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Water transport in Thai glutinous rice during soaking: investigation using ^{1}H-NMR relaxation

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Abstract

Glutinous rice requires a longer soaking time than other rice cultivars. In this study, water transport in Thai glutinous rice RD6 was compared with that in the non-glutinous cultivars Khao Dawk Mali 105 (KDML) and Chai Nat 1 (CN1) using time-domain nuclear magnetic resonance. The amylose and amylopectin percentages of the three rice cultivars were measured to study their potential links to the water absorption of the rice, which was measured after soaking for 0, 0.5, 1, 2, 3, 4, 5, and 6 h. CN1 became saturated with water within 1 h, whereas RD6 and KDML became saturated after about 2 h. Glutinous rice RD6 absorbed the greatest amount of water, whereas CN1 absorbed the least. The amount of water absorbed was inversely related to the amylose percentage of the three cultivars. A smaller amount of amylose in the amylopectin structure may provide more space to absorb water. The rate of water absorption of RD6 and CN1 was considered to have been greatest in the first half hour of soaking, where their free-water peaks dominated the T_2 spectra. Most of the absorbed water of the three rice cultivars was in the loosely bound state, corresponding to the water in the amorphous growth shells of granules. The physically bound water peak of RD6 gradually shifted to a higher T_2 value with increasing soaking time, indicating that the molecular mobility of water increases with soaking time.

Keywords: Rice soaking, Time-domain NMR, NMR relaxation, Glutinous rice, Khao Dawk Mali 105, Chai Nat 1

1. Introduction

Glutinous rice is primarily cultivated in Southeast, South, and East Asia, where it plays an important cultural and economic role. This is particularly the case in Southeast Asia, where it serves as both a staple food and a symbol of regional identity. For example, khao niew moon (sweet sticky rice with coconut milk) is traditionally prepared for merit-making festivals such as the Rocket Festival (Boon Bang Fai), while khao jee (grilled sticky rice) is a traditional food commonly consumed during the cool season. Glutinous rice is also a key ingredient in various traditional Thai dishes and desserts that are widely recognized internationally, such as mango sticky rice and khao lam (sticky rice roasted in bamboo). Outside these key regions, the global demand for glutinous rice-based products is rising with its increasing use in processed foods, including in gluten-free product formulations for health-conscious consumers. Glutinous rice is therefore not only culturally significant but also economically valuable, serving as an important agricultural commodity connecting local farming communities to global markets. It typically requires soaking for several hours before steaming, and its characteristic stickiness upon cooling gives rise to its name. It contains a low concentration of amylose but a high concentration of amylopectin, which contributes to its sticky texture [1]. Non-glutinous rice, in contrast, contains a considerably higher proportion of amylose, typically ranging from 15% to 30%, whereas glutinous rice generally contains less than 5% amylose and is predominantly composed of amylopectin [2].

