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Water Distribution in Tofu and Application of T_2 Relaxation Measurements in Determination of Tofu's Water-Holding Capacity

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ABSTRACT: Low-field nuclear magnetic resonance (LF-NMR) was introduced for the elucidation of tofu in the present study. After multiexponential analysis of relaxation decays, three water fractions centered at about 1.5−2.6, 24−114, and 132−305 ms were detected and identified as T_{2b}, T_{21}, and T_{22}, respectively. Principal component analysis (PCA) of the data revealed that sample aggregation was dependent on solubility of coagulants and contained anions. Stepwise centrifugation and microwave drying were employed as dehydration methods. Significant correlations were observed between T_{21} and T_{22} relaxation times and water-holding capacity (WHC) in both dehydration processes, which implied LF-NMR measurements could be an efficient method for determination and prediction of tofu's water-holding capacity. Ten linear equations that could be applied in prediction of WHC for tofu were reported. LF-NMR was suggested to be a powerful tool for the study of tofu.

KEYWORDS: *tofu, low-field nuclear magnetic resonance, water-holding capacity*

INTRODUCTION

Tofu or soybean curd is a gel-like food made by adding different coagulants to soymilk.[1] In order to optimize production technologies and expand understanding of the coagulant's effects on tofu formation, various physical and chemical properties of tofu have been studied.[2−4] The ability of tofu to retain water in its matrix, also known as the water-holding capacity (WHC), is of paramount importance as it relates to tofu's yield and sensory attributes. However, due to limited analytical techniques, WHC of tofu has generally been studied in isolation.

In recent years, many instruments have been applied to the study of tofu. Electrical impedance spectroscopy was introduced for the study of tofu's coagulation process by Li et al.[5] Hsieh et al.[6] studied proteomic profiling of the coagulation of soymilk proteins induced by magnesium chloride using two-dimensional polyacrylamide gel electrophoresis. Other technologies, such as scanning electronic microscopy (SEM) and sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) have been utilized in the characterization of tofu-making.[7−9] However, the measurement of tofu's WHC is still limited to centrifugation, which is a sample-consuming and destructive method.

Low-field nuclear magnetic resonance (LF-NMR) technologies have been widely applied in food study. Due to the contribution of Bertram et al.,[10,11] LF-NMR measurements have been widely applied in the evaluation of WHC of pork. Besides, the applications of LF-NMR have been successfully expanded to the effects of cooking and different freezing storage conditions on the quality of various meats.[12−15] The relaxation behaviors of different water fractions in water−casein system and milk have been studied in depth by Mariette and co-workers.[16−19] It has been demonstrated that LF-NMR measurement is a highly sensitive method to study changes of water mobility and corresponding water populations during the cooking process of potatoes.[20,21] Bosman and co-workers[22−24] reported that LF-NMR could be used as a powerful tool to evaluate various properties of different types of bread. Although the self-diffusion coefficient of water in $CaCl_2$-induced tofu was reported by Young et al.,[25] the number of water fractions and their spatial locations in different types of tofu are still unknown. Besides, due to the intimate connection between water distribution and microstructure, LF-NMR is assumed to build a bridge between water-related properties to structure-related properties of tofu. Therefore, in the present study, LF-NMR was introduced into the study of water distribution in different types of tofu. Centrifugation and microwave drying were performed to achieve a series of different WHC gradients for each type of tofu. The potential use of LF-NMR for determination and prediction of tofu's WHC were exploited.

2. MATERIALS AND METHODS

Materials. The coagulants of calcium sulfate (CS), magnesium sulfate (MS), calcium chloride (CC), magnesium chloride (MC), and glucono-δ-lactone (GDL) were obtained from a local food ingredient supplier. All the chemicals were analytical-grade. Commercial soybeans from northeast China were kindly provided by Key Laboratory of Biology and Genetic Improvement of Soybean, Ministry of Agriculture, P. R. China (Nanjing, P. R. China).

Preparation of Soymilk. Soybeans were rinsed and soaked in deionized water in a ratio of 1:3 (w/w) for 12 h at 20 °C. Once hydrated, soybeans were rinsed once more and placed into a household soymilk maker BL-747 (Deer Co., Ltd., Guangdong, China) and ground with deionized water in ratios of 1:10 and 1:5 (w/w), respectively, for salt-induced tofu and GDL tofu. After the soymilk maker cycle was completed, okara (soymilk insoluble fiber) was removed by filtering the soymilk through a strainer and passing twice through double layers of cheesecloth. The soymilk was then stored at 4 °C until further use.

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Preparation of Salt-Induced Tofu. Soymilk was heated to the boiling point, maintained for 3 min with stirring, and cooled to 90 °C. Dispersions of four divalent salt coagulants were prepared at concentration of 3% (w/w) in hot deionized water (90 °C). The prepared coagulant dispersions were added into the soymilk immediately to achieve a final coagulant concentration of 15 mM. As coagulants were added into soymilk, the mixtures were continuously stirred with a stainless steel spoon for 10 s. Then the soymilk was decanted into a water bath for heat preservation at 90 °C for 20 min for better coagulation of soybean proteins. After that, the mixtures were transferred to a wooden mold and pressed in order to obtain tofu curd and exclude the whey. In order to avoid the influences of different pressing pressures on water distribution of salt-induced tofu, all salt-induced tofu was pressed at 0.01 kg/cm^2 for 30 min. After pressing, tofu was stored in airtight containers at 4 °C before analysis.

