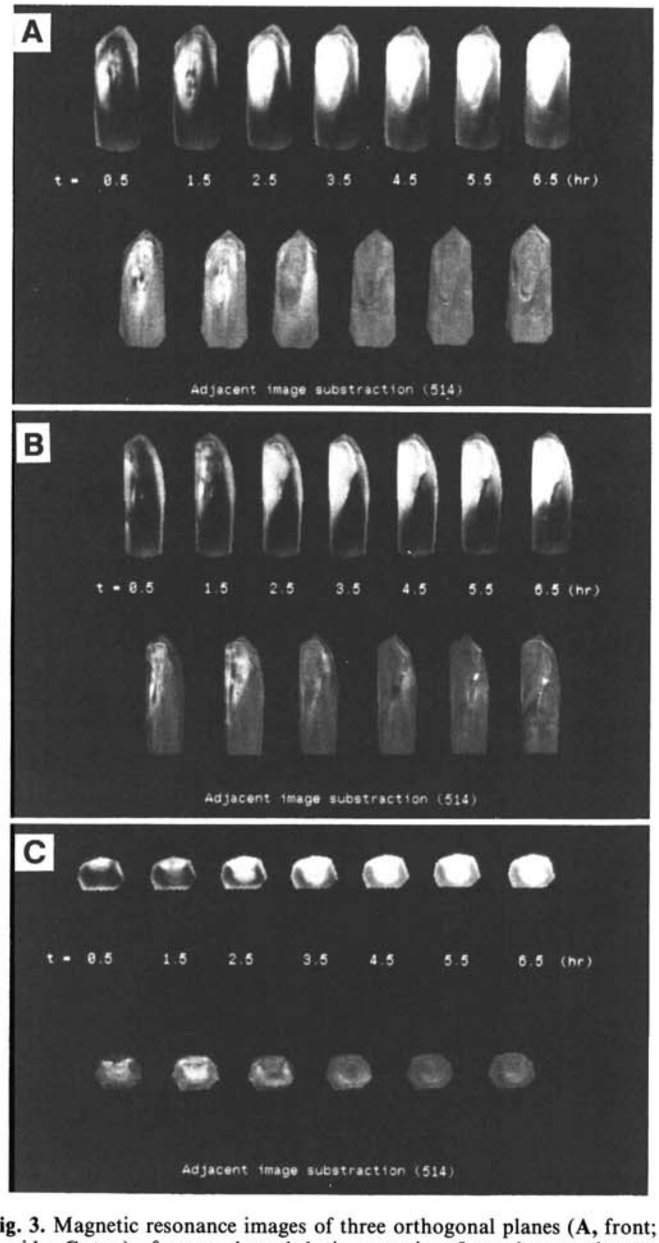

Fig. 3. Magnetic resonance images of three orthogonal planes (**A**, front; **B**, side; **C**, top) of a corn kernel during steeping. In each case, the top row is the region of interest, or the corn-kernel-only images; the bottom row is the adjacent-image subtractions. Steeping conditions: temperature, 53°C; lactic acid content, 0.55%; steeping time, 0.5–6.5 hr.

RESULTS AND DISCUSSION

Forty 2D (128 × 128) sequential slices showing the 3D data from the front to back of a kernel during steeping are shown in Figure 1. The spatial resolution of the 2D slices was about 100 μm. One slice of the 3D data (slice 59 in Fig. 1) is shown as a function of steeping time (Fig. 2). The ROI of the images, or in this case the corn-kernel-only images, was obtained (Fig. 3) to remove noise from the surrounding steepwater in the original images. Front, side, and top views of the ROI images show mass transfer in the kernel during steeping (Fig. 3). 2D contour images and 2D moisture profiles were produced from the 2D ROI images (Fig. 4A and B).

From Figure 3, steepwater first moved into the corn kernel through the tip cap to the space between the germ and endosperm and to the cross and tube cells of the pericarp layers. Steepwater diffused quickly into the germ and slowly into the endosperm. These results were similar to the diffusion paths of gaseous SO_2 (Eckhoff and Okos 1989). Moisture contents were never uniform inside the corn kernel during the test period (Fig. 5). In Figure 5C, the two low-moisture areas near the 1- and 4-mm locations correspond to the endosperm area around the germ, and the high-moisture areas near the 2- and 3-mm locations correspond to the germ area where moisture absorption was faster.

