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Water Status of Two Gelatin Gels during Storage as Determined by Magnetic Resonance Imaging

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ABSTRACT

A two-gelatin-gel model system was used to investigate the water status of two gels with different A_w , 0.968 (H-gel) and 0.828 (L-gel), using magnetic resonance imaging (MRI) techniques. A multi slice-multi echo pulse sequence was applied and the transverse relaxation time (T_2) of water protons was calculated. During storage the average T_2 values of H-gel decreased, while those of L-gel increased. However, at the end of storage (15 days) when the A_w of two gels were the same, the average T_2 values of L-gel were larger than those of H-gel, and heterogeneous T_2 values were observed in both gels. This was due to water migration between the two gels and redistribution of water in each gel during storage. The two-gelatin-gel model system and nondestructive MRI technique established in this study offer a good method to monitor moisture redistribution between gels with different A_w and to investigate the water status and structural changes in food systems during storage.

Key words: water status, gelatin gel, magnetic resonance imaging (MRI), transverse relaxation time (T_2), water activity (A_w)

INTRODUCTION

Water is the most important component in foods that affects their quality, stability, textural properties, and processing. Water status describes the chemo-physical state of water molecules and is associated with the interaction between water and other molecules. However, various foods with the same water content differ in stability and it has been recognized that water content alone is not an adequate indicator of food stability^(1,2). Scott introduced the concept of water activity (A_w) as an indicator of water status⁽³⁾. Although A_w has been widely used as a measurement for quality control of lipid oxidation⁽⁴⁾, nonenzymic browning⁽⁵⁾, nutritional quality⁽⁶⁾, texture⁽⁷⁾, and microbial growth⁽⁸⁾, several studies also indicated some of the theoretical and practical limitations of A_w ^(2,9,10,11). Pham and others found that water activity failed to predict mold germination with changing solid composition⁽¹¹⁾. They also claimed NMR was a better indicator of water status and different availabilities to mold spore germination. The NMR technique is based on the measurement of absorption or emission of electromagnetic radiation in the radio-frequency region⁽¹²⁾. When a nucleus is placed in an intense magnetic field, its energy levels split and the interaction between molecules influences the splitting of energy levels and the relaxation process of the excited nucleus.

It has been noted that food products are usually in non-equilibrium systems during processing and might be at a state of "pseudo" stability during storage⁽¹³⁾. Therefore, the food system's dynamic variables, including relaxation rate, molecular mobility, diffusion, viscosity, exchange rates, nucleation, crystallization, glass-rubber transitions,

and melting should be investigated rather than its equilibrium properties. The dynamic aspects of water status, such as relaxation rates, molecular mobility and diffusion, of water molecules in food systems have been successively measured by using nuclear magnetic resonance techniques⁽¹⁴⁾. In addition, proton (^1H), deuterium (^2H) and oxygen-17 (^{17}O) are the spin probes that are suitable for characterizing water mobility in heterogeneous and complex systems such as foods or biological systems^(15,16). The transverse (or spin-spin) relaxation time (T_2), longitudinal (or spin-lattice) relaxation time (T_1), and self-diffusion coefficient of water measured by NMR all imply non-invasive water dynamic information of foods during processing or storage^(17,18). The self-diffusion coefficient reflects the translational molecular motion, while T_1 and T_2 reflect complex interactions involving rotational motion and exchange⁽¹⁹⁾. Tang and others explored the relationship between T_2 and the microscopic distribution of water among the sub-granule compartment in various starch granules⁽²⁰⁾.

In recent years, magnetic resonance imaging (MRI) techniques are gaining increased applications in food science⁽²¹⁾, biomaterials⁽²²⁾ and biology⁽²³⁾. The basic principle of MRI is the same as NMR, but for MRI a gradient magnetic field is imposed to determine the location of protons in the space. MRI methods not only provide complementary information for molecular-dependent contrast parameters such as self-diffusion coefficient, water content and relaxation times (T_1 and T_2), which can be used to reflect the dynamic states of water just like NMR methods; but they also provide information on spatial structural changes in a complex system using noninvasive and nondestructive measurements⁽²⁴⁾. These parameters can be related to the spatial distribution of molecular mobility of

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water and the structural changes of the systems during processing and storage^(25,26). Toorn and others used MRI to follow the changes in time-dependent water status of tulip bulbs during storage for 12 weeks at 20°C and observed the redistribution of water between different bulb organs⁽²⁷⁾. Water absorption of rice kernels, pasta and noodles during cooking and water redistribution during storage investigated by MRI have also been reported^(25,26,28,29).