Linear chains of amylose tend to form compact helical structures that restrict water penetration into starch granules, resulting in slower hydration during soaking. By contrast, the highly branched structure of amylopectin provides numerous open chain ends that readily interact with water molecules, thereby facilitating faster diffusion of water into the granules. Consequently, the amylose-to-amylopectin ratio is key in determining both the rate and extent of water absorption. Rice varieties with lower amylose content, such as the glutinous cultivar RD6, demonstrate faster water uptake and greater swelling during soaking, whereas non-glutinous varieties with higher amylose content absorb water more slowly and remain firmer after cooking [3]. In traditional Thai culinary practice, glutinous rice (e.g., cultivar RD6) requires pre-soaking for approximately 5–6 hours prior to steaming for 20–30 minutes. During steaming, the pre-soaked grains are placed in a woven bamboo basket positioned above boiling water, allowing heat and moisture to be transferred by steam until the grains become fully gelatinized. Without adequate soaking, heat cannot effectively penetrate the inner portions of the grains, resulting in partially cooked or hard rice. Glutinous rice therefore needs to be soaked in water for a prolonged period before cooking to achieve complete gelatinization [4]. Conversely, non-glutinous rice, which has a higher amylose content, can be cooked directly in boiling water or in an electric rice cooker without pre-soaking, as water absorption and starch gelatinization occur simultaneously during the cooking process. This distinct difference in cooking methods highlights the contrasting water absorption and gelatinization behaviors between glutinous and non-glutinous rice. While glutinous rice normally requires a longer soaking time before cooking than other types of rice, the soaking duration varies even among glutinous rice cultivars. For instance, Khao Niew Khao Ngu (Chiang Rai glutinous rice), which is commonly used for mango sticky rice, generally requires soaking for about 3–4 hours, while the RD6 variety needs a soaking time of approximately 5–6 hours to achieve uniform softness and complete gelatinization. Soaking impacts the physiochemical characteristics of rice [5]. Knowledge of the various forms of water and its movement within the rice grains during soaking can be used to enhance rice cooking to achieve the desired final product. Generally, water in biological tissues can be considered to exist in three states: free water (FW), comprising intercellular water and water outside granules; physically bound water; and chemically bound water [6,7]. Physically bound water can be subdivided into two types: loosely bound water (LBW), which is water in amorphous growth shells, and strongly bound water (SBW), which is water in amylopectin channels [8,9]. Structurally, rice grains mainly comprise endosperm cells containing starch granules, which are composed of semicrystalline lamellae shells of amylopectin separated by amorphous growth shells of amylose [10,11]. During soaking, the absorbed water can alter the distribution of water in each part of the grain. Quantitative analysis of the water states during soaking can thus provide important information for regulating the cooking of glutinous rice.

Time-domain nuclear magnetic resonance (TD-NMR) is widely used by food scientists to study the phases of water and oil in various ingredients [12-14]. Conventional methods for determining moisture content, such as oven drying and Karl Fischer titration, can only provide the total water content of the sample and cannot distinguish between different physical states of water or characterize its molecular dynamics within rice grains [3]. In contrast, TD-NMR is a rapid, non-destructive technique that separates water populations according to their molecular mobility, as derived from their T_2 relaxation behavior. This allows the identification of free water, loosely bound water, and strongly bound water, providing deeper insight into water migration and distribution within rice grains during soaking. Low-field TD-NMR has the major benefit of not requiring special sample preparation procedures, and is a simple method of quality control in the food manufacturing process. Applications of TD-NMR in research on rice throughout its growing period [15], during storage [16], and even during cooking [17] have been reported. The NMR relaxation time of a protein in a biological material is significantly influenced by its molecular mobility and surrounding environment [18]. In biological tissues, the four water states exhibit distinct NMR relaxation times. Tang et al. [9] reported the distinct NMR relaxation times of the water components in starch granules, providing a valuable reference for investigating these in other starchy foods, including rice, by TD-NMR. In TD-NMR, the inversion recovery and Carr–Purcell–Meiboom–Gill (CPMG) pulse sequences are used to measure relaxation times T_1 and T_2, respectively. The CPMG pulse sequence is widely preferred for measuring T_2 because a very short time is required for measurement, making it suitable for quality control processes.

Previous studies have investigated water absorption and gelatinization in non-glutinous rice using TD-NMR or differential scanning calorimetry (DSC) [19-21], whereas glutinous rice, whose high amylopectin content leads to distinct water–starch interactions during soaking, has received limited scientific attention. Furthermore, the transition of different water states—free, loosely bound, and strongly bound—within glutinous rice grains during the soaking process has not been quantitatively characterized. Therefore, we aim to fill this gap by applying low-field TD-NMR to investigate the dynamic distribution and transformation of water states in Thai glutinous rice (cultivar RD6) during soaking, and compare it with two non-glutinous rice cultivars. This approach provides new insights into the molecular mobility of water and its relation to amylose–amylopectin composition, which have not been previously reported.