The rates of moisture change in the germ and endosperm during steeping were clearly different (Fig. 6). With steeping at 53°C and 0.55% lactic acid, the fastest period of moisture increase for the germ was during the first 2.5–3.5 hr of steeping, and the average rate of moisture increase during this period was about 10% per hour. However, for the endosperm, it took about 2.5 hr for the high rate diffusion to begin, and the highest average rate of moisture increase during this period was only 7% per hour (Figs. 5 and 6).

For the pericarp-damaged kernel, the side view and the top view at the pericarp-damaged plane are shown in Figure 7. Comparison of the 1D moisture profiles at the pericarp-damaged plane (Fig. 8B) with those at a plane above the damage (Fig. 8A) and with those at the same location as the pericarp damage but in a kernel with good pericarp (Fig. 8C) showed that pericarp did have the effect of resisting the movement of water into the kernel. With the section of pericarp removed, the endosperm attained 12% moisture content in about 0.5 hr, while with the pericarp intact it took about 1.5 hr to reach the same moisture content. This confirmed previous results showing that water absorption time could be shorten if the diffusion limitations, such as pericarp, were removed (Watson and Sanders 1961, Eckhoff and Tso 1991). However, about 2.5 hr were still required after the moisture content reached 10–15% to begin the fast moisture diffusion period, as mentioned previously (Fig. 6).

Comparison of Figure 9, the 1D moisture profiles during steeping of a corn kernel dried at high temperature, with Figure 5C showed that the high-temperature-dried corn kernel took longer to hydrate, so the rate of moisture increase was relatively lower, especially in the germ. For high-temperature-dried corn, the highest rate of moisture diffusion was only about 5% per

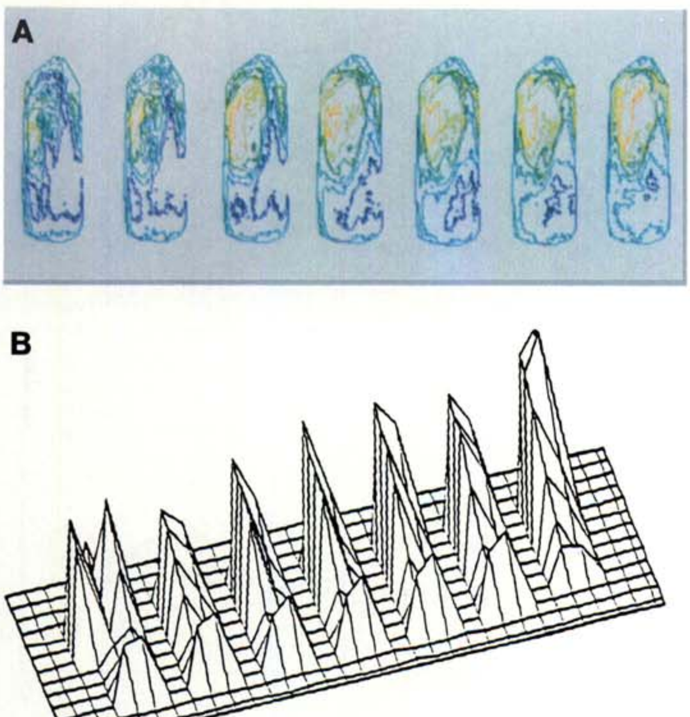

Fig. 4. Two-dimensional contour images (**A**) and moisture profiles (**B**) produced from the top row of Fig. 3A.

