Relaxation Time Distribution of Thai Rice Kernels During Growth Process

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ABSTRACT

Transverse relaxation time (T_2) distributions of Thai rice kernels during grain development were investigated using time-domain NMR technique and Inverse Laplace Transform (ILT) method for analysis. During the early stages, 4–7 days after flowering (DAF), the T_2 distributions revealed that most of water protons exhibit long relaxation times, while a small number of the protons show the short relaxation times. During the grain development from 10 to 16 DAF, the starchy endosperm in the grains increases. It was found that water protons showing long relaxation times decreased, while those exhibiting short relaxation times increased. From 16 to 49 DAF, the T_2 distributions showed a slight changes. At 52 DAF, the distribution in the grains was very sensitive and changed significantly, and the existence of protons of the macromolecules (starch) were presented highly at this stage.

Keywords: Relaxation time distribution, Thai rice kernel, NMR

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Introduction

Plants growing is a complex process which involves with physical and biological changes of living tissues. The high rate of cell division, and cell enlargement is occurred at the early stages of growth, and then slowly at the last stages [1]. It was reported that the enlargement of the fruit cells is accompanied by that of vacuole [2], resulting in increasing of water in the vacuoles. Our previous study has shown that water residing in the vacuole is increased during the early stages, and there are the water migrations from cell compartments to other spaces [3].

It is obvious that water plays an important role in growing of biological tissues. Capillary water or free water (FW) is known to be water in the intercellular space [4]. Loosely bound water (LBW) is that water residing in the cell, while strongly bound water (SBW) is that water occupying the space inside the cell wall [5]. Moreover, the proportion of water in different cell compartments (i.e. intercellular, intracellular and cell wall spaces) are different [6].

Transverse relaxation time (T_2) of water protons in each cell compartment has been investigated to study the morphological changes during the progressive processes such as drying [7], ripening [8] as well as growth process [3]. The T_2 relaxation time can lead to the water mobility; the ability of water molecules to rotate freely and move, which depends on the water-proton position in cell compartments. Water protons residing in different cell compartments provide the different T_2 relaxation values [9-10]. As a result, biological tissues exhibit multi-exponential T_2 relaxation behavior. Based on plant-based food materials, it was reported that the water in the cell wall spaces (SBW) showed the shortest T_2 relaxation, water residing in intercellular spaces (FW) had medium relaxation, and water occupying the intracellular spaces (LBW) had the longest relaxation [6].

During development and ripening, the mono-exponential relaxation model was still used to provide an approximate T_2 relaxation time of fruits [8, 11]. During growth process, the T_2 relaxation times of rice grains were examined and presumed as being one [12], two [13], and three [3] relaxation components in the grains. However, biological samples including rice grain tissues exhibit multi-exponential T_2 relaxation, which can be composed of three (or more) relaxation components.

In this work, we are therefore interested in investigation of T_2 relaxation time of rice kernels during growth process using multi-relaxation model to obtain the distribution of T_2 relaxation, consequently this finding could provide a better understanding of morphological changes of rice kernel during the process. Also, the experimental information from this study could be used to map the growth stage with its T_2 distribution pattern.

Methodology

Thai rice cultivar *Oryza sativa L.(RD6)*, which is consumed widely especially in the northeast of Thailand, was collected at 3-day intervals, namely 4, 7, 10,…, 52 days after flowering (DAF). The details of the grains were presented in our previous study [3]. The experimental setup is shown in figure 1. Five rice kernels at each stage were placed in a 5 mm diameter test tube, and then the

sample tube was inserted into an NMR probe to measure T_2 relaxation time. The relaxation measurements were performed on a 21.6 MHz time-domain (TD)-NMR instrument (TeachSpin's Pulsed NMR, New York, USA). The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used to measure the relaxation signals decaying with T_2 time constant, known as the CPMG curve. The signal averaging of 8 samples on the oscilloscope was used to obtain a good signal-to-noise ratio (SNR). This can eliminate the random noise which occurs in the CPMG signal measurement.

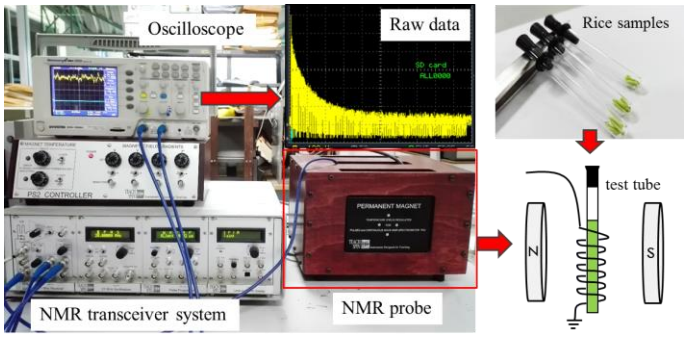

Figure 1 The experimental setup with TeachSpin's Pulsed NMR

The acquired data had their noise backgrounds removed, as shown in figure 2(A). The resulted data were determined their echo amplitudes as a function of time (echo time), using MATLAB. The treated data is now called the CPMG curve, as shown in figure 2(B). The signal amplitude at the echo time (t) is expressed by:

$$A(t) = \sum_i A_{0,i} e^{-\left(\frac{t}{T_{2,i}}\right)} + c$$

where $A_{0,i}$ is the signal amplitude at the beginning, which is proportional to the local spin density of the i^{th} relaxation component, $T_{2,i}$ represents the transverse relaxation time of the i^{th} component, and c is a constant value representing any error such as the residue noise background and the DC offset. The CPMG curves were analyzed using Inverse Laplace Transform (ILT) method to obtain the distribution of T_2 relaxation, as shown in figure 2(C).

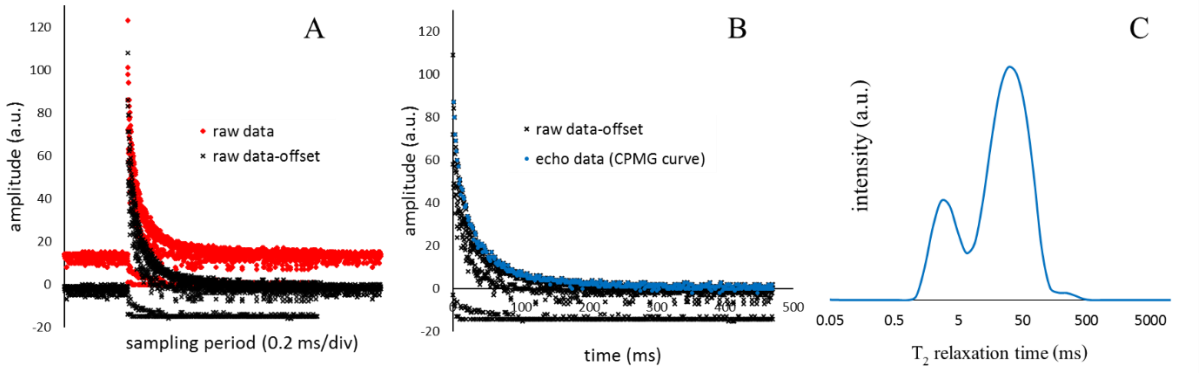

Figure 2 (A) Raw data before and after eliminating the noise backgrounds, (B) the raw data without the noise and the echo data as a function of the echo time, and (C) the distribution of T_2 relaxation time obtained using ILT

Results and Discussion

Figure 3 shows some CPMG curves of the kernels at each grain stage. These curves were then analyzed using the ILT method, and the results are the distribution of T_2 relaxation times as shown in figure 4. It was clearly shown that there are more than three components of T_2 relaxation time existing in the grain samples. Two main peaks are found and the relaxation time value for the highest intensity at each peak was given.

It was reported that T_2 relaxation time below 1 ms shows the presence of protons of macromolecules [14-15]. The T_2 relaxation value between 1 and 10 ms can be assigned to water molecules which tightly associated with macromolecules, such as protein and starch [16]. The T_2 value between 10 and 100 ms represents the protons in weakly bound water such the water in intercellular spaces [3]. At 4 DAF, the kernels contained a little of milky liquid (starchy endosperm), most of the water protons exhibit the long relaxation times (30.1-100 ms), while a small number of the protons show the short relaxation times (3.7-12.2 ms). Here, we therefore believed that those of long relaxation components are mainly from the water protons in intercellular spaces, while the remaining components indicate the water molecules tightly bound with starch macromolecules.

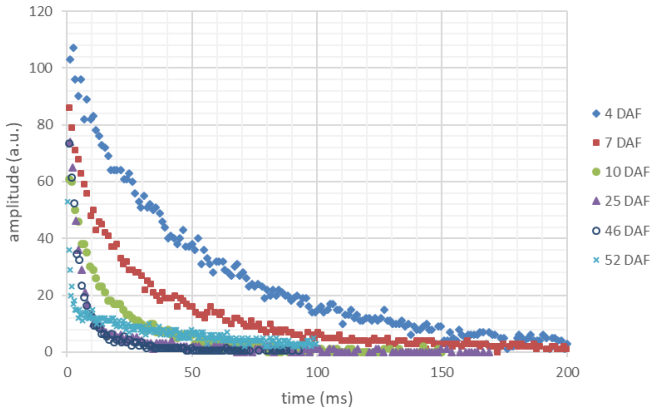

Figure 3 Examples of the CPMG curves of different rice stages

During 7-13 DAF, it was clearly demonstrated that the population of the protons exhibiting short relaxation times was increasing, while the number of protons showing long relaxation was declining. This result could be due to an enlargement of the endosperm and an increasing of the milky liquid during the period. Yang et al. reported that cell division rate in the rice grains can be occurred until 14 days after anthesis (DAA), while the grain filling rate are stopped at approximately 27 DAA [1]. Hence, it is reasonable to state that the number of water molecules which were tightly bound with the macromolecules is increasing during these stages, and this results in the increasing of the protons showing short relaxation times.

