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Self-Diffusion Coefficient of Water in Tofu Determined by Pulsed Field Gradient Nuclear Magnetic Resonance

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The self-diffusion coefficient of water in soybean protein dispersion and tofu was measured by pulsed field gradient (PFG) NMR. A soy protein isolate (SPI) dispersion (6 and 12%, w/w) in water, calcium cross-linked precipitate, and tofu were used for comparison. The self-diffusion coefficient of water (D) in the SPI dispersion, 2.23×10^{-9} m^2/s, was estimated lower than that of free water, 2.6×10^{-9} m^2/s at 25 °C, and decreased as the SPI concentration increased. It further decreased by the addition of calcium chloride, reflecting the obstruction effect induced by the precipitates in addition to the hydration and hydrodynamic interaction in the protein dispersion. The two water regions in tofu were interpreted by the two-site Kärger model: D_1 and D_2 of soft tofu were 2.26 ($\pm$0.11) $\times 10^{-9}$ and 6.84 ($\pm$0.34) $\times 10^{-11}$ m^2/s, respectively. The relative amount of proton (water) was $p_1 = 0.98$ and $p_2 = 0.02$ at 100 ms of diffusion time. The self-diffusion coefficients of water decreased in pressed tofu, and their relative amounts of water changed to $p_1 = 0.93$ and $p_2 = 0.07$. It was suggested that D_1 corresponded to obstructed water in the network structure and D_2 corresponded to hydrated water on the surface layer of pores formed in the protein network of tofu. The pore sizes estimated from the diffusion length of obstructed water were 21.3 μm in soft tofu and 20.8 μm in pressed tofu. The removal of fat from pressed tofu led to a decrease in D_2 from 6.26 ($\pm$0.31) $\times 10^{-11}$ to 3.53 ($\pm$0.18) $\times 10^{-11}$ m^2/s, and the relative amount of hydrated water increased from 0.07 to 0.14, which indicated hydrophobic hydration.

KEYWORDS: Tofu; soybean curd; soy protein isolate (SPI); water self-diffusion coefficient; PFG-NMR

INTRODUCTION

The behavior of water in a food matrix is an important factor determining the shelf life and sensory quality of food. The moisture content and hydration water amount around food macromolecules (protein, carbohydrate, and occluded water in a gel structure) have been measured quantitatively by gravimetric methods, sorption isotherm measurements, and viscometric methods (*1−3*). However, the water mobility and distribution in a food matrix have been difficult to measure.

Pulsed field gradient (PFG)-NMR is a powerful tool for the measurement of a water molecule's movement within biological cells, tissues, and food matrixes. The PFG-NMR technique is the most suitable method of water diffusion investigation of biological systems, due to its remarkable sensitivity to molecular displacement in the range of 10 nm to 100 μm and its noninvasive character. This technique has been used to study water mobility in different protein and polysaccharide systems such as wheat starch gels (*4, 5*), cheese (*6*), and even in nanoporous materials such as zeolite NaX (*7*). The diffusion coefficients of bound water in cotton fiber and plant tissue (*8, 9*) were measured by spin−echo NMR technique (*10*). We have

also measured the self-diffusion coefficients of water in yeast and chlorella cells and the changes in the diffusion coefficient of the growth phase and heating processes of yeast using PFG-NMR(*11−13*).

In this study, soybean protein dispersion and soybean curd (tofu) were selected for the model study to measure the water mobility and distribution in a food matrix. Soybean curd is made from soymilk by coagulating the protein with calcium or acids. Added calcium ions provide intermolecular bonds between proteins, which results in a thickening of the suspension with low concentrations of calcium (*14*) and curd formation due to a sufficient amount of calcium and heat-denatured proteins (*15*). The thickening process and gelation of the protein matrix to entrap water will alter the mobility of water molecules. To elucidate the water mobility in protein matrix using soybean curd as a model, we have measured the changes of the self-diffusion coefficient and of the population water molecules created by calcium addition to the dispersion of soybean protein.