In this study, a two-gelatin-gel model system was designed to investigate the moisture migration and water status during storage of two gelatin gels with different A_w . The water content and A_w of the two gelatin gels were also determined during storage for comparisons.

MATERIALS AND METHODS

Gelatin and sorbitol used for gel preparation were purchased from Nacalai Tesque Inc. (Kyoto, Japan) and Showa (Tokyo, Japan). Parafilm® (American National Can, Chicago, USA) was used as a film in the control model system because it is a good water barrier. The thickness of Parafilm® was 130 μm .

A two-gelatin-gel model system was designed as shown in Figure 1. This system was used to investigate water status of two different A_w gels during storage with or without film by using an MRI technique. The model system included two Teflon cylinder moulds (3.5 cm in diameter \times 3.0 cm in height), which had a rectangular well of 1.5 \times 1.0 cm inside each mould for setting of the gel. The pit on the surface of the Teflon mould was designed for inserting a tube of reference solution (for example, CuSO_4 solution) or standard material to evaluate the variations of magnetic homogeneity during measurements. Gelatin gels with different A_w were prepared by adding adequate amounts of sorbitol into the gelatin sol (gelatin:water = 24:76, w/w). The ratios of gelatin:water:sorbitol of two gels used in this study were 24:76:5 for the gel with $A_w = 0.968$ and 24:76:100 for the gel with $A_w = 0.828$ (at 20°C). For gel preparation, gelatin and sorbitol were first dissolved separately in water at 60°C for 2 hr, then mixed together and kept at 80°C for another 1 hr. The mixture was cooled down to about $45 \pm 5^\circ\text{C}$, and then poured into the rectangular well of the Teflon mould with tape sideboards. The moulds were then stored at 4°C for 2 days. Before performing the MRI measurements, the tape sideboards were removed from the Teflon mould and the gelatin gel was leveled off with a sharp knife. Then two Teflon moulds with different A_w were sealed face-to-face with Teflon tape. The above two-gelatin-gel model system was used for the MRI measurements at $17 \pm 2^\circ\text{C}$. After each MRI measurement, the whole model system was stored at 4°C until the experiment was complete. Two test model systems, one without a film and another with Parafilm® in between the gels, were investigated during the storage.

Changes of water status in the two gelatin gels during storage were investigated by measuring T_2 maps. A Bruker

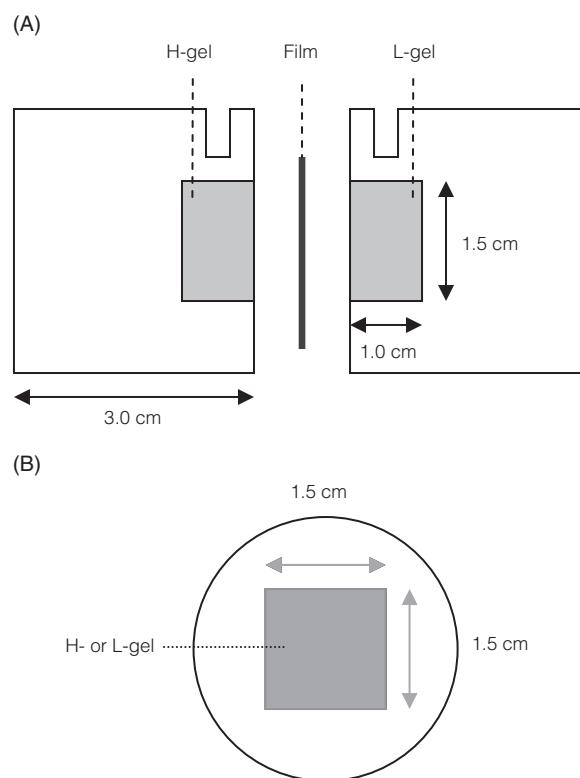


Figure 1. Schematic diagrams of two-gelatin-gel model system: (A) side view of two Teflon moulds filled with gels and a film in between gels. H-gel and L-gel are gelatin gels with higher and lower A_w , respectively; (B) side view from another side of Teflon mould with a well having a surface area of 1.5 \times 1.5 cm and a depth of 1.0 cm (not shown in the Figure).

