In vitro NMR Relaxation Study of Water Protons in the Intracellular Water of Eggplant

[1]M. A. Rahman, [1]A.K.M.S. Islam, [2]B.K. Bala and [3]A. Khair
[1]Bangladesh Rice Research Institute, Gazipur, Bangladesh
[2]Bangladesh Agricultural University, Mymensingh, Bangladesh
[3]Jahangirnagar University, Dhaka, Bangladesh

Abstract: Xenon treated eggplant fruit was studied to examine the formation of structured water through nuclear magnetic resonance (NMR) measurements. Spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2), were carried out immediately after sample preparation and one day of xenon application, and continued for 15 days at the same temperature. It was found that the mean relaxation times T_1 (921 ms) and T_2 (324 ms) were shorter in the xenon treated samples compared with the control ones, T_1 (995 ms) and T_2 (344 ms), respectively. Two phase behaviours were observed for both T_1 and T_2. T_2 was also found to be independent to temperature. Browning of flesh was developed in the control sample after 6 days, while no sign of flesh browning was developed after 17 days in the treated sample. Formation of structured water by xenon gas results in suppression of the metabolic activity. Thus, xenon application was found to be effective in extending the storage life and maintaining the quality of fresh agricultural products.

Key words: Xenon gas, eggplant, NMR relaxation times(T_1, T_2), intracellular water, browning

Introduction

Water constitutes the major components of all living systems and its vital function in the life process is well known. There is conclusive evidence that water does not simply act as an inert medium but also participates at the molecular level basic biological interactions and in fundamental biological processes. The quantity and the mobility of the water reflect cellular activity as because biological reactions occur in water phase (Clegg, 1979). Nuclear magnetic resonance (NMR) spectroscopy is a powerful, non-invasive technique for studying the structure of water in various biological systems; in particular, mobility, self-interaction and extent of order water (Xin *et al.*, 1986). NMR technology enables us to observe changes in the properties of water in a single sample throughout the storage period. The most useful NMR parameter in the study of water are the spin-lattice relaxation time (T_1) and the spin-spin relaxation time (T_2). The NMR relaxation time of tissue in biological systems is assumed to be influenced by abnormal states in cells and tissues. It has been reported that the T_1 of water protons in biological systems can be affected by a variety of changes in the conformational state of macromolecules, such as water-membrane and water-protein interactions and in other factors affect the chemical environment (Mathur-DeVre, 1984). Such changes are associated with respiratory and energy metabolism (Iwaya-Inoue *et al.*, 1996). In general, lower T_1 and T_2 values reflect the slower motion of water molecules in the system. The dissolution of xenon gas, a non-polar gas, gives rise to a change in water structure to a clathrate-like structure and yields an increase in population of hydrogen-bonded water molecules (Tanaka and Nakanishi, 1991). The water in this state is called as "structured". The suppression mechanism of enzyme reaction could be understood through the physical state of water. The rate of enzyme reaction is regulated by the diffusion of substrate. Reduction in diffusion rate of substrate suppresses the biochemical reaction of metabolic activity of living products. Relaxation times in tissues, especially T_1, are dominated by the change of water viscosity (Mauss *et al.*, 1992). In many systems two T_1 or T_2 values can be obtained from the magnitude decay curve in NMR-pulse experiments, indicating regions of different mobility and different bond lengths of the water molecules to the biopolymer. Proton spin-lattice relaxation time (T_1) is reported as approximately to self-diffusion coefficient and viscosity (Simpson and Carr, 1958). Therefore, the objective of the present study was to investigate the suppression of metabolic activity by the formation of structured water in eggplant by measuring the proton NMR relaxation times, T_1 and T_2.

Materials and Methods

The study was conducted during the month of September' 1997 in the NMR laboratory room, Department of Biological and Environmental Engineering, University of Tokyo, Japan. *In vitro* study was performed at 20 °C in a tesla magnet corresponding to the proton resonance frequency of 25 MHZ. Eggplant fruits were collected from the farmers field at the Chiba prefecture, Japan. Fruits were stored within 1-2 hr of harvest in constant temperature chamber at 15 °C, before out-set of the experiment. Samples without defects were selected. The samples were cut by the size of 5x5x30 mm³. Immediately after cutting, the samples were placed into the two NMR pressure tubes (10 mm in dia and 200 mm in length) and were tightly closed by the rubber cork. NMR relaxation measurements were performed with Jeol NMR spectrometer (Pulsed JNM-MU 25 A, Jeol Co., Japan). This spectrometer was interfaced with a microcomputer for curve fitting. The whole magnetization decay curve was used for the calculation of the relaxation times. After initial measurement of T_1 and T_2, xenon gas was applied in one of the NMR tube for 15 minutes at 0.40 MPa at constant room temperature of 20 °C. In other NMR tube, no gas pressure was applied and was treated as control. Samples were stored at 20 °C constant room temperature. T_1 values were determined by a repeated 90°-t-90°, saturation pulse sequence method where t is the time interval between two pulses and T_2 values were determined by 90°x-t-180°y-2t-180°y-2t Curr-Purcell-Meiboon-Gill (CPMG) pulse sequence method (Martin *et al.*, 1980). The temperature was controlled with the accessory temperature control unit of the spectrometer and had an accuracy of about 4 °C± 1. All T_1 and T_2 values were the average of two independent measurements of 15 days. The relationship of the relaxation time (T_1) with self-coefficient (D) and viscosity (η) is as follows;