MATERIALS AND METHODS

Preparation of Soybean Protein Dispersions and Curds. The soybean protein dispersion (6 and 12%, w/w) was prepared by mixing soybean protein isolate (SPI, 91% protein, Dupont, United States) with distilled water. The soybean protein calcium precipitate was made by

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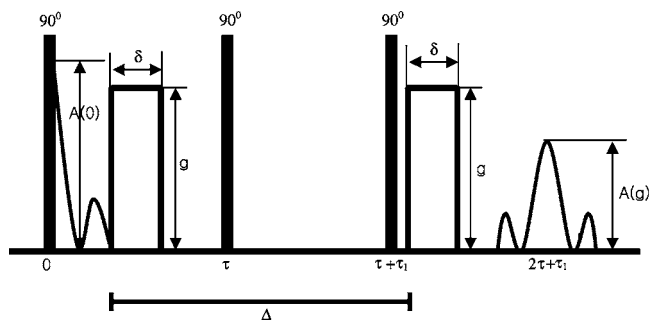
Figure 1. Stimulated echo pulse sequence with the magnetic field gradient pulses. Here, τ is the time interval between the first and the second RF pulses, and τ_1 is the time interval between the second and the third ones. ΔZ is the interval between the gradient pulses, δ is the duration of the magnetic field gradient pulses, and g is the amplitude of the gradient pulse. The gradient pulse is rectangular and oriented along the Z-axis.

adding 0.4% (w/w) $CaCl_2$ to the SPI dispersion while stirring. It was placed at 4 °C for 1 day. The sedimented layer of the precipitate was transferred to a 5 mm standard tube (Sigma-Aldrich, MO) for PFG-NMR measurements.

The soft and pressed tofus were obtained from Pulmuone Co. (Seoul, Korea). Commercial soybean curd products (tofu) are made by adding a coagulant complex, which includes $CaCl_2$, to soybean milk. Soybean milk is made from whole soybean and heated before adding the coagulant. Pressed tofu is made by pressing the curd, and soft tofu is made after coagulation (*16*). Defatted tofu was prepared in the laboratory from pressed tofu by treating with hexane extraction. The soybean oil of pressed tofu was extracted using hexane for 1 day in a shaking water bath, and then, the hexane was evaporated completely at 25 °C. Pressed, soft, and defatted tofus were transferred to a 5 mm standard tube after they were cut carefully into a rectangular size for PFG-NMR measurements.

PFG-NMR Measurements. The self-diffusion coefficient measurements were carried out with a home-built PFG-^{1}H NMR machine (*13*). The NMR frequency for protons was 63 MHz, and the maximum field gradient amplitude (g) was 50 T/m. The measurements were all computerized. The diffusion time (t_d), the interval between magnetic field gradient pulses, could be varied from 2 ms to 2 s. The duration time (δ) of magnetic field gradient pulse varied from 20 μs to 5 ms. The temperature of measurement varied from 20 to 200 °C with stability within 30.0 ± 0.5 °C. The signal of the free induction decay (FID) was converted by a Fourier Transformation program installed in the computer.

The stimulated spin−echo sequence with the magnetic field gradient pulses was used as described in our previous papers (**Figure 1**) (*11*−*13*). During the measurement of echo signal amplitude evolution, time intervals τ and τ_1 were fixed, and only the dependence of A on g was analyzed, which was called the diffusional decay. The diffusional decay was expressed by the following equation.

$$A(g) = \sum_{i=1}^{m} p_i \exp(-\gamma^2 \cdot g^2 \cdot \delta^2 \cdot t_d \cdot D_{si}) \qquad (1)$$

where γ is the gyromagnetic or magnetogyric ratio (rad $T^{-1} S^{-1}$), t_d (ms, $\Delta - \delta/3$) is the diffusion time, and D_{si} (m^2/s) is the self-diffusion coefficient of the i-th component, p_i is the population or relative amount of water proton of i-th component, δ (μs) is the duration time of the gradient, g (T/m) is the amplitude of the gradient pulse, and Δ is the interval between the gradient pulses.