Preparation of Glucono-δ-lactone Tofu. For GDL tofu, soymilk was heated to the boiling point, maintained for 3 min with stirring, and cooled to 80 °C, and then 2% (w/w) GDL solution was added to achieve a final concentration of 0.4% (w/w). The mixtures were incubated in a water bath at 80 °C for 30 min. The coagulation of GDL tofu could form automatically without external pressures. The final tofu curd was stored in airtight containers at 4 °C before analysis.

Vacuum Freeze-Drying Pretreatment of Tofu Samples. Vacuum freeze-drying (VFD) was employed to pretreat the tofu samples used for evaluation of influence of fat on T_2 measurements. The samples were prefrozen at −18 °C for 24 h and then decanted to a Labconco freezone 12 L console freeze-dry system (Labconco Co., Ltd., Kansas City, MO) with temperature of −50 °C for 30 h.

Centrifugation Treatments of Tofu Curds. Thirty grams of cylindrical samples were obtained from the central portion of three tofu curds (CS, MC, and GDL) with a stainless cylindrical cutter and placed into a CT15RT centrifugal filter system (Techcomp Co., Ltd., Shanghai, China). Stepwise centrifugation was performed at 420*g*, 650*g*, 1660*g*, 2600*g*, 3740*g*, 5000*g*, 6640*g*, 8400*g*, 10380*g*, 12560*g*, and 14940*g* for 15 min at 20 °C (for GDL and CS tofu). The centrifugation of MC tofu only reached 8400*g* since no significant change of WHC was observed at higher centrifugation force. The weight of released water was subsequently measured. Water-holding capacity was expressed as the ratio of sample weight after centrifugation to initial weight:

$$\text{WHC}\ (\%) = \frac{(A - B)}{A} \times 100 \tag{1}$$

where B is the weight (grams) of water lost from the tofu sample during centrifugation and A is the initial weight (grams) of the sample. Each measurement was performed in triplicate.

Microwave Treatments of Tofu Curds. Microwave treatments were employed to provide another way to generate a series of different WHC gradients of tofu. Samples of CS and MC tofu were cut into cuboids of 1.5 cm thick, 15 cm long, and 1.5 cm wide and placed in an EM-309Eb1 microwave oven (Sanyo Co., Ltd., Osaka, Japan). Samples were heated for 30, 45, 60, 75, 90, 105, 120, 135, 150, or 165 s at 600 W. Three replicate tests were carried out for each heating time. WHC was calculated according to eq 1.

Low-Field Nuclear Magnetic Resonance Measurements. Low-field nuclear magnetic resonance measurements (LF-NMR) were performed on a 22.4 MHz NMR Analyzer PQ001 (Niumag Co., Ltd., Shanghai, China). After storage at 4 °C for 1 h, the tofu samples were thermostated to 32 °C in a water bath for 20 min. Approximately 2 g of different tofu samples were taken from the middle of tofu curd and placed into 15 mm NMR glass tubes. Then the tubes were inserted in the NMR probe. T_2 relaxation times were measured by use of a Carr−Purcell−Meiboom−Gill sequence. The T_2 measurements were performed with a τ-value of 150 μs (time between 90° and 180° pulse). Data from 3000 echoes were acquired as 16 scan repetitions. The repetition time between two successive scans was 3 s. The relaxation measurements were performed at 32 °C.

MultiExp Inv Analysis software (Niumag Co., Ltd., Shanghai, China) was employed for data analysis. This software performs distributed exponential curve fitting. In the time domain, spin−spin relaxation data is presumed to be a sum of exponentials:

$$R_{\text{mag}}(t) = \sum_{j=1}^{n} p_{2j} \exp\left(\frac{-t}{T_{2j}}\right) + e(t) \tag{2}$$

where R_{mag} is the residual magnetization as a function of acquisition time t; P_{2j} and T_{2j} are the transverse relaxation amplitude and time, respectively, of the jth component; and e is residual error. MultiExp Inv Analysis software performed multiexponential fitting analysis on the relaxation data according to a modified inversion method of Xiao et al.[26] Plots of relaxation amplitude versus relaxation time were obtained. The relaxation time T_{2i} and its corresponding water population (signal area ratio) M_{2i} were recorded.

Statistical Analysis. Principal component analysis (PCA) was performed with the software Simca-P+ (Umetrics AB). PCA was achieved on T_2 relaxation data to identify cluster patterns. Statistical analyses were performed with SPSS version 18.0. Linear regression analysis was performed with Origin 8.1. Adjust determination coefficients (adj-R^2) were used to evaluate the fitting degree and are defined as

$$\text{adj-}R^2 = 1 - \frac{(n - 1)(1 - R^2)}{n - m} \tag{3}$$

where R^2 is the original determination coefficient and n and m are the number of samples and variables, respectively. The significance levels are defined as (*) $p < 0.05$ and (**) $p < 0.01$.

RESULTS AND DISCUSSION

LF-NMR Analysis of Different Tofu Curds. Since oil can be detected by LF-NMR,[25,27] the influence of oil on tofu's T_2 measurements should be evaluated before further studies are performed. Although soybean is rich in oil, the final oil content in tofu curd is very limited. Previous data showed that the oil content of final tofu matrix ranged from 1.4% to 2.2% when similar proportion of soybean and water was used.[7] In order to evaluate the influence of constant oil content on tofu's T_2 measurements, tofu samples were pretreated by vacuum freeze-drying (VFD) and then measured by LF-NMR. Figure 1 shows the multiexponential fitting relaxation curves of three types of tofu before and after VFD treatments. After pretreatment by VFD, samples presented two weak peaks. The component centered at about 86−100 ms was likely to be the signal of oil in tofu, which accounted for less than 1% compared to that of samples without VFD treatment. Therefore, the influence of oil in tofu on its T_2 relaxation measurements is negligible.