In this work, we used the CPMG pulse sequence and T_2 relaxation times to compare the water states of one glutinous and two non-glutinous rice cultivars during soaking. A limitation of the CPMG pulse sequence is that it cannot detect the short relaxation time signal of chemically bound water, which has a T_2 relaxation time of approximately 10 ms [9]. Thus, only FW, LBW, and SBW could be monitored from their T_2 relaxation times by using the CPMG pulse sequence. The relation between the state of water and its transportation to amylose and amylopectin is also discussed. Our main objectives were to (i) examine changes in moisture distribution within rice grains at different soaking durations, (ii) compare water absorption characteristics across rice cultivars with varying amylose–amylopectin ratios, and (iii) elucidate the relationship between the transformation of water states (free, loosely bound, and strongly bound) and starch composition to explain the similarity in T_2 spectra observed among glutinous rice varieties with closely related amylose contents.

2. Materials and methods

2.1 Rice samples

The glutinous rice cultivar RD6 was compared with the non-glutinous cultivars Khao Dawk Mali 105 (KDML) and Chai Nat 1 (CN1). All three cultivars were planted in Khon Kaen province in the northeast of Thailand and were stored for a year before being used in the study. Samples of each rice variety were separated into nine groups, each subdivided into five samples of three grains that had had the entire husk removed by hand. Each sample had a total mass of approximately 68.0–69.0 mg (a total of 45 samples for each rice variety), and each group was soaked in water for a different duration (0, 0.5, 1, 2, 3, 4, 5, and 6 h). Before the NMR experiment, each sample was wiped and weighed to determine the mass of water absorbed by the grains. Each rice sample was then placed in a 5-mm-diameter test tube for the NMR relaxation signal measurement, as described in Section 2.2. After the measurement, the sample was oven-dried at 110 °C for 6 h and weighed again. The dry mass was used to calculate the water absorption rate of the sample.

2.2 Measurement of T_2 relaxation signal

The NMR T_2 relaxation signal of each sample was measured by using the CPMG pulse sequence on a PS2-B 0.5 T system (TeachSpin, New York, USA) with a laboratory-built RF transmission system and data acquisition software [22]. The CPMG pulse sequence parameters used in data collection were a repetition time (TR) of 3000 ms, an echo time (TE) of 2 ms, 500 echoes, and an average of 200 signals. The NMR receiver amplifier gain was fixed to the same value for every sample to compare the proton density related to the quantity of water absorption.

2.3 Determination of T_2 spectra

The NMR relaxation signal of a biomaterial is typically a combination of multiple exponential decay curves from protons in different environments with differing molecular mobility, and it is thus referred to as a multi-exponential relaxation signal. Let S(t) be a T_2 multi-exponential decay signal measured using a CPMG pulse sequence. For a sufficiently large number of exponential decay components, $S(t)$ can be expressed as the following integral [23]:

$$S(t) = \int F(T_2) e^{-1/T_2} dT_2 \tag{1}$$

The function $F(T_2)$ is called the T_2 spectrum. Because Eq. (1) is a type of Laplace transform, $F(T_2)$ for a multi-exponential relaxation $S(t)$ can be determined from the inverse Laplace transform. In this study, the n umerical open-source inverse Laplace transform program (OSILAP) developed by Ohkubo [24], which is based on the Butler–Reeds–Dawson algorithm [25], was used to compute the T_2 spectra of all samples. Each output spectrum from OSILAP was multiplied by the amplitude of the first echo of the corresponding relaxation signal to reflect the sample's proton density.

2.4 Measurements of amylose and amylopectin

The amylose and amylopectin percentages of each rice cultivar were measured by UV–visible spectroscopy (Agilent 8453, Santa Clara, USA) to investigate their potential links with the water absorption and transportation of the three water components.

3. Results and discussion

3.1 Amylose and amylopectin percentages

Table 1 lists the measured amylose and amylopectin percentages of the three rice cultivars. The glutinous rice RD6 had the lowest amylose percentage, and CN1 had the highest. This information was used in the subsequent analysis.