For our PFG-NMR instrument, the maximum values of g and δ are 50 T/m and 5×10^{-3} s, respectively. It gives us the opportunity to measure diffusion coefficients from 10^{-8} to 10^{-15} m^2/s and to observe diffusional decay changing in 3 orders of magnitude. In this experiment, g was incremented to 8.6 T/m for water and t_d increased from 5 to 180 ms. The pure water self-diffusion coefficient was measured to be 2.6 $\times 10^{-9}$ m^2/s at 25 °C.
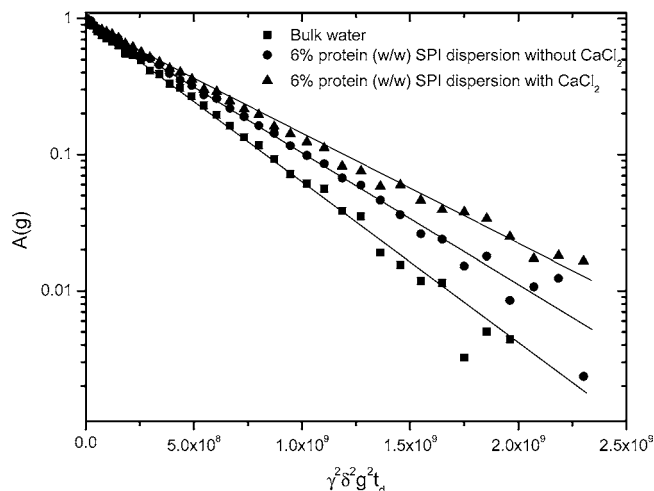

Figure 2. Echo attenuation for water in 6% (protein, g/g) SPI dispersions with and without $CaCl_2$ as a function of $\gamma^2 \cdot g^2 \cdot \delta^2 \cdot t_d$. This diffusion curve is monoexponential, and the slope means the self-diffusion coefficient of water from eq 1.
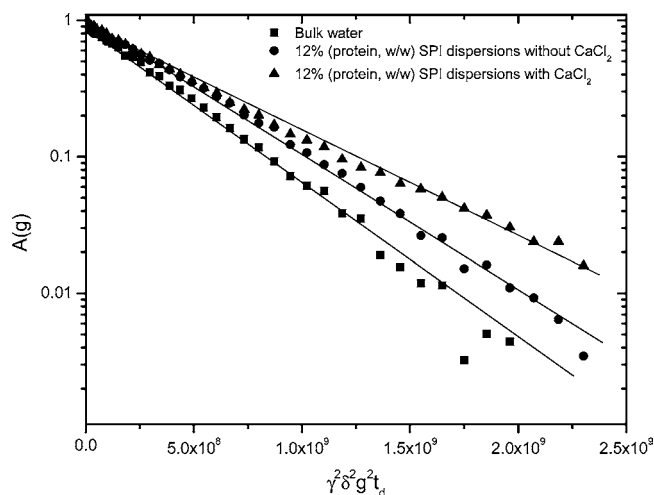

Figure 3. Echo attenuation for water in 12% (protein, g/g) SPI dispersions with and without $CaCl_2$ as a function of $\gamma^2 \cdot g^2 \cdot \delta^2 \cdot t_d$. This diffusion curve is monoexponential, and the slope means the self-diffusion coefficient of water from eq 1.

RESULTS AND DISCUSSION

Self-Diffusion Coefficient of Water in SPI Dispersions. **Figures 2** and **3** show the diffusion decay of water in SPI dispersions of 6 and 12% (protein, w/w) with and without 4% CaCl2, respectively. The monoexponential decay means that only one self-diffusion coefficient of water was able to be measured in the dispersions as estimated by eq 1. The self-diffusion coefficienta of water in 6 and 12% SPI dispersions without $CaCl_2$ were 2.23×10^{-9} and 2.0×10^{-9} m^2/s, respectively, which were significantly lower than that of bulk water, 2.6×10^{-9} m^2/s. The reduction in the self-diffusion coefficient depended on the protein concentration.

Hansen (*17*) reported that in the case of soybean protein concentrate water up to 0.07 g/g of solid is tightly bound (BET monolayer) and water up to 0.25 g/g of solid is more loosely bound, indicating the presence of water molecules hydrated with tightly bound hydration groups and water molecules associated with polar protein and carbohydrate groups, respectively. It was presumed that there is a wide distribution of molecular mobilities for the water species around the protein powder. However, with SPI water dispersion having 6−12% protein, the hydrodynamic
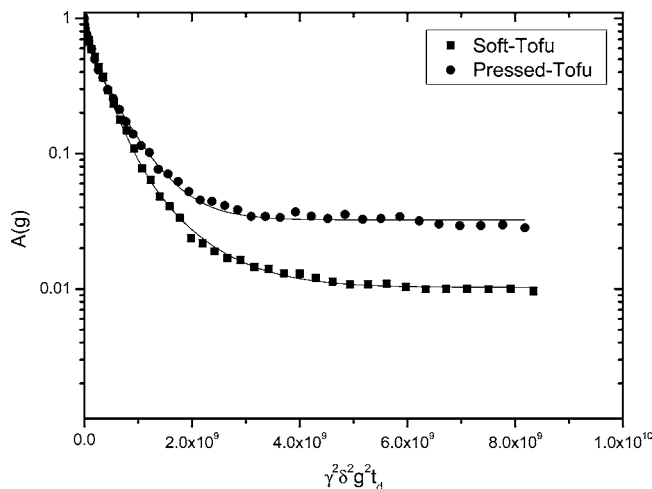
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Figure 4. Biexponential diffusion decay of water in pressed and soft tofu. The slow-diffusing component corresponds to hydrated water, and the fast-diffusing component corresponds to obstructed water, which is divided by eq 2.