A representative LF-NMR T_2 measurement distribution of five types of tofu curds (without dehydration treatment) after multiexponential fitting is presented in Figure 2. All five types of tofu samples showed three peaks, and these peaks were identified as T_{2b}, T_{21}, and T_{22} according to previous assignments.[10,11] The number of fractions was similar to the number detected in meat but lower than that of potato and other plant tissues.[10,11,20,27] T_{2b} was a minor fraction with a relaxation time between 1.5 and 2.6 ms and accounted for only 1−2.5% of total signal. The mean value of T_{2b} relaxation time was about 2 ms regardless of the type of tofu (data not shown). T_{21} was regarded as the major fraction (about 90% of total signal) with a relaxation time of 24−114 ms, while T_{22} was the slowest fraction with a relaxation time of 114−305 ms.

LF-NMR results after multiexponential fitting are shown in Table 1. T_{21} values descended in the following order: GDL > CS > MS > MC > CC. Pronounced differences in T_{21} relaxation times were observed between GDL tofu and salt-induced tofu, which might be explained by their different coagulation

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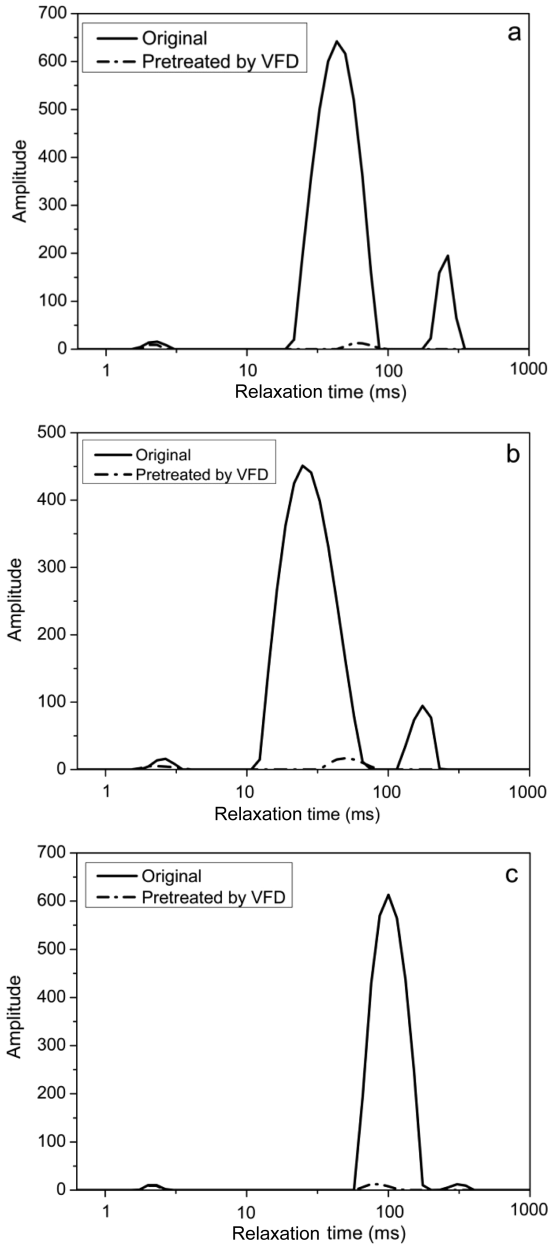

Figure 1. LF-NMR T_2 relaxation curves of three types of tofu samples before and after VFD treatment: (a) CS, (b) MC, and (c) GDL tofu.

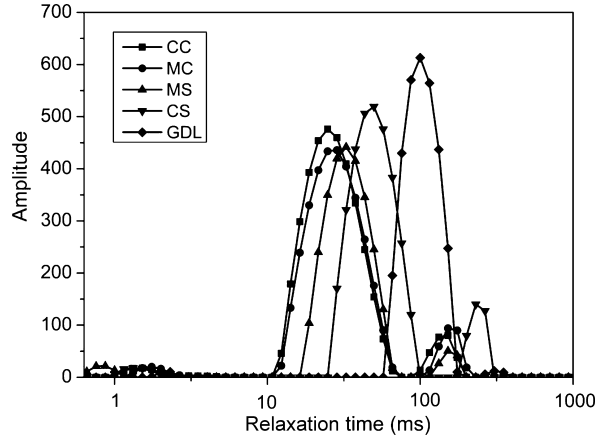

Figure 2. LF-NMR T_2 relaxation curves of five types of tofu samples.

mechanisms and final microstructures. The formation of salt-induced tofu is usually considered to be driven by salt-bridge effects between soybean proteins and coagulants.[28] Due to the pH reducing effect, mechanism of GDL tofu's formation is generally regarded as a special form of acid-induced coagulation.[29] Noh et al.[7] reported a globular stacked (or honeycomb) microstructure of salt-induced tofu which proteins were integrated into clumps. Bin et al.[30] found a well-connected network microstructure of GDL tofu. T_{21} water fraction of GDL tofu, which accounted for 98% of total signal, was higher than the other samples, while salt-induced tofu samples shared similar T_{21} water populations of approximately 90%; nevertheless, T_{21} water fraction was the dominant population in all types of tofu. T_{22} relaxation times of different tofu samples presented the same tendency as T_{21} relaxation times but with inverse population order among different samples. GDL tofu showed the lowest value of T_{22} water population of 1.73%, while salt-induced tofu shared similar values of about 7%.