Table 1 Amylose and amylopectin percentages of rice cultivars RD6, KDML, and CN1.

Rice cultivar	Amylose (%)	Amylopectin (%)
RD6	9.52	90.48
KDML	16.04	83.96
CN1	23.40	75.42

3.2 Water absorption

Photographs of samples of the three cultivars after different soaking times are shown in Figure 1. In the case of the 6-h soak, some of the KDML and CN1 rice grains cracked after approximately 3–4 h, while no cracked grains were observed for RD6. The average water absorptions of the three rice varieties are shown in Figure 2; the graph indicates that RD6 glutinous rice had the highest water absorption rate, with saturation occurring after approximately 2 h. KDML had the lowest water absorption rate in the first hour of soaking. However, its water absorption rate subsequently exceeded that of CN1 and the samples became saturated after about 2 h. CN1 achieved saturation in the shortest time, after about 1 h, and also absorbed the smallest amount of water. The water absorption trends of the three rice cultivars appeared to be inversely proportional to that of their amylose percentages (Table 1). The present study found that RD6 glutinous rice exhibited a higher rate of water absorption and reached saturation faster than non-glutinous varieties [26]. This observation supports the established understanding that rice with lower amylose content allows for the greater molecular mobility of water, facilitating faster diffusion into the starch granules [27]. Similar patterns of water redistribution during soaking have been reported in recent studies, where the rapid uptake of water was attributed to diffusion-driven transport governed by the internal microstructure of rice grains [28,29]. These findings collectively confirm that the water absorption process is predominantly controlled by the diffusion mechanism associated with starch morphology and composition [28,30]. According to the current understanding of starch granule structure [10,11], amylose chains are infiltrated in the semicrystalline structure of amylopectin and the growth shells. A smaller amount of amylose in the amylopectin structure may result in more space available for water absorption.

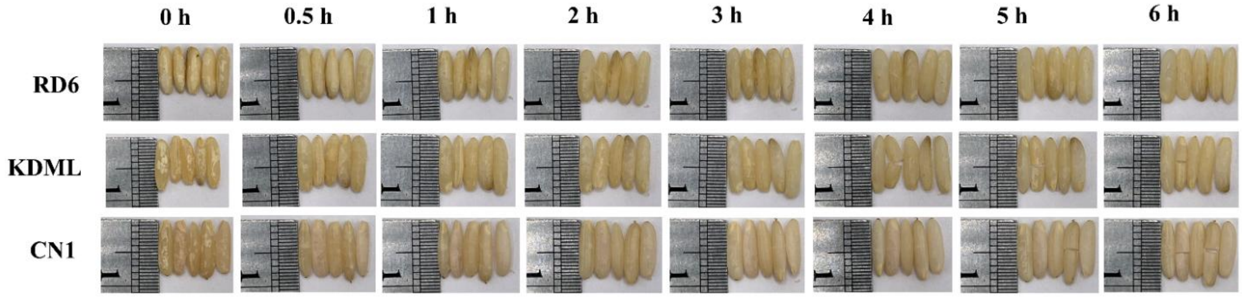

Figure 1 Photographs of samples of the three rice cultivars after different soaking times.