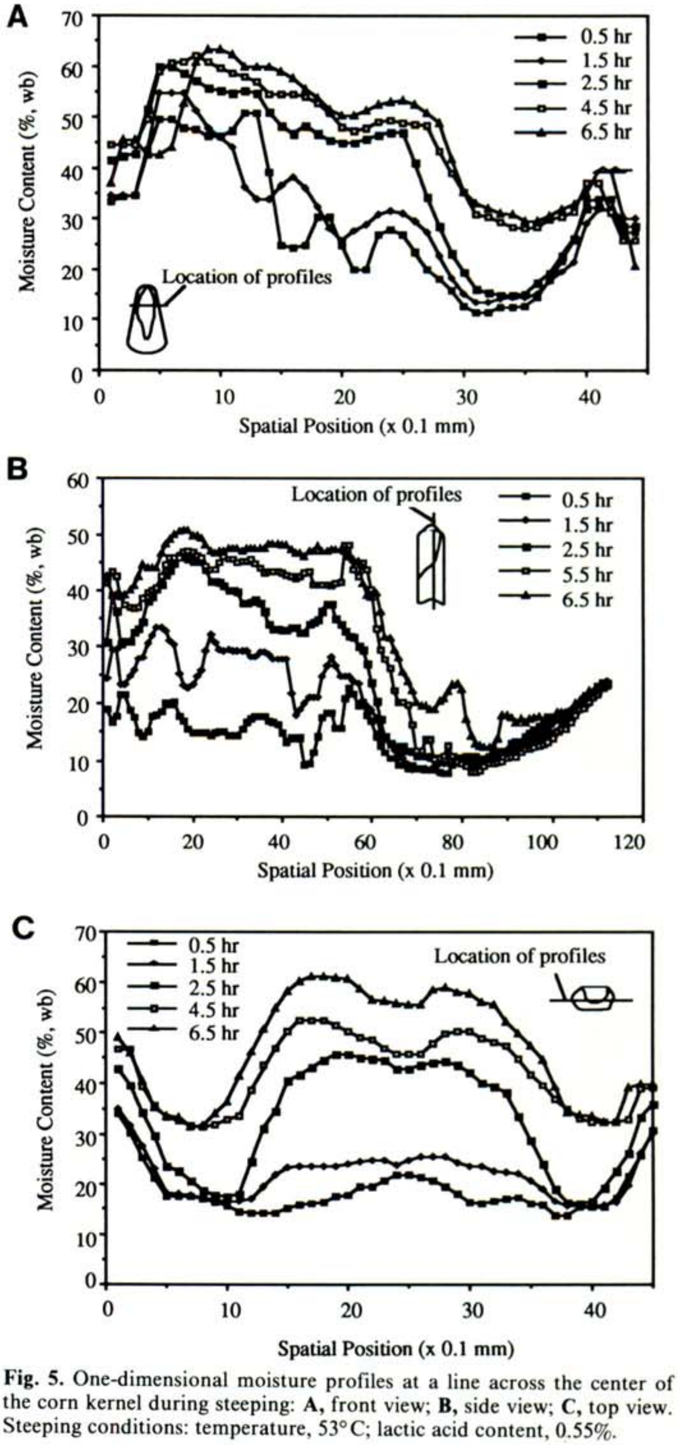

Fig. 5. One-dimensional moisture profiles at a line across the center of the corn kernel during steeping: **A,** front view; **B,** side view; **C,** top view. Steeping conditions: temperature, 53°C; lactic acid content, 0.55%.

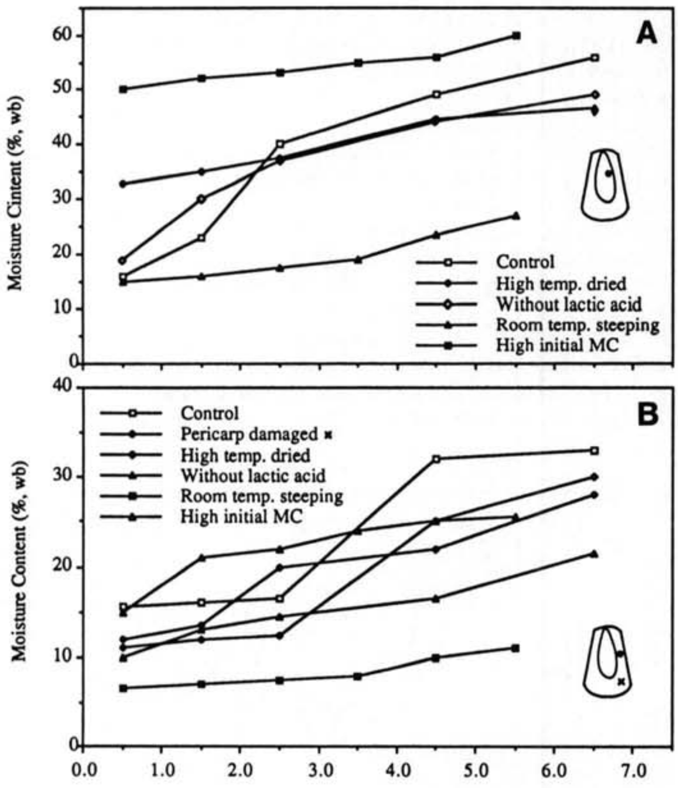

Fig. 6. Moisture changes inside corn kernels during steeping at different conditions at a point in the germ (**A**) and a point in the endosperm (**B**). The control was a corn kernel with low initial moisture content that was dried at low temperature and steeped at 53°C with 0.55% lactic acid.