$$T_1 \propto D \propto T/\eta \propto \exp(-E/RT) \qquad \text{Eq. (1)}$$

with a common activation energy E, within the temperature (T) change between 0 and 40 °C and R is the gas constant (Simpson and Carr, 1958). From the equation (1), the increase in viscosity can be estimated through the decrease in T_1.

Results and Discussion

Spin-lattice relaxation time (T₁): Table 1 shows the T_1 of intracellular water in eggplant. Initially, T_1 of both xenon treated and control samples are same. T_1 of treated sample gradually decreased up to 11 days and then again slightly increased up to 15 days, however remains lower than the initial value. Overall, T_1 value was significantly low after eight days in the xenon treated sample than in control. In the control sample, the T_1 value was increasing constantly after 7 days. The final value (1190 ms) was 24 % higher than it's initial value (947 ms). The increasing T_1 values indicate that eggplant cell was deteriorating with passage of time. So that fluidity of cell was increasing due to the damage of cell membrane by increasing metabolic activities, and resulting in the increasing of the relaxation time. The mean relaxation time was 7 % lower in xenon treated sample (921 ms) than in control (995 ms). Consequently, individual T_1 times in xenon treated sample were generally lower than that of control at various times throughout the measurement, although, there were exceptions, most notably from 6 to 8th day of storage. Therefore, it could be understood that in the xenon treated sample, relaxation characteristics were altered. This means that cell in xenon treated eggplant maintained its viability. The values of T_1 presented here are similar to those documented for biological tissues (Clark and Macfall, 1997 and Morris, 1987). It is reported that the spin-lattice relaxation time, T_1 of xenon in tissues is generally short. Two phase behaviour was observed in T_1 relaxation plot for the xenon treated and untreated samples. Two phase behaviour might be due to high moisture content as reported by Zimmerman and Brittin (1957). The phases of water depend on the moisture content, nature of the molecule and temperature (Leung *et al.*, 1976; Belton *et al.*, 1973). Two phase behaviours I.,e two components of T_1 in xenon treated and control samples are also shown in Fig. 1. It can be seen from the Fig. 1, that the second component of $T_1(2)$ was significantly lower than that in the first component [$T_1(1)$] for both xenon and control treatments. It also indicates that the second component, $T_1(2)$ in the xenon treated sample was lower comparing with the control treatment although some variation was observed between 2 to 11 days. The two phase behaviour or multiple relaxation time of water can be interpreted as showing an exchange of protons or water molecules between the regions of water. Spin-lattice relaxation time, T_1 depends on the viscosity of water. In our experiment the decrease in T_1 could be attributed to the increase in viscosity of free water (Mauss *et al.*, 1992). This was due to the formation of structured water with xenon dissolution.

Spin-spin relaxation time (T₂): Table 2 shows the T_2 of intracellular water in eggplant. Initially, T_2 value was almost same for both xenon treated and the control samples. T_2 value of xenon treated and control sample did not show significant difference up to 7 days. However after 7th day, it was observed that T_2 value was significantly low in xenon treated samples than in the controls. The mean relaxation time, T_2 was 324 ms in the xenon treated sample and 344 ms for the control sample. It is interesting to note that the final value of T_2 was higher in xenon and control treatments than that in the initial value, while only T_1 was higher in the control than the initial value. The T_2 value depends on deterioration of cells as well as moisture content of the cells (Tsang and Khan, 1990). Two phase behaviour of T_2 was observed for the xenon treated and the control ones. Similar trend was observed for the second component of $T_2(2)$ of both xenon treated samples and the control ones, that it was lower than that in the first component of the same as described earlier for relaxation time for T_1. It is very interesting to note that the $T_2(2)$
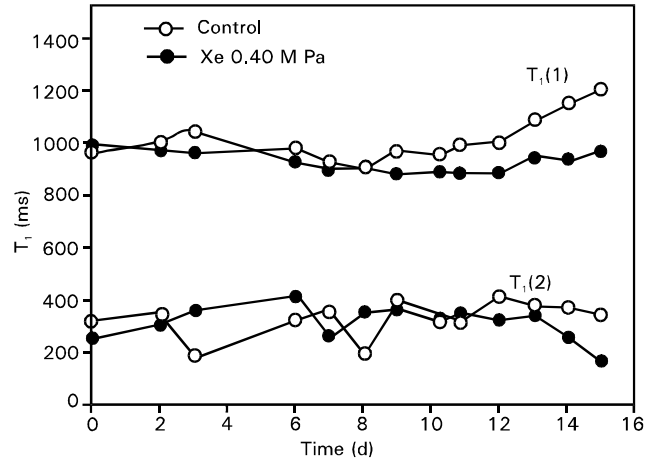