medspec 3T system (Ettlingen, Germany) with a micro-gradient system (35 mm in inner diameter, using a multi slice-multi echo (MSME) pulse sequence was provided by the manufacturer (Bruker, Ettlingen, Germany). Parameters of the MSME sequence were repetition time (TR) = 3000 ms, 20 echo times (TE) starting from 15 ms with 15 ms increment, pixel matrix size = 128 \times 64, field of view (FOV) = 28 mm, slice thickness = 5 mm and number of excitations (NEX) = 4. The acquiring time was 12.8 min for one acquisition. The receiver gain was constant for each scan. The changes in T_2 values of the two-gelatin-gel model system during storage were measured in duplicate and the standard deviations of the T_2 values at the same position was 4-20 ms in both experiments.

T_2 is responsible for the loss of spin coherence of magnetization in the xy-plane and is associated with water mobility^(30,31). The T_2 map of water protons was constructed from a series of MR images acquired at different echo times. The map was fitted by the following equation: $S = S_0 \exp(-TE/T_2)$, where S is the pixel signal intensity measured at echo time (TE), and S_0 is the signal intensity at $TE = 0$ ⁽³²⁾.

Several sets of the two-gelatin-gel model systems were also prepared for the measurements of moisture content and A_w at different storage time. The gelatin gels from two

Table 1. Changes of composition, moisture content, Aw and T₂ value of two gels during storage

Storage time (day)	H (Aw = 0.968 initially)				L (Aw = 0.828 initially)			
	Moisture content (%) ^a	Aw	T ₂ (ms)		Moisture content (%) ^a	Aw	T ₂ (ms)	
			Range ^b	Average ^c			Range ^b	Average ^c
With Parafilm [®] in between gels								
0	71.59	0.968	139-145	143	32.08	0.828	41-44	42
15	71.87	0.970	133-143	138	34.33	0.828	41-50	43
Without Parafilm [®] in between gels								
0	71.59	0.968	124-151	146	32.08	0.828	31-63	45
3	48.49	0.964	65-102	88	45.60	0.888	56-96	77
7	47.97	0.931	53-76	63	52.24	0.914	71-94	85
15	45.64	0.914	43-73	56	54.50	0.913	67-97	89

^aThe moisture content of gelatin gel during storage was recalculated based on the weight of total components of the gel, on the assumption that water was the only component redistributed in the model system.

^bT₂ range is the range of T₂ values along the central line in each gel.

^cT₂ average is the mean value of T₂ along the central line in each gel.

Teflon moulds were removed separately, and their moisture contents were determined by pre-drying in a 60°C oven for 24 hr and drying with P₂O₅ in a desiccator for one week. The moisture content of gelatin gel during storage was calculated by taking the weight differences before and after drying, divided by the weight of gelatin gels. The Aw of gels were measured by using an AquaLab CX-3 (Decagon Devices Inc., Washington, USA). The standard deviations of moisture content and Aw measurements were 0.21-1.44% and 0.001-0.008, respectively.