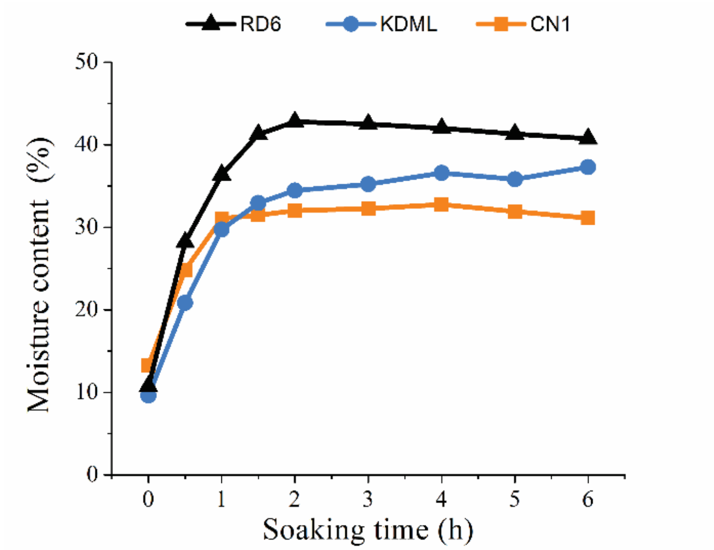

Figure 2 Water absorption of RD6, KDML, and CN1 rice cultivars during 6 h of soaking.

3.3 NMR relaxation analysis

The measured T_2 relaxation signals of the three rice cultivars are shown in Figure 3. Each relaxation curve is an average for five samples of the same cultivar with the same soaking time. Our primary observation was that a longer soaking time resulted in a stronger relaxation signal, indicating a higher proton density in the absorbed water. On average, the amplitude of the relaxation signals for RD6 was greater than that for KDML and CN1, which aligns with the water absorption curves in Figure 2. The T_2 spectra corresponding to the T_2 relaxation signals of the cultivars in Figure 3 are illustrated in Figure 4; note that the apparent broadening and partial overlap of the LBW and SBW peaks in Figure 4 arise from the intrinsic limitation of the 0.5 T low-field TD-NMR spectrometer rather than from insufficient graphical resolution. Because the instrument operates at a relatively low magnetic field with a fixed echo time of 2 ms, its effective spectral resolution ($\Delta 1/T_2$) is inherently restricted. Consequently, signals from water components with closely spaced relaxation times—such as LBW and SBW—cannot be fully separated and thus appear as merged peaks. Nevertheless, the relative peak positions and their gradual rightward shift with soaking time were reproducible across all replicates ($n = 5$), confirming that the observed trend reliably reflects increased molecular mobility of bound water rather than noise or plotting artifacts. The NMR signals from chemically bound water and carbohydrates decay rapidly—in approximately 0.1 and 0.05 ms, respectively [9]. These exponential decay components could not be detected using our instrument; thus, only physically bound water and FW were observed in this study. According to the studies of Tang et al. [9] and Zhu et al. [31], the FW peak should be located at approximately 50 ms, indicated by the vertical dashed line labeled FW in Figure 4. The relaxation components located above 100 ms correspond to the relaxation of residual water inside the test tube and on the grain skin. The relaxation peaks of SBW and LBW, the two types of physical water, are located very close to each other; for example, they are at 1 and 8 ms for potato starch, respectively [9], and we expected the SBW and LBW peaks for the three rice cultivars to be in this region. The peaks on the left of all spectra in Figure 4 are those of the physically bound water, since they are in this region. Tang et al. [9] proposed that most physically bound water is LBW. At room temperature, the peaks of SBW and LBW merge due to the self-diffusion of water. This information was applied to the T_2 spectra of the unsoaked rice to estimate the location of the SBW and LBW peaks. The peaks for the physically bound water are at approximately 2 ms, from which we estimated the location of the LBW peak to be 0.3 ms and that of the SBW peak to be 0.8 ms (20% of the peak height on the left side), indicated by the vertical dashed lines labeled LBW and SBW, respectively.