volume moving with protein molecules appears to exceed the water volume, meaning no free water exists, in terms of hydrodynamic nature.

By adding $CaCl_2$, the self-diffusion coefficient of water further decreased to 2.0×10^{-9} m^2/s in 6% SPI and 1.8×10^{-9} m^2/s in 12% SPI suspension, respectively. The addition of calcium ions to unheated SPI dispersion (consisting mostly of globular proteins), forms aggregates, and the particles were precipitated (*15*).

To explain the water diffusion in polymer solutions, at least two effects should be considered as follows: (i) the obstruction effect induced by the impenetrable slow-moving polymer molecules and (ii) the hydration effect, i.e., the lowering of the water diffusion on account of water–protein interactions. These models based on different physical concepts such as obstruction effects, free volume concepts, and hydrodynamic interactions in a system containing spherical particles have been proposed to describe the reduction of the water mobility (*18*–*20*). In our case, we found only one self-diffusion flux of water in all SPI dispersions. The reduced water self-diffusion coefficients in the SPI dispersions can be described by hydration and hydrodynamic interactions, while in the SPI–Ca precipitate suspension the obstruction effect is added (*21*).

Self-Diffusion of Water in Tofu. Figure 4 shows the nonlinear diffusion decay of water in pressed tofu and soft tofu as a function of $\gamma^2 \cdot g^2 \cdot \delta^2 \cdot t_d$ (s/m^2). A two-site model for molecular transport and exchange in composed systems has been presented by Kärger et al. (*22*). In the Kärger model, it is assumed that the exchanging species undergoes free diffusion in each of the two equivalent domains; one domain is normally referred to as the internal phase and the other as the external phase. According to this two-site Kärger model, eq 1 can be expressed

$$A(g) = p_1 \exp(-\gamma^2 \cdot g^2 \cdot \delta^2 \cdot t_d \cdot D_1) + p_2 \exp(-\gamma^2 \cdot g^2 \cdot \delta^2 \cdot t_d \cdot D_2) \quad (2)$$

where $p_1 + p_2 = 1$, D_1 and D_2 are the self-diffusion coefficients, and p_1 and p_2 are the population fractions (relative signal intensities) of water in the two domains, respectively.

As shown in **Figure 4**, the D_1 and D_2 values in soft tofu were 2.26×10^{-9} and 6.84×10^{-11} m^2/s, respectively, and their relative amounts or populations correspond to each $p_1 =$
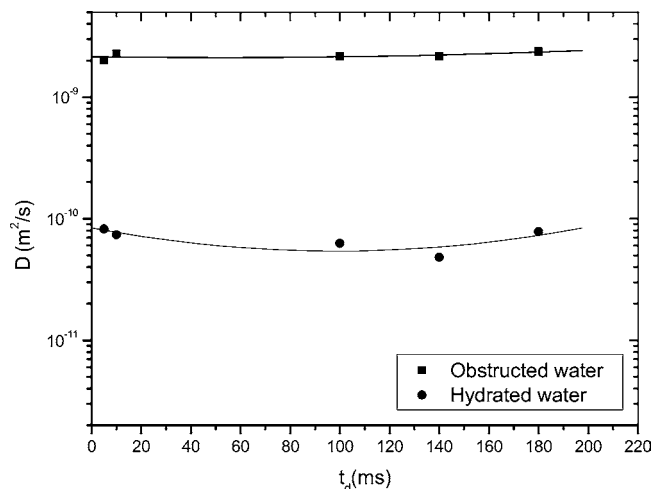

Figure 5. Plots for self-diffusion coefficients of obstructed and hydrated water in pressed tofu as a function of diffusion time t_d (ms).