RESULTS AND DISCUSSION

I. Water Status of Gels during Storage

Table 1 shows the changes in water status of two gels in the model system during storage, including moisture content, Aw and T₂ value. In the two-gelatin-gel model system with Parafilm[®] in between, there were no significant changes in moisture content, Aw and T₂ value during 15 days of storage. Each gel maintained its own equilibrium state and there was no change in the dynamic aspects of water molecules through the entire period of storage. On the contrary, the moisture content, Aw and T₂ value of two gelatin gels without a film in between changed with time, as expected. The moisture content, Aw and T₂ value of the gel with high Aw (H-gel) and a small amount of sorbitol decreased as storage time increased. In contrast, those samples of the gel with low Aw (L-gel), which contained a large amount of sorbitol (20 times that of H-gel) and initially had low Aw (0.828), increased with increasing storage time. The Aw of two gelatin gels without a film in between approached one another by the end of storage (15 days), but the moisture contents and T₂ values were still quite different. Moisture content is the quantity of water molecules, whereas Aw is the ability of water molecules to evaporate from liquid state to gas state. The interaction of water molecules, solutes and macromolecules, and capillary effect of gelatin gel structure will influence the Aw of a gel system. The T₂ values measured by NMR or MRI

primarily represent the chemical environments of protons of water molecules, which are also influenced by the interactions of water molecules, solutes and macromolecules.

It was found that the average T₂ value of L-gel increased from 45 ms to 89 ms due to driving water inwards from the H-gel, and the average T₂ value of the H-gel decreased from 146 to 56 ms due to transferring water out of H-gel. It is worth noting that different T₂ values were observed in both gels even after storage for 15 days. A larger T₂ value of L-gel at the end of storage indicated that water molecules were more mobile in the L gel than in the H-gel. The different T₂ values of the two gels and the wide range of distribution of T₂ values in each gel indicated continuous water redistribution, even after storage for 15 days.

II. MRI T₂ Maps of Gels during Storage

T₂ maps of two-gelatin-gel model systems without and with Parafilm[®] in between the gels during storage are shown in Figures 2(A)-(D) and Figures 2(E)-(H), respectively. The brighter maps with higher T₂ values indicate that there were more mobile water molecules in gelatin gels than those in the darker maps⁽²⁷⁾. The T₂ maps of the two-gelatin-gel model system without a film in between show that the mobility of water molecules of H-gel decreased with increasing storage time, whereas the water mobility of L-gel increased with increasing storage time (Figures 2(A)-(D)). After 7 days of storage, the H-gel became darker, while the L-gel became brighter. This indicates that the water molecules were more mobile in the L-gel than in the H-gel. The maps also show that the L-gel swelled significantly, whereas the H-gel shrank significantly, thereby the interface of the two gels shifted upward from the center. The swelling of L-gel is attributed to water migration from H-gel to L-gel, which contained 20 times more sorbitol than the H-gel. The different brightness of maps of the two gels after 15 days of storage (Figure 2(D)) reveals that (1) both gels were not at true equilibrium based on the dynamic aspect as determined by their water mobility, even after storage for 15 days; and (2) the water reabsorbed by L-gel had weak interactions with the sorbitol or gelatin gel

network.

Fullerton and others proposed a hydration model of globular protein molecules and divided water into three different states, i.e. bulk water, structured water, and bound water⁽³³⁾. The T_2 values are the sum of all fractions of water states with their fraction weighted⁽³²⁾. In the gelatin gel of this model system, the mobility of water molecules was also hypothesized to have several status due to the various degrees of interaction among water, solute (sorbitol), and the gelatin gel matrix. The same A_w but different T_2 values found in a two-gelatin-gel model system without a film after 15 days of storage indicated that water mobility was strongly associated with the interactions of water molecules and solute, as well as the environmental interactions of molecules. In the L-gel, the water absorbed from the H-gel resulted in an increase of bulk water

fraction but a decrease of structured and bound water fractions. In the H-gel, water was driven away by the L-gel, resulting in an increase of the fractions of structured and bound water but a decrease of the fraction of bulk water. Therefore, the gel maps after 7 days of storage are attributed to the net effect of the T_2 value because the L-gel had a larger average T_2 value. The redistribution of water molecules not only changed the amount of water, but also modified the chemical and/or physical environmental interactions of sorbitol and gelatin gels. This also resulted in the significant changes in the gel structure and appearance during storage.

The T_2 maps of the two-gelatin-gel system separated by Parafilm[®] show no significant change in the brightness and shape of both gels during the storage period (Figures 2(E)-(H)). This further indicates that the Parafilm[®] had a good water resistance that prevented migration of water between the two gels, and so is suitable for use as a control system.