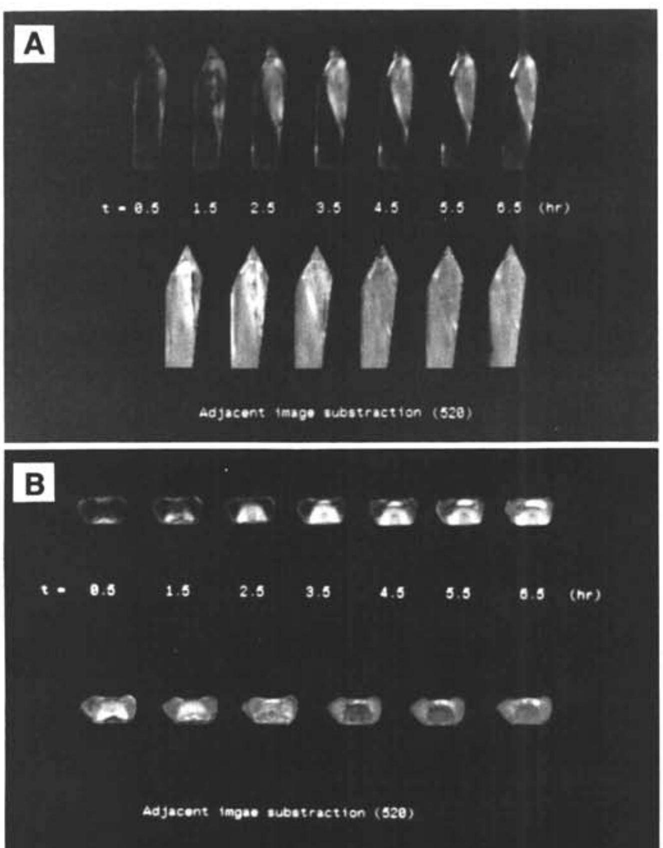

Fig. 7. Region-of-interest images of a corn kernel with damaged pericarp, during steeping. **A,** side view; **B,** top view of the pericarp-damaged plane. Steeping conditions: temperature, 53°C; lactic acid content, 0.55%.

hour in the germ, whereas for low-temperature-dried corn, it was about 18% per hour (Fig. 6). This might be because the high drying temperature denatured the protein and produced case hardening, so the moisture sorption characteristics were changed (Freeman 1973). However, the initial water absorption rate was slightly higher (Fig. 6), which might be due to the small cracks inside the kernel caused by the high drying temperature.

Comparison of Figure 10, the 1D moisture profiles of a corn kernel steeped with no lactic acid, with Figure 5A showed that 0.55% lactic acid accelerated the rate of moisture absorption, especially in the floury and vitreous endosperms. Furthermore, the lactic acid seemed to help the corn kernel attain a higher moisture content during the first 6.5 hr of steeping (Fig. 6). Similar results were found by Cox et al (1944) for steeping in sulfurous acid solution. This might be because the acid increased the water mobility or altered the material's sorption characteristics.

Comparison of Figure 11, the 1D moisture profiles of a corn kernel during room-temperature steeping, with Figure 10 demonstrated the effect of steeping temperature. Moisture movement