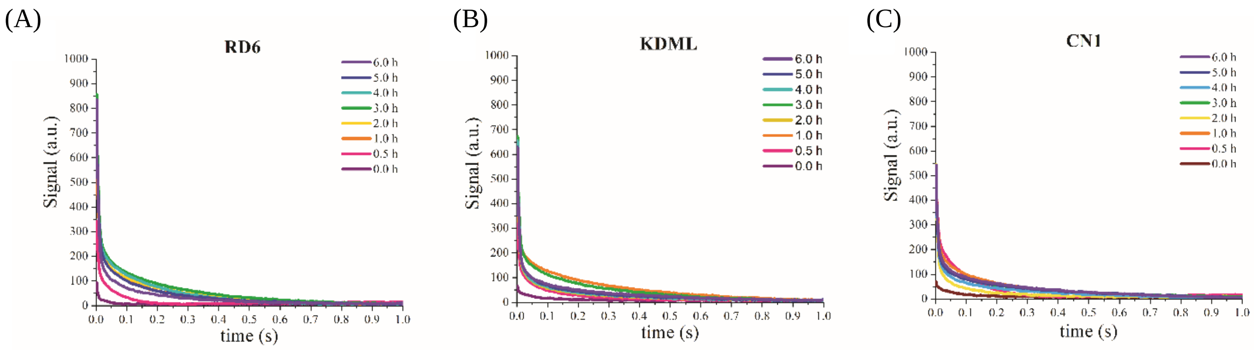

Figure 3 T_2 relaxation signals of (A) RD6, (B) KDML, and (C) CN1 rice cultivars measured using the CPMG pulse sequence.

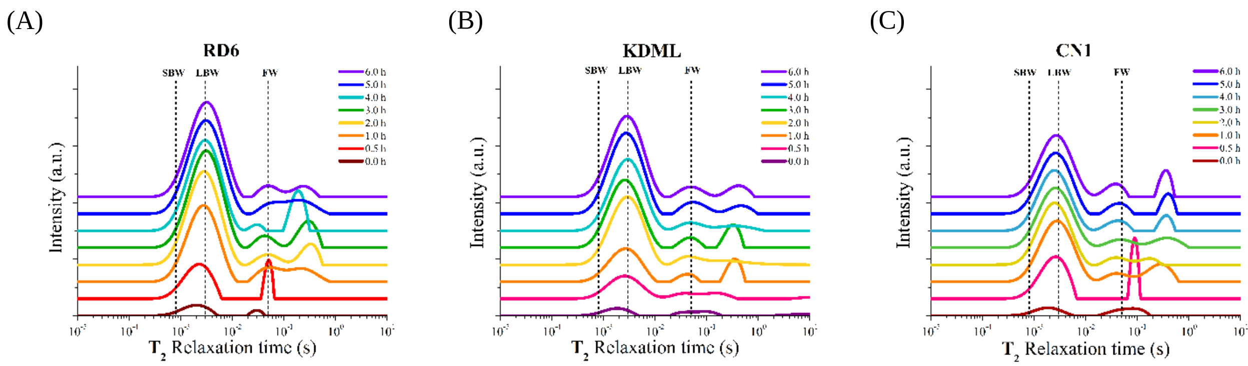

Figure 4 T_2 spectra of (A) RD6, (B) KDML, and (C) CN1 rice cultivars for soaking times of 0–6 h.

In the spectra for the unsoaked samples, the FW peaks of KDML and CN1 extended further in the direction of higher T_2 values than that of RD6, indicating that the FW of KDML and CN1 has a higher molecular mobility than that of RD6. The FW peaks of RD6 and CN1 in the spectra for 0.5 h soaking were larger than that for KLDL; thus, the water absorption rates of these two rice cultivars are higher than that of KDML, consistent with Figure 2. In addition, the FW peak of CN1 was shifted to about 80 ms, indicating high FW molecular mobility. All five samples of CN1 exhibited this behavior in the 0.5-h soak, which we are currently unable to explain. After 1 h of soaking, the FW peaks of the three rice cultivars were reduced in height and the absorbed water was changed to water in the physically bound state. During the same soaking period, the physically bound water peaks were smallest for KDML and largest for RD6, which is also consistent with Figure 2. For all three rice cultivars, the physically bound water peaks were slightly shifted to the right, indicating that the preferential state of the absorbed water was LBW. In the 2-h soak, the peak for physically bound water was largest for RD6 and smallest for CN1, in agreement with the water absorption amounts in Figure 2. After the samples were saturated with water, the physically bound water peaks for RD6 slowly shifted to the right (i.e., to higher T_2 values). This suggests that a long soaking time increases the molecular mobility of water, since water with higher molecular mobility has a longer T_2 relaxation time [18].