0.98 and $p_2 = 0.02$ at 100 ms of diffusion time. The D_1 and D_2 values in pressed tofu were 2.16×10^{-9} and 2.74×10^{-11} m^2/s with $p_1 = 0.93$ and $p_2 = 0.07$, respectively, at the same diffusion time.

Soybean curd is characterized to have entrapped water in the protein network of its gel structure. When it is pressed to make pressed tofu, the entrapped water is exuded. Mariette et al. (*23*) suggested the presence of two water regions for protein concentrations above the close packing limit of casein gels. These authors divided the two regions into pure water and water close to the protein molecules or the micelle aggregates. However, these authors could not find a lower self-diffusion coefficient corresponding to hydrated water in casein gel, probably due to the lower maximum gradient strength (2.9 T/m) used in their studies. For measurements of small diffusivities in systems where the transverse relaxation times (T_2) are short due to the inherent small molecular mobility, magnetic field gradient pulses should be generated with a very high strength and extremely short rise and fall times (*24*, *25*). In our study using 8.6 T/m as the maximum gradient strength, the lower self-diffusion coefficient from short relaxation time for water could be measured. Therefore, we suggest that D_1 corresponds to obstructed water and D_2 to hydrated water on the surface layer of pores formed in the protein network of tofu. In other words, D_1 was reduced by obstruction effect and D_2 by hydration effect through water–protein interactions.

Figure 5 shows the changes of self-diffusion coefficient of obstructed and hydrated water in pressed tofu for different diffusion times in the range from 5 to 180 ms. This demonstrates that the obstructed water can diffuse over a length to 29.2 ($\pm$1.46) μm, given by the relation $\langle r^2 \rangle = 2 \cdot D \cdot t_d$, while the hydrated water can diffuse over a shorter length to 5.31 ($\pm$0.27) μm. The self-diffusion coefficient of obstructed water was independent of diffusion time, while the self-diffusion coefficient of hydrated water changed slightly. This means that the pores and their surface layer within the tofu matrix are inhomogeneous. The obstruction effects and the hydrated effects should be considered to explain the reduction of the water mobility in gels or polymer solutions, using models based on different physical concepts such as free volume concepts and hydrodynamic interactions. Mariette et al. (*23*) reported that there are two water regions in a casein aggregate (micelle) as a spherical particles using the cell model: water molecules inside of the micelles showing the obstruction effects and water molecules outside of the micelles involved in water–protein interactions.
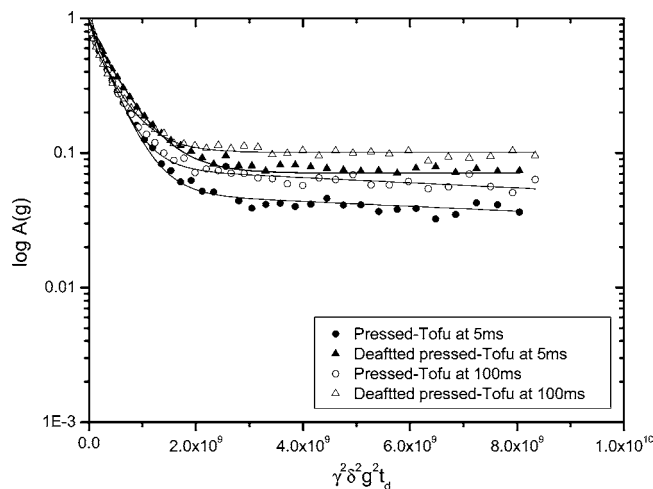
Figure 6. Diffusion decay for water in tofu after and before removing the soybean oil using hexane. The diffusion times t_d were 5 and 100 ms.

At the same time, these authors also explained the two water regions for protein concentration above the close packing limit, suggesting the pure water and water molecules close to the protein molecules (or the micelle aggregates in case of a gel). Recently, Gottwald et al. (26) reported about the bound water and free water on casein gels, describing the obstruction effects and hydration effects on the reduction of water diffusion coefficients and the chemical exchange between two water states using water diffusion and spin–spin relaxation times (T_2), respectively. The models, i.e., the cell model (23) and the Wang model (26), have been successfully applied to describe the two regions involved in the water diffusion, including the fast diffusion coefficients of water over 10^{-9} m^2/s. However, the two water states within tofu in this study were distinguishable directly, without above models. The slow water diffusion coefficients below 10^{-11} m^2/s could be measured by our PFG-NMR, showing the microscopic water diffusion. The high maximum gradient strength should be used to measure the water diffusion coefficients below 10^{-9} m^2/s due to the fast exchange between exchangeable protons and water molecules as reported in our previous study (the water exchange in surfactant–water micelle systems) (27). Therefore, the two water states around the protein matrix were well-characterized by the Kärger model (22), even though the chemical exchange on the pore surfaces with water molecules in the tofu was not measured due to the inhomogeneity.