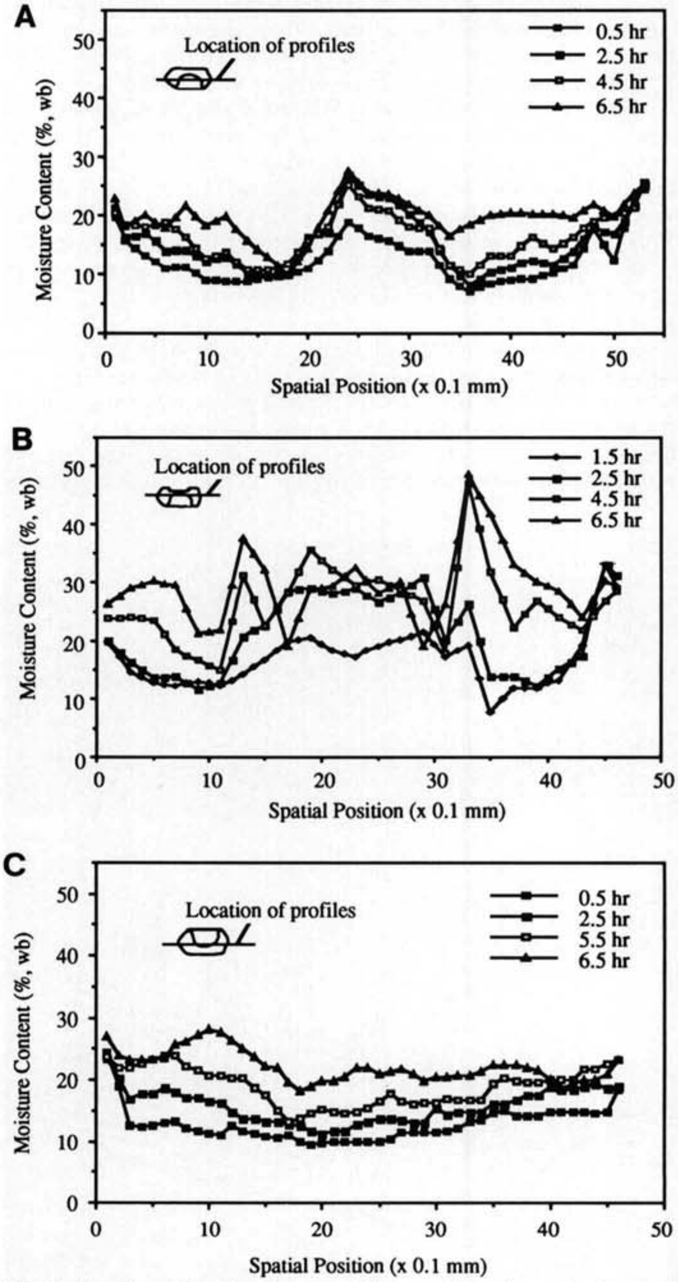

Fig. 8. One-dimensional moisture profiles at a plane above the damaged pericarp (**A**), at the pericarp-damaged plane (**B**), at the same location as **B**, but in in a corn kernel with good pericarp (**C**). Steeping conditions: temperature, 53°C; lactic acid content, 0.55%.

was dramatically reduced at the low steeping temperature. The moisture content of the endosperm did not reach the 10–15% range during the entire test period, and no high diffusion rate period was observed (Fig. 6). After 5.5 hr of steeping, the volume expansion of the high-temperature-steeped corn kernels was about 11%, whereas that of the low-temperature-steeped corn kernel was less than 4%. Higher steeping temperature may add the thermal expansion effect that helps to decrease the density and open spaces for water absorption. These results illustrate, by way of imaging, a phenomena that has been demonstrated previously, namely that effective diffusivity in a corn kernel increases with temperature.

Comparison of Figure 12, the 1D moisture profiles of a corn kernel with higher initial moisture content (26%, wb) during steeping, with Figure 5A clearly showed that corn kernels with higher initial moisture content have a lower moisture absorption rate in the germ during the first 4.5 hr of steeping. However, the high-rate moisture absorption in the endosperm occurred earlier; in fact, it started at the beginning of the steeping test (Fig. 6), which might be because the moisture content of the endosperm was already at least 10–15% before the steeping test.

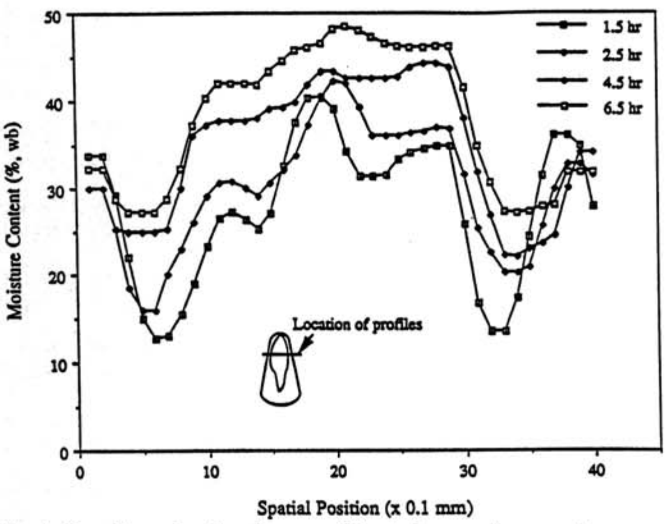

Fig. 9. One-dimensional moisture profiles during steeping, at a line across the center of a corn kernel dried at high temperature. Steeping conditions: temperature, 53°C; lactic acid content, 0.55%; initial drying temperature, 100°C.