These results align with recent studies demonstrating that the molecular mobility and relaxation behavior of water in rice are predominantly governed by starch composition and microstructural rearrangements that occur during hydration and gelatinization. Investigations on non-glutinous rice with varying amylose contents have revealed that prolonged soaking enhances the mobility of loosely bound water as starch granules swell and gradually lose crystallinity, resulting in a rightward shift of the T_2 peaks [32,33]. Although these previous studies were conducted on non-glutinous rice, a similar trend was observed in the present work for the RD6 glutinous rice samples, where extended soaking caused a gradual shift of the physically bound water peak toward higher T_2 values. This phenomenon suggests that prolonged hydration promotes greater molecular mobility within the starch matrix of RD6, likely due to partial relaxation of the semi-crystalline amylopectin domains, consistent with earlier reports of starch swelling and crystallinity loss during hydration [29,34]. Moreover, in situ low-field NMR analyses have confirmed that the transition of water from free to bound states follows a diffusion-controlled mechanism influenced by the amylose–

amylopectin ratio and internal grain microstructure [34]. Collectively, these findings support the interpretation that the progressive rightward displacement of bound-water peaks with increasing soaking time reflects the enhanced diffusivity and redistribution of water molecules within the swollen starch network [35].

In addition, a recent study titled "Effects of starch gelatinisation and water migration on the microstructure of rice" [29] reported that during gelatinization, part of the absorbed water becomes trapped within the starch matrix, thereby reducing its molecular mobility. This phenomenon helps explain why, after prolonged soaking, the LBW and SBW peaks of RD6 shifted toward higher T_2 values—indicating that a portion of the water molecules gained greater mobility within the newly formed voids of the gelatinized starch structure. These comparisons further support the utility of TD-NMR for providing a modern and powerful method of investigating water states (FW, LBW, and SBW) in glutinous rice, whereas conventional thermal or gravimetric methods can only measure total moisture content without differentiating between molecular interactions.

We quantitatively analyzed the three water states by selecting a spectrum high at the positions of the three water states and calculating their percentages. Although this method does not provide the best quantitative analysis, it should capture trends of the changes in the three water components with increasing soaking time. The obtained percentages of the different states of water for the three rice cultivars are shown in Figure 5. The figure indicates that most of the absorbed water was transferred to the LBW, which is the water in the amorphous growth shells of rice granules. The RD6 and CN1 samples soaked for 0 and 0.5 h had the highest FW percentages. During the first half hour of soaking, the water absorption rates of RD6 and CN1 should have been the greatest.

The investigation of water saturation time provides a basis for understanding the gelatinization process of rice grains with high precision. Since glutinous rice cultivars differ slightly in amylopectin content, their water absorption and gelatinization behaviors also vary accordingly. The application of TD-NMR to monitor the transformation of water states during soaking could provide valuable insights for determining the optimal soaking duration required for complete hydration of each cultivar. Such information will help guide further studies linking T_2 relaxation characteristics to the gelatinization process and may ultimately be applied to improve both traditional steaming practices and the industrial processing of glutinous rice, leading to more consistent texture, desired stickiness, and efficient energy use during cooking.