The biexponential diffusion decays for water in pressed tofu and defatted tofu are shown in **Figure 6**. The self-diffusion coefficient of hydrated water in pressed tofu D_2, 6.26 ($\pm$0.31) $\times$ 10^{-11} m^2/s, decreased to 3.53 ($\pm$0.18) $\times$ 10^{-11} m^2/s in defatted tofu. The population fraction or relative signal intensity p_2 of hydrated water in defatted tofu increased to 0.14 as compared with p_2 of 0.07 in original pressed tofu. This means that the amount of hydrated water in defatted tofu increased more than two times by the removal of the fat fraction from the protein matrix.

According to Ide et al. (28), the probability or degree of hydrogen bonding between water molecules was associated with an increase around hydrophobic side chains with formation of water cluster and decrease around hydrophilic side chains with attractive interaction between the side chains and the water. From our results, we assume that the area of hydrophobic side chain on the surface layer of pores in tofu is exposed to water solvent and increased by removing soybean oil while that of hydrophilic side chains were not changed; therefore, the population fraction of hydrated water p_1 was increased due to the increase in hydrophobic side chains, which could form water clusters. It may be referred to as hydrophobic hydration. The decrease in self-diffusion coefficient of hydrated water in defatted tofu is in good agreement with the finding that hydrophobic groups of globular proteins give the main contribution to the hindering of water rotational mobility (29).

This increase in hydrated water and the decrease in self-diffusion coefficient on the surface layer of defatted pressed tofu are also supported by Lee et al. (3). These authors found that the amount of hydration of the protein in water solution is increased and the intrinsic viscosity also increased with the reduction of glycerol concentration in water.

LITERATURE CITED

(1) Hansen, J. R. Hydration of soybean protein. *J. Agric. Food Chem.* **1976**, *24*, 1136–1141.

(2) Lee, C. H.; Rha, C. K. Rheological properties of proteins in solution. In *Food Texture and Rheology*; Sherman, P., Ed.; Academic Press: New York, 1979.

(3) Lee, C. H.; Kim, C. S.; Yang, H. C. Microstructure and hydrodynamic properties of soybean protein bodies in solution. *J. Food Sci.* **1983**, *48*, 695–720.

(4) Callaghan, P. T.; Jolley, K. W.; Leievre, J.; Wong, R. B. K. Nuclear magnetic resonance studies of wheat starch pastes. *J. Colloid Interface Sci.* **1983**, *92*, 332–337.

(5) Ohtsuka, A.; Watanabe, T.; Suzuki, T. Gel structure and water diffusion phenomena in starch gels studied by pulsed field gradient stimulated echo NMR. *Carbohydr. Polym.* **1994**, *25*, 95–100.

(6) Callaghan, P. T.; Jolley, K. W.; Humphreys, R. J. Diffusion of fat and water in cheese as studied by pulsed field gradient nuclear magnetic resonance. *J. Colloid Interface Sci.* **1983**, *93*, 521–529.

(7) Kärger, J.; Stallmach, F.; Vasenkov, S. Structure-mobility relations of molecular diffusion in nanoporous materials. *Magn. Reson. Imaging* **2003**, *21*, 185–191.

(8) Zakhartchenko, N. L.; Skirda, V. D.; Valiullin, R. R. Self-diffusion of water and oil in peanuts investigated by PFG NMR. *Magn. Reson. Imaging* **1998**, *16*, 583–586.

(9) Anisimov, A. V.; Sorokina, N. Y.; Dautova, N. R. Water diffusion in biological porous systems: A NMR approach. *Magn. Reson. Imaging* **1998**, *16*, 565–568.

(10) Stejskal, E. O.; Tanner, J. E. Spin diffusion measurements: Spin–echoes in the presence of a time-dependent field gradient. *J. Chem. Phys.* **1965**, *42*, 288–292.