III. Profiles of Water Mobility in Gels during Storage

Figure 3 shows the T_2 profiles along the central line (A), as indicated in the T_2 maps of two-gelatin-gel model systems with or without Parafilm[®] during storage. During storage, there was no significant change in T_2 profile for either L- or H-gel in the model system when Parafilm[®] separated the gels (Figure 3(D)). Further more, there was no moisture transfer or redistribution between the two gels nor within each gel when Parafilm[®] was used as a barrier. In contrast, right after storage for 1.83 hr, a decrease of T_2 value in the H-gel and an increase of T_2 value in the L-gel were observed near the interface of the two gels which were not separated by film (Figure 3(C)). Due to gradual water transfer the interface of the two gels moved from the position of 0 mm to -4 mm. The significant migration of water from H-gel to L-gel and re-equilibration of water mobility, especially at the interface of the two gels, were observed after 3 days of storage. This was attributed to the greatest driving force at the intersection of the two gels resulting from the greatest environmental difference of sorbitol contents. It is also noteworthy that the T_2 values of H-gel varied, but showing a gradient from the lowest T_2 value to the highest T_2 value from the edge to the interface of the two gels after storage for 7 days. The T_2 value of L-gel increased gradually from the interface on the swollen areas, but remained constant on other areas of the gel. This implies that the non-equilibrium state of the two gels not only existed in many water states but also caused different physical properties, even though the two gels had the same A_w . Generally, equilibrium is reached when the components of a system have reached the same A_w ⁽³⁴⁾, as observed in the two-gelatin-gel model systems without a film after storage for 15 days in this study (Table 1). However, the two gels were not in dynamic equilibrium and had not been stabilized, based on the water mobility determined by the T_2 values of the gels, even though they had

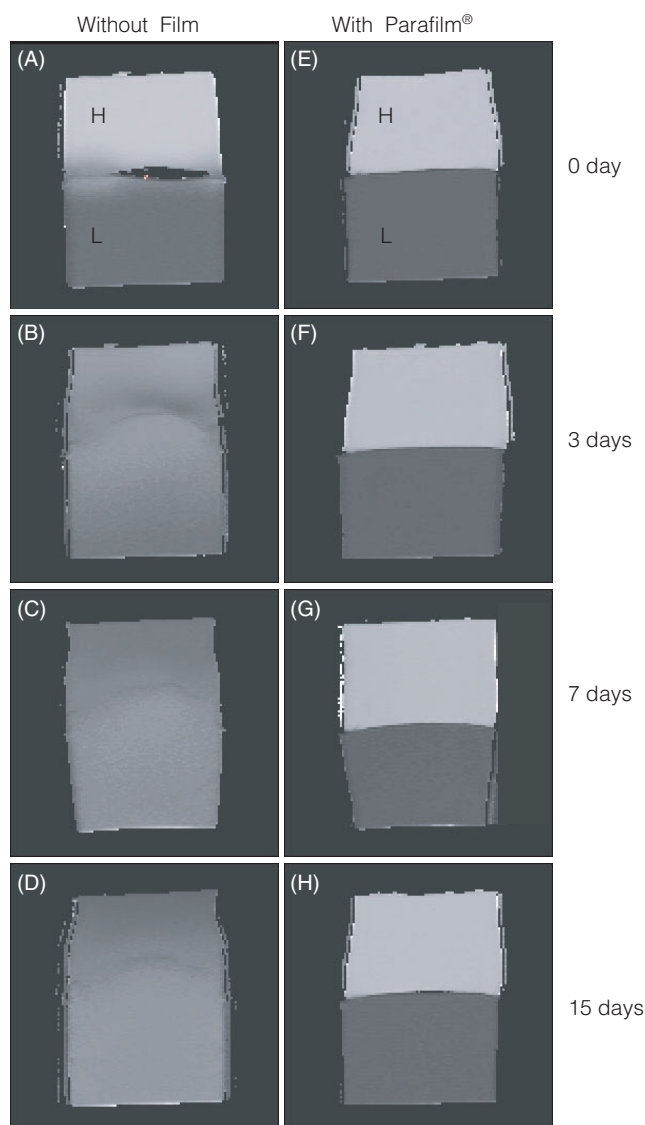


Figure 2. T_2 maps of gelatin gels during storage. Maps (A) to (D) in the left column are top views of the two gels without a film in between. Maps (E) to (H) in the right column are those of two gels with Parafilm[®]. Top (H-) gel had high A_w and bottom (L-) gel had low A_w initially.