Interpretation of T_2 transverse relaxation of heterogeneous systems has been extensively discussed. On the basis of the diffusive and chemical exchange model of heterogeneous system established by Hills et al.,[31−33] different water fractions in many biological tissues have been identified. Three water fractions detected in meat are generally regarded as T_{2b} (water bound to proteins), T_{21} (water trapped within the myofibrils), and T_{22} (water outside the cell).[10,11] In the case of plant tissues, the component with the slowest relaxation time (550−650 ms) is ascribed to vacuolar water, while the second (200−250 ms) and third components (50−60 ms) are assigned to cytoplasmic and extracellular water, respectively. The component with the shortest relaxation time, about 5−10 ms, is considered to be water associated with the cell wall.[27] As suggested by Hills et al.,[31] proton relaxation measurements reflect the state of biopolymers and morphology rather than the state of water. Due to the existence of membrane systems, the microstructures of food matrixes mentioned above are different from that of tofu. Tofu is formed by aggregation of soybean protein and lacks a membrane system. However, cheese, a membrane-free milk protein matrix induced by rennet, is much more similar to tofu compared to biological tissues. Therefore, the studies of different water fractions in cheese may be pertinent to the present study. Water distribution in milk protein mixtures has been widely studied.[34,35] In general, transverse water proton relaxation in heterogeneous systems like cheese and tofu is influenced by the network structure of food matrix because water is influenced by its surroundings. The distance between water and the surrounding protein network is the key factor giving rise to the different relaxation times of three water fractions in tofu. Gianferri et al.[34] reported four fractions in mozzarella cheese. Except for the fraction derived from fat droplets, the other three fractions were considered to be water fractions located in different spatial areas of cheese's microstructure. The slowest fraction with a relaxation time of 488 ms was ascribed to water free of the influence of protein surface. The intermediate fraction was considered to be water molecules entrapped in meshes of gel. The fastest fraction, with a relaxation time centered at about 7.2 ms, was attributed to water molecules trapped within casein junction zones.[34] Although the microstructures of salt-induced tofu and GDL tofu are different, they still show some similarities. Thus, both salt-induced tofu and GDL tofu are characterized by the presence of big pores and small meshes in the microstructure.[2,7,30] Based on the identification of different water

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Table 1. T_2 Relaxation Times (T_{21}, T_{22}) and Populations (M_{21}, M_{22}) of Five Types of Tofu[a]

coagulant (n = 15)	T_{21} (ms)	M_{21} (%)	T_{22} (ms)	M_{22} (%)
GDL	109.98 ± 7.31 a	98.16 ± 1.12 a	301.46 ± 6.38 a	1.73 ± 0.11 a
CS	45.61 ± 3.16 b	90.75 ± 1.81 b	251.77 ± 7.45 b	7.44 ± 0.70 bc
CC	24.54 ± 0.86 c	90.05 ± 0.67 b	133.61 ± 5.29 c	7.57 ± 0.21 b
MS	32.18 ± 1.50 d	91.66 ± 0.23 c	226.99 ± 10.58 d	7.07 ± 0.21 d
MC	27.02 ± 2.26 e	90.22 ± 0.55 b	174.75 ± 6.58 e	7.16 ± 0.32 cd

[a]Results are presented as the mean ± standard deviation. Lowercase letters (a–e) in the same column indicate significant differences (p < 0.05).

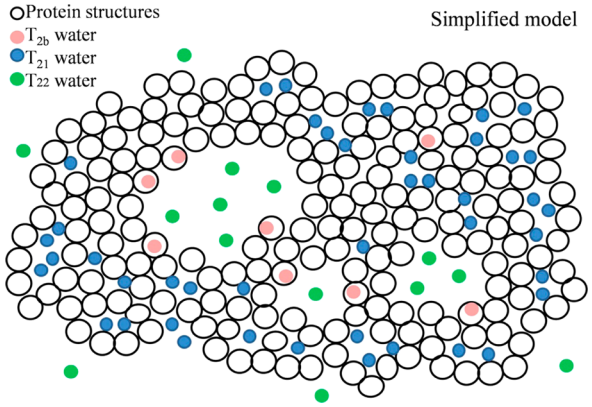

Figure 3. Simplified model of tofu's microstructure. Pores and meshes with different sizes exist in tofu's three-dimensional network. Color-coded circles represent water molecules of different fractions.

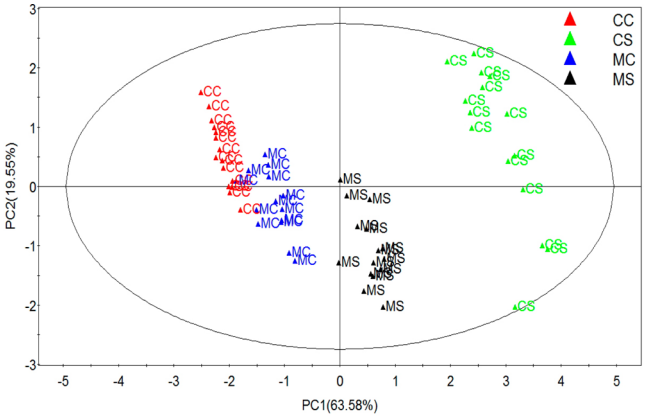

Figure 4. PCA plot (n = 60) obtained on distributed NMR T_2 relaxation data of four types of salt-induced tofu samples. CC, MC, CA, and MA represent the samples containing these coagulants.