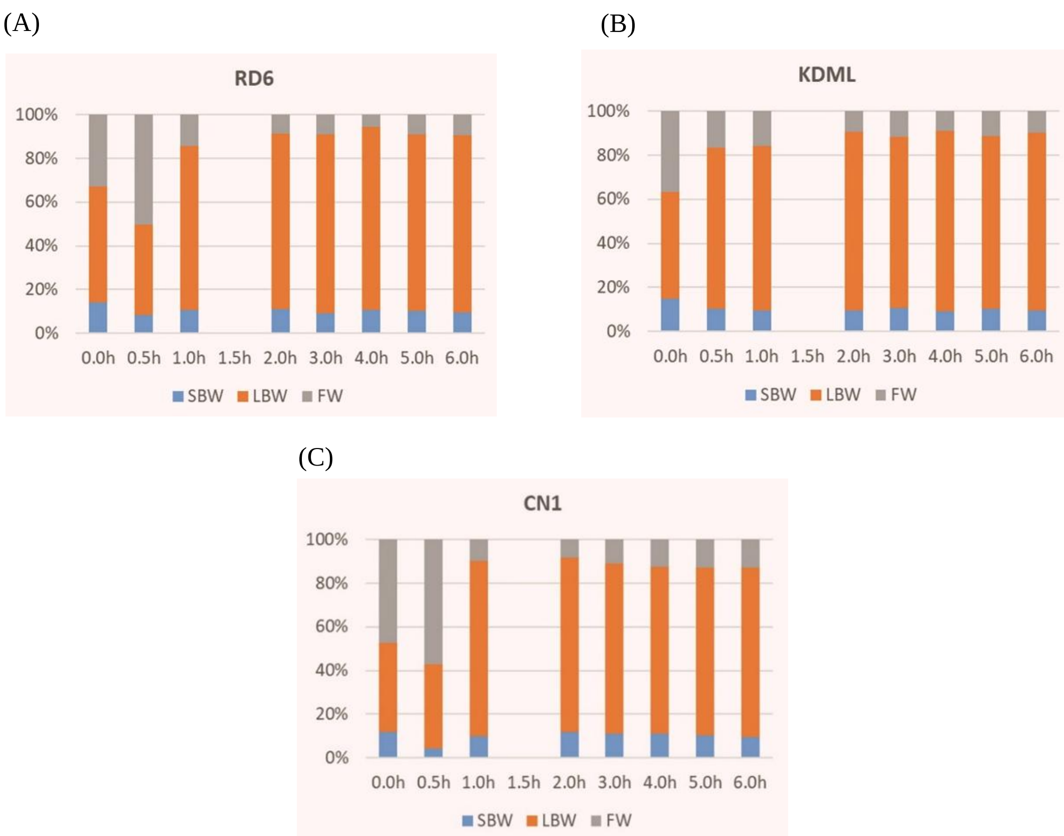

Figure 5 Percentages of FW, LBW, and SBW for (A) RD6, (B) KDML and (C) CN1 rice cultivars soaked for 0–6 h.

4. Conclusions

The results of our water absorption experiment suggest that rice cultivars with a lower amylose percentage can absorb more water, possibly because a smaller amount of amylose in the amylopectin structure provides more space for the absorption of water. The water absorption rates of cultivars RD6 and CN1 were considered to be greatest during the first half hour of soaking, because their FW percentages were highest for the samples soaked for 0 and 0.5 h. CN1 achieved water saturation in the shortest time (within 1 h), whereas RD6 and KDML became saturated with water after about 2 h. After saturation, most of the water in the three rice cultivars was in the LBW state, corresponding to water inside the amorphous growth shells of the rice granules. For saturated RD6, the peak corresponding to physically bound water gradually shifted to a higher T_2 value, indicating that a longer soaking time increases the water's molecular mobility. Although the low-field TD-NMR used in this study provides limited spectral resolution, the consistent trend of peak displacement and the reproducibility among replicates ensure the reliability of the observed relaxation behavior.

5. Acknowledgments

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6. Author statement

Nattawoot Maleelai: Investigation, Data Curation, Formal Analysis, Visualization, Writing – Original Draft.; Wiwat Youngdee: Conceptualization, Methodology, Supervision.; Nath Saowadee: Methodology, Data Curation, Formal Analysis, Writing – Review & Editing.

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