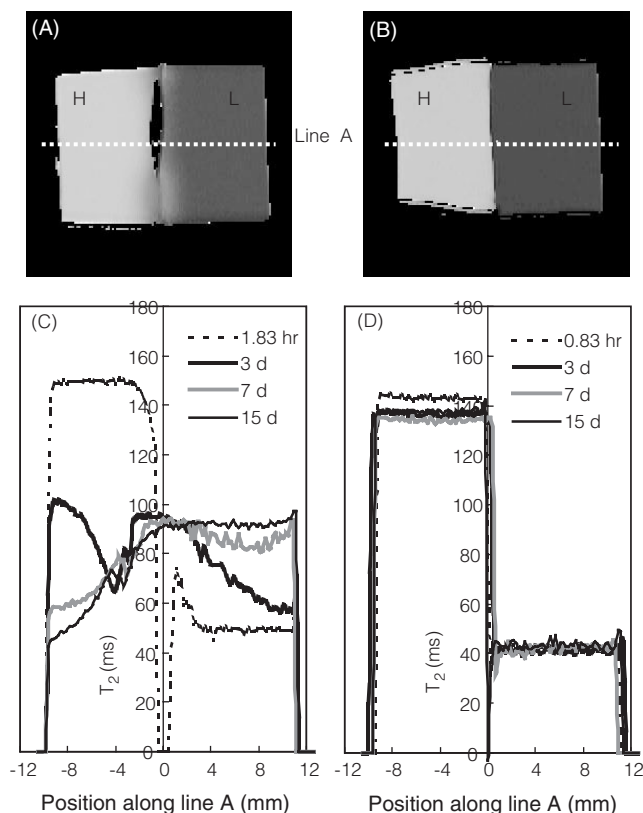


Figure 3. T_2 profiles along the central lines, as indicated in the T_2 maps of two-gelatin-gel model systems without a film (A), and with Parafilm® (B). Figures (C) and (D) are the T_2 profiles along the line, as indicated in (A) and (B), respectively.

reached the same A_w . The T_2 values of water molecules which were significantly influenced by the chemical environments (for example, chemical bonds of water and gelatin or sorbitol) and/or physical conditions of gel structures may take a very long time to reach an equilibrium or stability, based on considerations of the dynamic aspect⁽¹³⁾.

The driving force of water molecules in the two-gelatin-gel model system is a function of solute concentration, chemical interactions of water/solute/gelatin, physical structure of gelatin gel, and time. The concentration imbalance of solute (for example, sorbitol in this study) caused the water transfer from the gel of low solute content (H-gel) into the gel of high solute content (L-gel). The greatest driving force was observed at the interface of the two gels as indicated by the largest relative difference in local solute content. The water molecule was considered to be a plasticizer that reduces the friction force of solutes, increases the free volume, and lowers the glass transition time⁽³⁵⁾. Water transfer and redistribution are continuous processes that occur both between gels and inside each gel. The heterogeneous T_2 profiles after 15 days of storage indicate that water migration between gels and the redistribution of water molecules in a gel were still proceeding, but much more slowly due to the decrease in driving force, which resulted from the decrease of the relative difference of sorbitol content between the gels when the storage time increased.