fractions in cheese and the microstructure of tofu, the following assignments to the three fractions in tofu's microstructure are proposed. The slowest fraction T_{22} is ascribed to water molecules remote from the protein network whose motion can be little affected by the protein network. In conjunction with the microstructure of tofu, the big pores and gaps in tofu's three-dimensional network may provide space for T_{22} water fraction. The intermediate component T_{21} can be ascribed to water molecules near to the surface of protein network and is influenced by the interaction between protein network and water molecules. Due to the Brownian movement of water molecules, T_{21} water fraction diffuses from the bulk to protein interface and its T_2 value is controlled by diffusive exchange. Small meshes in tofu's microstructure and gaps in the space of protein's tertiary and quaternary structures may provide space for the T_{21} water fraction. The fastest fraction T_{2b}, with a relaxation time about 2 ms, can be assigned to the water molecules tightly associated with protein and can be regarded as an integral part of the protein structure. Its transverse relaxation time is essentially dominated by a fast chemical exchange with exchangeable protons of hydrophilic groups, such as hydroxyl, carboxyl, and sulfydryl, resulting in a short T_2 relaxation time. According to previous research,[10] the influence of T_{2b} fraction on physical characteristics and WHC is minor, and it will therefore not be further discussed in this paper. On the basis of assignments of different water fractions in tofu matrix, we established a simplified model of tofu's microstructure (Figure 3). Pores and meshes with different sizes exist in tofu's three-dimensional network, leading to a heterogeneous microenvironment and multiple-exponential relaxation behavior of water in it.

The effects of divalent salts on the coagulation process of soymilk have been intensively studied, but no convincing conclusion has been obtained yet.[28,36] Both cation and anion seem to affect the coagulation process as well as the physical properties of final matrix.[37] Parameters such as viscosity[38] and turbidity[39] have been introduced for coagulation studies of tofu. Although differences were observed between GDL tofu and salt-induced tofu, the differences among four types of salt-induced tofu were not intuitively revealed. In order to obtain an overview of variation in the multivariate LF-NMR relaxation data of salt-induced tofu, PCA analysis was performed. As shown in Figure 4, PC1 explained 63.58% of the variation in the data and discriminated clearly according to the different divalent salts of tofu. PC2, explaining 19.55% of the variation in the data, was considered to be largely due to the variation between replicates. A clear separation of CS tofu from other samples in PC1 was observed and might be explained by low water solubility of CS. The amount of cations and anions available for reactions in CS–soymilk mixtures were lower than the other three salt–soymilk mixtures due to the low water solubility of CS, which might lead to different coagulation speeds, microstructures, and final water distribution. It is noteworthy that the separation of MS tofu and chloride salt-induced tofu in PC1 seemed to be determined by type of anion. Previous studies have demonstrated that anions affect the coagulation speed and textural properties of tofu,[38,39] which is in accordance with our results.

Relationship between WHC and LF-NMR. Water-holding capacity defines the capacity of tofu to retain water in its microstructure and is an important parameter. Centrifugation is a process that removes water through physical pressure without any chemical reaction between water and macromolecules. Due to a similar principle, stepwise centrifugation can simulate different pressures under which salt-induced tofu is made. CS, MC, and GDL tofu was stepwise centrifuged (Table 2). Both T_{21} and T_{22} relaxation times of all three tofu samples decreased with increases in centrifugation force. Similar findings were observed in a study of centrifugation

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Table 2. T_{21}, T_{22}, and WHC of Three Tofu Samples at Different Centrifugation Forces[a]

	GDL (n = 3)			$CaSO_4$ (n = 3)			$MgCl_2$ (n = 3)		
centrifugation (g)	T_{21} (ms)	T_{22} (ms)	WHC (%)	T_{21} (ms)	T_{22} (ms)	WHC (%)	T_{21} (ms)	T_{22} (ms)	WHC (%)
420	100.00 ± 0.00 a	297.56 ± 6.89 a	86.86 ± 1.84 a	41.41 ± 3.26 a	242.54 ± 11.53 a	86.15 ± 1.24 a	26.01 ± 0.14 a	174.75 ± 0.00 a	91.56 ± 1.21 a
650	95.66 ± 7.52 b	292.13 ± 12.96 ab	73.91 ± 0.96 b	39.53 ± 1.01 a	192.20 ± 8.72 b	83.32 ± 1.04 b	24.77 ± 0.00 bc	167.17 ± 7.58 a	88.74 ± 0.68 b
1660	91.31 ± 7.86 bc	278.87 ± 7.25 bc	72.53 ± 3.02 b	34.38 ± 2.83 b	183.48 ± 5.11 bc	80.27 ± 1.74 c	22.62 ± 1.86 cd	145.39 ± 11.42 b	87.75 ± 0.08 b
2600	86.97 ± 0.00 cd	265.61 ± 8.72 c	65.44 ± 0.76 c	32.75 ± 0.00 b	174.75 ± 5.74 bcd	75.24 ± 0.28 d	20.61 ± 1.62 de	138.79 ± 6.59 bc	84.56 ± 0.36 c
3740	79.42 ± 6.54 de	254.08 ± 9.97 c	62.67 ± 0.57 d	31.26 ± 2.64 bc	167.17 ± 7.58 d	73.45 ± 1.48 e	18.05 ± 3.03 ef	131.29 ± 5.73 cde	77.83 ± 0.45 d
5000	72.36 ± 5.69 e	242.54 ± 11.53 cd	58.86 ± 1.16 e	28.48 ± 0.00 cd	159.58 ± 13.14 ef	69.50 ± 1.93 ef	15.59 ± 1.22 fg	126.45 ± 9.94 def	74.05 ± 1.02 e
6640	60.08 ± 4.94 f	220.98 ± 10.03 de	56.30 ± 0.58 f	26.01 ± 2.14 de	145.39 ± 11.42 efg	64.97 ± 1.60 fg	14.88 ± 1.06 g	120.71 ± 6.42 ef	73.07 ± 0.81 e
8400	59.57 ± 5.45 f	210.95 ± 11.37 de	53.63 ± 0.16 g	24.77 ± 0.00 ef	138.79 ± 6.59 fgh	64.58 ± 0.29 g	14.17 ± 0.00 g	115.91 ± 4.99 f	71.28 ± 1.28 f
10380	52.22 ± 0.76 gf	202.23 ± 8.15 ef	48.15 ± 1.31 h	23.69 ± 1.86 fg	132.19 ± 11.76 fgh	62.84 ± 0.96 h			
12560	49.77 ± 0.00 f	192.20 ± 9.11 f	45.39 ± 0.86 i	21.54 ± 0.00 gh	126.45 ± 9.94 gh	60.73 ± 0.53 i			
14940	45.45 ± 3.74 f	174.75 ± 0.00 g	42.13 ± 0.49 j	19.63 ± 1.62 h	120.71 ± 9.97 h	58.17 ± 0.55 g			