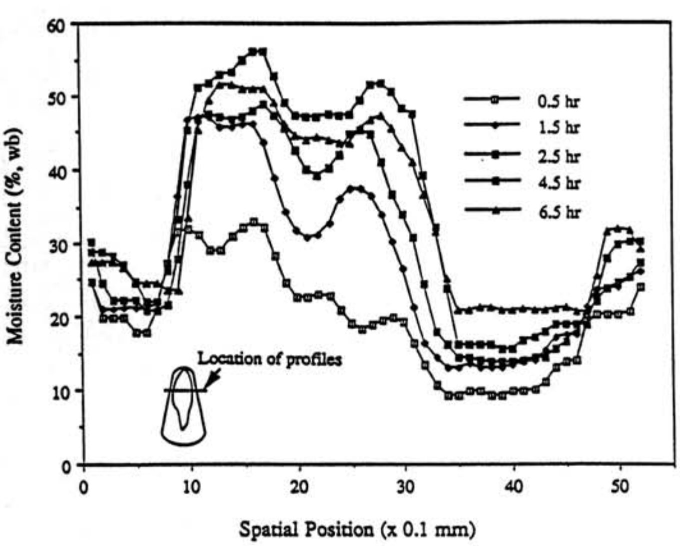

Fig. 10. One-dimensional moisture profiles at a line across the center of a corn kernel during steeping with no lactic acid in the steepwater. Steeping conditions: temperature, 53°C; lactic acid content, 0%.

2D images with the moisture profiles of an alkali-debranned corn kernel during steeping clearly showed stress crack development (Fig. 13). The stress crack was initiated and grew at the high-moisture-gradient region, which is similar to results obtained for drying-induced cracking (Song and Litchfield 1991; Song et al, *in press*). The alkali might have dissolved some of the structural matrix inside the corn kernel so that the strength of the corn kernel decreased.

CONCLUSIONS

The 3D images and transient moisture profiles of corn kernels at different steeping conditions (with and without pericarp damage, with high and low initial drying temperature, with high and low initial moisture content, with and without lactic acid, and with high and low steeping temperature) were obtained by MRI. The effects of these factors on each component of the corn kernel during steeping could be observed directly from 2D images, ROI images, contour images, image subtractions, and one- and two-dimensional moisture profiles produced from images. More replicate experiments with statistical analysis must be done to confirm these results, but the results clearly show the power of MRI as a tool with which to better understand the steeping process.

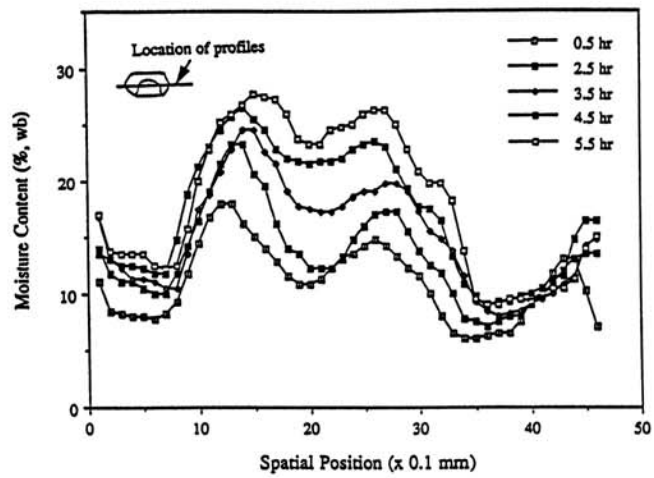

Fig. 11. One-dimensional moisture profiles at a line across the center of a corn kernel during room-temperature steeping. Steeping conditions: temperature, 25°C; lactic acid content, 0%.