From 16 to 49 DAF, there is no significant change of the proportion of the water in cell wall and intracellular spaces, but there is a slightly uncertain change of the proportion of the water in intercellular spaces [3]. Here, it is therefore appeared that there is no change in the short relaxation components (1.5-9.1 ms) which were ascribed to the water molecules tightly bound with the

macromolecules; consequently this type of water is not easily to be transported. Moreover, that slight change of the intercellular water which is freely to move, resulting in the uncertainly changing of the long relaxation components over the stages.

However, the T_2 distribution changed significantly when the grains became white and dry at 52 DAF. At this stage, the grain size is also reduced. It was clearly found that the short relaxation components were more decreased and the components are between 0.2 and 2.7 ms. This result demonstrates that the protons in this solid stage are more tightly bound. As mentioned previously, this range of short relaxation components are showing the existence of protons of macromolecules [14-15]. Therefore, it was believed that those of short relaxation were from the protons of the starch macromolecules. Moreover, there were an increase of the protons exhibiting long relaxation time (22.3-100 ms). It was also reported that migration of cellular water has a significant effect on tissue shrinkage and structure collapse [10, 17-18], food tissue is deformed due to the migration of cell wall water [18], and the cell wall water completely moved out of its compartment to the other paces (i.e. intercellular spaces) and then it acts as characteristics of the water in those spaces [3]. Here, we believe that those of the long relaxation components are from the water residing in intercellular spaces, which moved from the former spaces of cell wall. This movement caused the shrinkage of the grain tissue, consequently the reduction in grain size at 52 DAF.

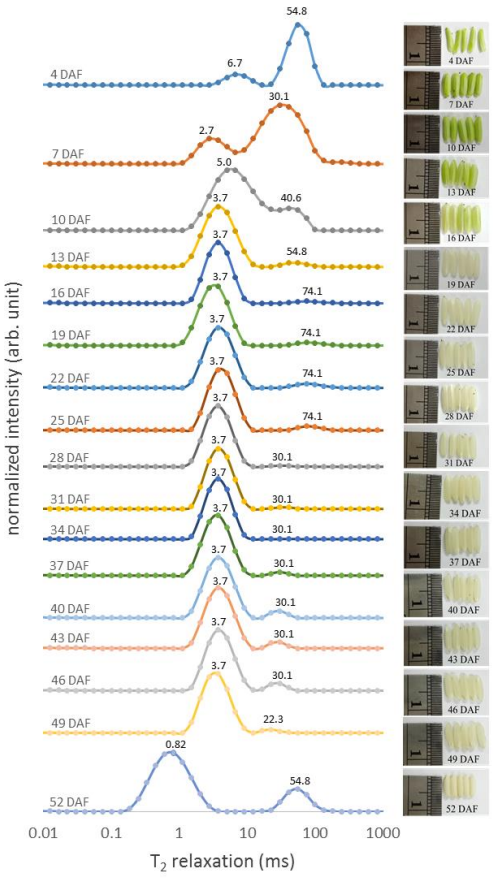

Figure 4 T_2 relaxation times distribution curves of Thai rice kernels at different grain stage

Conclusions

The physical and biological changes of Thai rice kernels *Oryza sativa L.(RD6)* during growing can be well understood by evaluating of the T_2 relaxation distribution obtained using TD-NMR technique and the ILT method. From 4 to 7 DAF, it was revealed that most of water protons exhibit long T_2 relaxation times, while a small number of the protons show the short relaxation times. However, these fractions are changed over time due to an increasing of the macromolecules, resulting in an increase of the protons showing short relaxation times. At the last stage, 52 DAF the highest level of protons of the macromolecules were presented in the grains, which caused the 52 DAF grains exhibited shortest relaxation times, compared to the others. The T_2 distribution for the 52 DAF grains also reveals that there is the movement of cell wall water from its spaces to the intercellular spaces. Moreover, the results were found that the patterns of T_2 distribution were obviously different during the early stages (4-13 DAF) and the 52 DAF stage, but slightly different during the middle stages. These differences in the T_2 distribution pattern are advantageous, which can be used to map the growth stage of rice with its T_2 distribution pattern.

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