[a]Results are presented as the mean ± standard deviation. Lowercase letters (a–j) in the same column mean significant differences ($P < 0.05$).

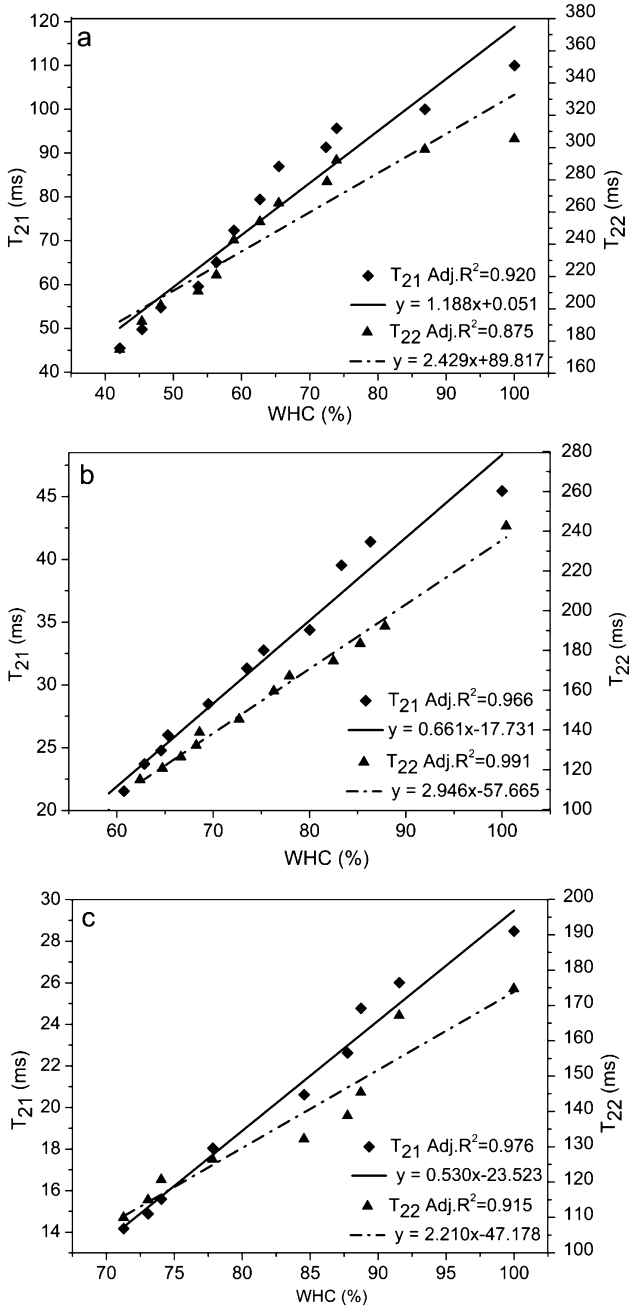

Figure 5. Linear regression equations to explain the linear relationships between WHC and T_{2i} relaxation times during the process of centrifugation. (a) GDL tofu (n = 12); (b) CS tofu (n = 12); (c) MC tofu (n = 9).

of pork.[10,11] As the centrifugation force increased, tofu formed a more compact microstructure, which shortened the distance of water molecule diffusion from the bulk to protein network interface, resulting in less mobility of water and lower values of T_{21} and T_{22} relaxation times.

Due to potential correlations between T_{21} and T_{22} relaxation times and WHC of tofu, Pearson correlation analysis was carried out, and the results are shown in Table 3. In the case of centrifugation, T_{21} relaxation times presented significant correlations with WHC. This was in agreement with the results derived from continuous distribution analysis of pork reported by Bertram et al.[10] However, in the present study, the correlations between T_{22} relaxation times and WHC were

Table 3. Pearson Correlation Analysis and Levels of Significance for Correlations between WHC and T_{21}, T_{22}, M_{21}, and M_{22}[a]

parameter	centrifugation	microwave	parameter	centrifugation	microwave
		GDL Tofu			
T_{21}	0.963**		M_{21}	0.659*	
T_{22}	0.942**		M_{22}	−0.455	
		CS Tofu			
T_{21}	0.984**	0.996**	M_{21}	0.374	0.806**
T_{22}	0.996**	0.995**	M_{22}	0.539	−0.712*
		MC Tofu			
T_{21}	0.990**	0.994**	M_{21}	0.642*	0.920**
T_{22}	0.962**	0.978**	M_{22}	0.425	−0.591

[a]Levels of significance are defined as (*) $p < 0.05$ and (**) $p < 0.01$.