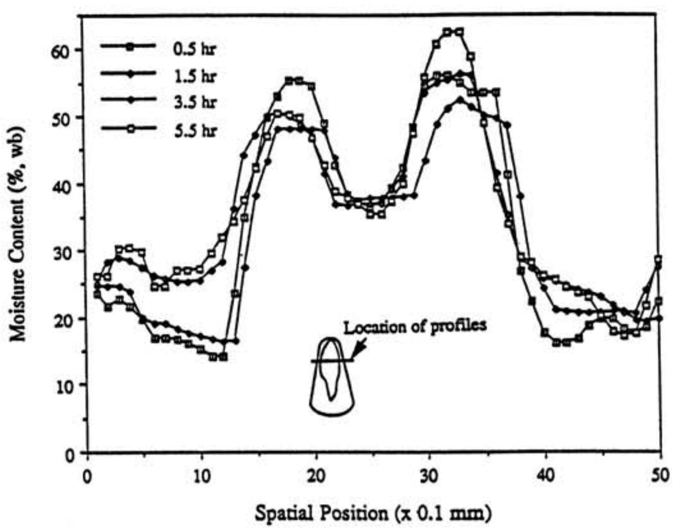

Fig. 12. One-dimensional moisture profiles during steeping, at a line across the center of a corn kernel with high initial moisture content. Steeping conditions: temperature, 53°C; lactic acid content, 0.55%, initial moisture content, 26% (wb).

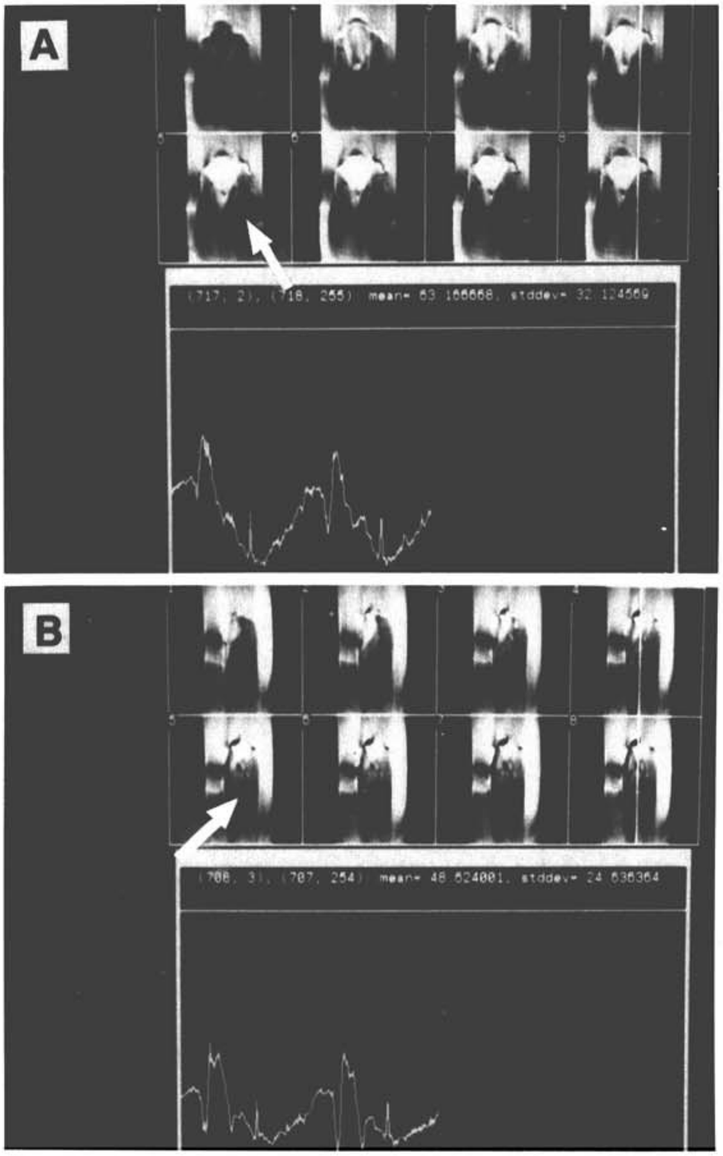

Fig. 13. Magnetic resonance images of two orthogonal planes (upper part) and local moisture profiles (lower part) of an alkali-debranned corn kernel during steeping, showing stress crack development in front view (**A**) and side view (**B**). Slices 1–8 are for steeping times of 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, and 7.5 hr, respectively. The stress crack was first visible from both views in slice 5 (arrows). Steeping conditions: temperature, 53°C; lactic acid content, 0.55%.

ACKNOWLEDGMENTS

This study was funded by the National Science Foundation, NSF grant CBT-8808748. Appreciation is expressed to the Biomedical Magnetic Resonance Laboratory at the University of Illinois, Paul C. Lauterbur, director, for the use of its facilities. Thanks are also extended to Clint Potter, National Center for Supercomputing Applications, for providing the image processing software.

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[Received July 18, 1991. Accepted May 8, 1992.]