(11) Suh, K. J.; Hong, Y. S.; Skirda, V. D.; Volkov, V. I.; Lee, C. Y.; Lee, C. H. Water self-diffusion behavior in yeast cells studied by pulsed field gradient NMR. *Biophys. Chem.* **2003**, *104*, 121–130.

(12) Cho, C. H.; Hong, Y. S.; Kang, K.; Volkov, V. I.; Skirda, V.; Lee, C. Y. J.; Lee, C. H. Water self-diffusion in *chlorella sp.* studied by pulse field gradient NMR. *Magn. Reson. Imaging* **2003**, *21*, 1009–1017.

(13) Hong, Y. S.; Suh, K. J.; Yoon, S. W.; Skirda, V. D.; Volkov, V. I.; Lee, C. H. Effect of heat treatment on water permeability of yeast cells as measured by pulsed field gradient (PFG) NMR spectroscopy. *Food Sci. Biotechnol.* **2004**, *13*, 586–590.

(14) Lee, C. H.; Rha, C. K. Thickening process of soy protein with calcium. *J. Texture Stud.* **1977**, *7*, 441–449.

(15) Lee, C. H.; Rha, C. K. Microstructure of soybean protein aggregates and its relation to the physical and textural properties of the curd. *J. Food Sci.* **1978**, *43*, 79–84.

(16) Kao, F. J.; Su, N. W.; Lee, M. H. Effect of calcium sulfate concentration in soymilk on the microstructure of firm Tofu and the protein constitutions in Tofu whey. *J. Agric. Food Chem.* **2003**, *51*, 6211–6216.

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(17) Hansen, J. R. Hydration of soybean protein—2. Effect of isolation method and various other parameters on hydration. *J. Agric. Food Chem.* **1978**, *26*, 301−304.

(18) Muhr, A. H.; Blanshard, J. M. V. Diffusion in gels. *Polymer* **1982**, *23*, 1012−1016.

(19) Jönsson, B. H.; Wennerström, H.; Nilsson, P. G.; Linze, P. Self-diffusion of small molecules in colloidal systems. *Colloid Polym. Sci.* **1986**, *264*, 77−88.

(20) Masaro, L.; Zhu, X. X. Physical models of diffusion for polymer solutions, gels and solids. *Prog. Polym. Sci.* **1999**, *24*, 731−775.

(21) Venu, K.; Denisov, V. P.; Halle, B. Water ^{1}H magnetic relaxation dispersion in protein solutions—A quantitative assessment of internal hydration, proton exchange, and cross relaxation. *J. Am. Chem. Soc.* **1997**, *119*, 3122−3134.

(22) Kärger, J.; Pfeifer, H.; Heink, W. Principles and applications of self-diffusion measurements by nuclear magnetic resonance. *Adv. Magn. Reson.* **1988**, *12*, 1−89.

(23) Mariette, F.; Topgaard, D.; Jönsson, B.; Soderman, O. ^{1}H NMR diffusometry study of water in casein dispersions and gels. *J. Agric. Food Chem.* **2002**, *50*, 4295−3402.

(24) Stallmach, F,; Kärger, J. The potentials of pulsed field gradient NMR for investigation of porous media. *Adsorption* **1999**, *5*, 117−133.

(25) Galvosas, P.; Stallmach, F.; Seiffert, S.; Kärger, J.; Kaess, U.; Majer, G. Generation and application of ultrahigh-intensity magnetic field gradient pulses for NMR spectroscopy. *J. Magn. Reson.* **2001**, *151*, 260−268.

(26) Gottwald, A.; Creamer, L. K.; Hubbard, P. L.; Callaghan, P. T. Diffusion, relaxation, and chemical exchange in casein gels: A nuclear magnetic resonance study. *J. Chem. Phys.* **2005**, *122*, 34506−34510.

(27) Hong, Y. S.; Kim, K. C.; Volkov, V. I.; Skirda, V. D.; Lee, C. H. Structural and dynamic properties of polyoxyethylene sorbitan monooleate micelle in water dispersion studied by pulsed field gradient NMR. *Appl. Magn. Reson.* **2005**, *29*, in press.

(28) Ide, M.; Maeda, Y.; Kitano, H. Effect of hydrophobicity of amino acids on the structure of water. *J. Phys. Chem. B* **1997**, *101*, 7022−7026.

(29) Vorob'ev, M. Bound water measurements for aqueous protein solutions and food gels. *Colloids Surf., B* **2003**, *31*, 133−140.