Table 4. T_{21}, T_{22}, and WHC of CS and MC Tofu at Different Microwave Drying Times[a]

	$CaSO_4$ (n = 3)			$MgCl_2$ (n = 3)		
drying time (s)	T_{21} (ms)	T_{22} (ms)	WHC (%)	T_{21} (ms)	T_{22} (ms)	WHC (%)
30	41.41 ± 1.88 a	254.61 ± 9.23 a	94.53 ± 1.45 a	23.69 ± 1.86 a	171.85 ± 9.72 a	93.83 ± 0.56 a
45	39.53 ± 3.25 a	242.07 ± 8.97 ab	85.82 ± 0.44 b	20.61 ± 1.62 b	165.32 ± 12.53 bc	86.16 ± 0.25 b
60	34.38 ± 1.63 b	220.55 ± 7.29 bc	79.74 ± 1.25 c	19.67 ± 0.93 b	158.98 ± 7.37 bc	80.24 ± 0.30 c
75	28.67 ± 2.30 c	210.51 ± 6.37 cd	72.23 ± 0.70 d	16.40 ± 2.28 c	151.65 ± 6.24 cd	71.32 ± 0.76 d
90	26.01 ± 1.23 c	192.98 ± 7.37 d	66.95 ± 1.47 e	14.27 ± 1.98 cd	137.22 ± 5.12 de	67.10 ± 0.88 e
105	20.61 ± 1.62 d	167.58 ± 4.14 e	60.85 ± 0.13 f	11.79 ± 0.92 de	131.48 ± 4.12 e	61.94 ± 0.55 f
120	17.92 ± 1.41 de	159.36 ± 6.43 ef	55.20 ± 0.38 g	10.39 ± 1.22 ef	129.17 ± 7.13 ef	53.61 ± 0.65 g
135	14.97 ± 1.32 ef	145.79 ± 8.03 efg	49.62 ± 0.48 h	8.92 ± 0.70 fg	125.89 ± 3.48 fg	50.41 ± 1.47 h
150	13.25 ± 0.92 fi	138.39 ± 3.78 fg	46.43 ± 0.17 i	6.44 ± 0.53 gh	124.75 ± 0.00 fg	45.43 ± 0.41 i
165	9.33 ± 0.53 i	132.59 ± 2.42 g	41.38 ± 1.26 j	5.87 ± 0.46 h	116.98 ± 2.29 h	44.69 ± 0.29 i

[a]Results are presented as the mean ± standard deviation. Lowercase letters (a−j) in the same column indicate significant differences ($p < 0.05$).

also significant. Besides, Bertram et al.[10] revealed that T_{2i} populations presented closer relationships with WHC compared with T_{2i} relaxation times in the determination of WHC in pork, while T_{2i} populations of three types of tofu were almost unchanged during the centrifugation process (data not shown) and the correlations were insignificant. We deduce that the dissimilar changes of T_2 parameters between tofu and pork meat during centrifugation processes can be ascribed to their different microstructures. T_{21} and T_{22} water fractions of meat exist in different compartments of muscular tissues. Cell membranes are considered to act as physical barriers for the different water fractions, giving rise to their different contribution to centrifugation water loss and significant correlations between T_{2i} populations and WHC. Compared with meat, the water transfer in tofu during centrifugation can be faster and unhindered because, unlike a membrane system, water in tofu is present in a system regardless of obstacles. Water transfer in tofu during centrifugation is most likely a dynamic process. Due to shrinkage of tofu matrix, both T_{21} and T_{22} water fractions could be expelled outside without the obstacles of cellular tissues during centrifugation.

According to previous studies, linear relationships existed between T_{2i} relaxation times and WHC of different food matrixes.[11,15] In order to analyze the quantitative relationships between T_{2i} relaxation times and WHC, mean values of T_{21} and T_{22} relaxation times and WHC were used to establish simple linear equations. Six equations were obtained from linear regression (Figure 5). High adj-R^2 values (0.875−0.991) indicated high fitting degrees of prediction equations. Accordingly, LF-NMR could be an effective method to monitor and predict the WHC of tofu during centrifugation.

Microwave drying was introduced as another method of dehydration. Unlike centrifugation, microwave drying achieved dehydration by heating the tofu matrix integrally. Moreover, microwave induced a high temperature condition which might cause some chemical and physical changes of soybean proteins. GDL tofu was not included because severe destruction of texture occurred during microwave drying. Table 4 shows the LF-NMR results of microwave drying of tofu made with CS and MC. T_{21} and T_{22} relaxation times decreased along with the microwaving, which was similar to the centrifugation results. With increasing drying time, T_{21} populations decreased while T_{22} populations increased, which might indicate the density of water in tofu's matrix changed due to potential changes of tofu microstructure during microwave drying. It is noteworthy that the relaxation time of the major water fraction decreased to below 10 ms, which was generally regarded as the boundary relaxation time between T_{2b} and T_{21}. In centrifugation, limits of T_{21} relaxation time (above 10 ms) and WHC seem to exist when centrifugation force reached a threshold. Like MC tofu, when centrifugation reached 8400g, no significant changes of WHC and T_{2i} relaxation times were observed at centrifugation forces exceeding this level. However, even though the water loss was much more than that of centrifugation, the T_{21} relaxation time and WHC had not reached their limits during microwave drying. In microwave drying, both the microstructure and chemical properties of protein network might extensively alter due to the high temperature induced by microwave. Thus, different mechanisms of these two dehydration methods are considered to be the reason for the dissimilar changes of T_2 parameters and WHC. Although the relaxation time of the major water component was below 10 ms, this portion was still likely to be responsible for further water loss from 46% to 41% (CS tofu) and from 53% to 44% (MC tofu). As mentioned before, T_{2b} water could hardly contribute to water loss of food matrix. Besides, the T_2

relaxation time of the major component was still significantly slower than tofu's T_{2b} (0.80−2.6 ms). So it seems to be reasonable that the major portion of water was assigned as T_{21} water fraction.