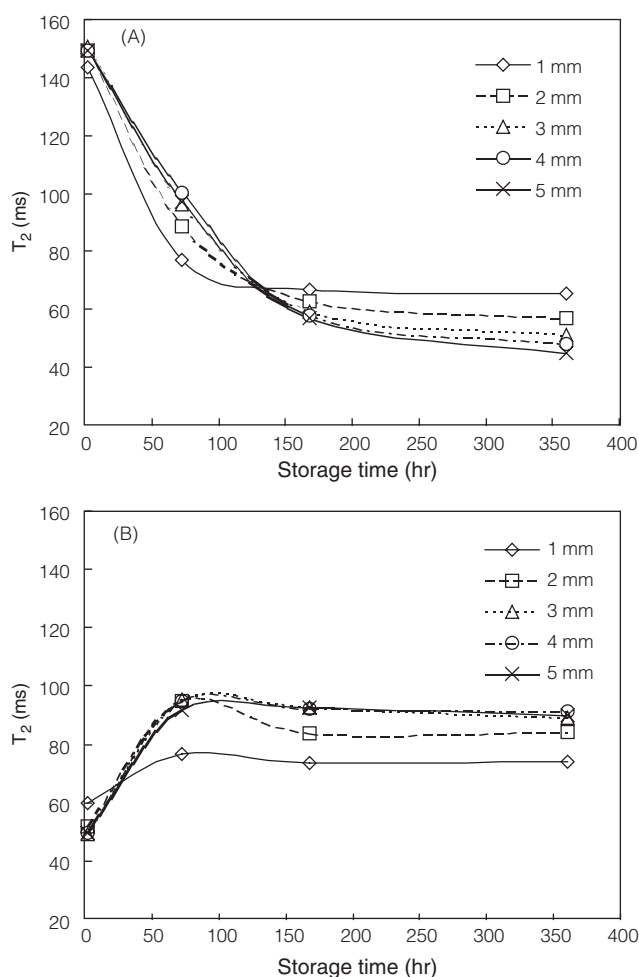


Figure 4. Effects of storage time on the T_2 values at five positions along line A in Figure 3(A); H-gel (A) and L-gel (B). Five successive positions 1 mm apart starting from the interface of the two gels were chosen.

IV. Water Redistribution in Gels during Storage

To investigate the water redistribution in each gel, five successive positions 1 mm apart starting from the interface of the two gels along the line A in Figure 3 were chosen. Changes of T_2 values during storage are plotted in Figure 4. It was found that in the H-gel T_2 decayed quickly in the first 125 hr, but slowed down thereafter (Figure 4(A)). The rates of T_2 decay decreased when moving away from the interface of the two gels after the first 125 hr, but reversed results were observed thereafter. This phenomenon was attributed to the difference in driving force, which was determined mainly by the sorbitol content at the specific time and position. Increases of T_2 value with time were observed for all five positions in the first 72 hr in the L-gel, but there were no significant differences in the rates at positions 2, 3, 4 and 5 mm away from the interface of the two gels (Figure 4 (B)). After storage of 72 hr, slight decrease of T_2 values at these positions indicated that water molecules were continuously moving to the interior of the L-gel. The T_2 values at the position 1 mm away from the

gels' interface were 65.6 ms (H-gel) and 73.9 ms (L-gel). This indicates that equilibrium was quickly achieved at the interface of both gels, whereas equilibrium in each gel required a longer time.

CONCLUSIONS

The dynamic stability of two different A_w gels during storage with or without a film in between was determined by the measurements of MRI T_2 values in a two-gelatin-gel model system. The T_2 maps and profiles revealed both the detailed changes in water mobility and the changes in gel structure during storage. After storage for 15 days, the A_w of the two gels were the same, but their water contents and T_2 values differed. The two-gel system showed pseudo stability based on the equilibrium of A_w at the end of storage. However, it also showed dynamic instability within each gel and between the two gels based on measurements of the T_2 values. The driving force of water migration and the rate of redistribution changed with time because the sorbitol contents and gelatin gel structure changed continuously after the two gels were kept together. According to the rate of T_2 changes at different positions of each gel, the dynamic stability was quickly achieved at the interface of the two gels, but the dynamic stability within each gel might require a longer time.

The two-gelatin-gel model system and nondestructive MRI technique established in this study offer a good method to monitor moisture redistribution between two gels with different A_w . This model system can be used to investigate the water status in food systems during storage, as well as evaluate water resistant properties of packaging materials between two different food systems. Compared with the traditional water vapor permeability measurement between two different relative humidity environments, the two-gelatin-gel model system combined with MRI technique not only provides information on the water barrier properties of packaging materials between different food systems, but also monitors the water redistribution and structural changes during storage.

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