It is noteworthy that when drying time exceeded 60 s (165 s for CS tofu), a new fraction, of which the relaxation time was 72−37 ms, was observed and identified as T_{21n} (Figure 6). The population of T_{21n} increased from 1% to 14% (for MC tofu) along with prolonged drying time. A previous study demonstrated that microwave treatment could transform soybean protein dispersions to a gel-like curd.[40] Consequently the formation of this new fraction may be explained by further changes of tofu's microstructure driven by microwave drying. During the microwave drying process, some proteins experienced thermal denaturation and formed special microstructures, which could retain the water molecules expelled from inner spaces. This portion of water might become the source of T_{21n} water.

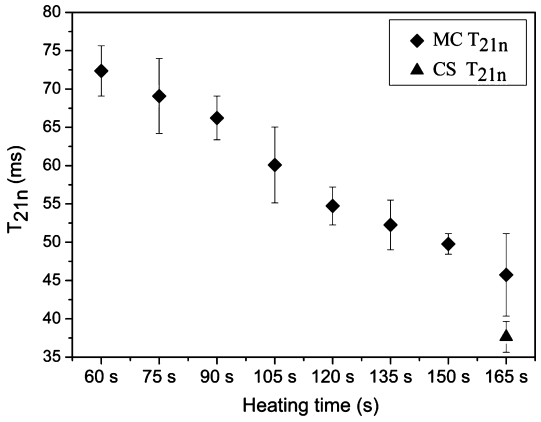

Figure 6. Relaxation time of new water component T_{21n} in MC and CS tofu samples.

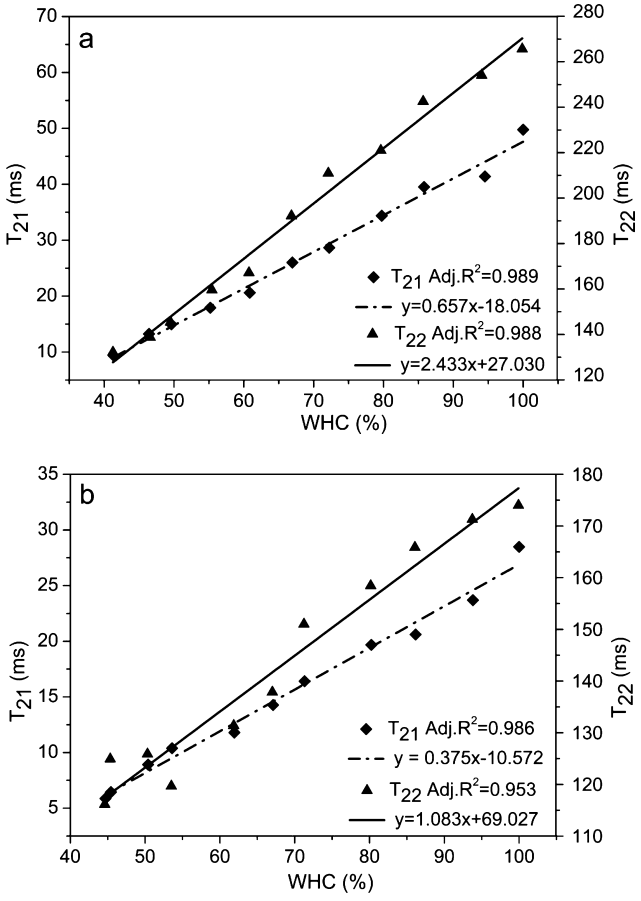

Figure 7. Linear regression equations to explain the linear relationships between WHC and T_{2i} relaxation times during the process of microwave drying. (a) CS (n = 11); (b) MC tofu (n = 11).

Pearson's correlations between T_2 data and WHC are shown in Table 3. Significant correlations between T_{2i} relaxation times and WHC were observed in the case of centrifugation. Although the mechanism of decrease of T_{2i} relaxation times may be complex due to overlying effects in microwave drying, it still seems to be feasible to make a prediction of tofu's WHC by its T_{2i} relaxation times because of the significant correlations between them. Therefore, linear regression analysis was performed on mean values of T_{2i} relaxation times and WHC (Figure 7). Four equations with adj-R^2 ranging from 0.953 to 0.989 were obtained to explain the correlations between T_{2i} relaxation times and WHC.

In conclusion, we consider that the T_{21} and T_{22} relaxation times can be good parameters in monitoring tofu's WHC during microwave dehydration.

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Notes

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ABBREVIATIONS USED

Low field nuclear magnetic resonance (LF-NMR); water holding capacity (WHC); calcium sulfate (CS); magnesium chloride (MC); Glucono-delta-lactone (GDL); vacuum freeze-drying (VFD); and adjust determination coefficient ($Adj.R^2$)

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