Fig. 1: Changes in two phase behaviours of spin-lattice relaxation times, T_1 in intracellular water of eggplant fruit. Values in parenthesis indicate the phase.
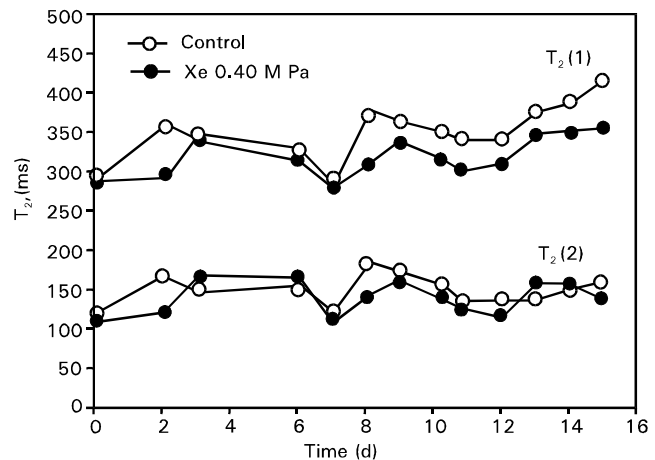

Fig. 2: Changes in two phase behaviours of spin-spin relaxation times, T_2 in intracellular water of eggplant fruits. Values in parenthesis indicate the phase.

behaviour patterns are similar to $T_2(1)$ both in xenon treated and control samples (Fig. 2). It indicates that spin-spin relaxation time is temperature independent and it supports the findings of Mauss *et al.* (1992). The phase behaviours depend on the moisture content (Lechert and Henning, 1976).

The relaxation times, T_1 and T_2 values were increased by the increase in soluble metabolite concentration and paramagnetic component (Ling, 1989 and Clark and Macfall, 1997). Hazlewood (1979) reported that when T_2 is decreased, the self-diffusion coefficient of water is also decreased. Therefore, the decrease in T_2 in the xenon treated samples indicated the viscosity increase due to the dissolution of xenon gas. Similar findings of decreased T_1 and T_2 in fruit and vegetables as well as T_2 value in animal cells were reported by Oshita *et al.* (1998). On the contrary, increase of T_1 value depends on the increase in the amount of free water movement in the plant and animal cells (Hazlewood, 1979 and Mathur-DeVre, 1984). It is known that an increase in water viscosity decreases the diffusion rate of substrate, resulting in decrease of metabolic activities. Therefore, the metabolic activity can be suppressed by reducing water availability for

Table 1: Changes in spin-lattice relaxation times, T_1 (ms) in intracellular water of eggplant fruits

Observation Nos.	Time (d)	Control (0.1 MPa)	Xenon (0.4 MPa)	
1	0.013	947	980	
2	2.02	984	962	3
	3.06	1030	952	
4	6.00	912	967	
5	7.00	887	914	
6	8.01	897	896	
7	8.99	954	868	
8	10.22	945	877	
9	10.83	979	872	
10	11.98	994	876	
11	13.01	1075	930	
12	14.00	1140	927	
13	14.96	1190	954	
Mean		995	921	

Table 2: Changes in spin-spin relaxation times, T_2 (ms) in intracellular water of eggplant fruits

Observation Nos.	Time (d)	Control (0.1 MPa)	Xenon (0.4 MPa)
1	0.013	289	295
2	2.02	359	298
3	3.06	343	348
4	6.00	319	330
5	7.00	285	290
6	8.01	375	313
7	8.99	367	339
8	10.22	352	319
9	10.83	343	304
10	11.98	344	311
11	13.01	379	349
12	14.00	390	352
13	14.96	419	358
Mean		344	324

enzyme reaction by formation of structured water. Oshita *et al.* (1997) mentioned that control the state of water by means of structured water formation could extend the postharvest life of fruits and vegetables without lowering the refrigeration temperature.

Browning: Browning was observed in the control sample after 6 days but xenon treated sample remained in good state even after 15 days of storage. Suppression of browning was claimed to be due to the formation of structured water, where a similar mechanism was reported for broccoli and persimmon (Rahman, 1996 and Oshita *et al.*, 1997).

In conclusion the spin-lattice and spin-spin relaxation times, (T_1 and T_2) were lower in xenon treated eggplant tissues than the control. The values of T_1 are to be found similar to those documented for biological tissue of persimmon fruit. Two phase behaviours were observed for T_1 and T_2. The spin-spin relaxation time, T_2 was found to be independent to temperature. The decrease in T_1 and T_2 showed that the water viscosity increased in xenon treated sample, which confirmed the structured water formation. Moreover, xenon treatment also suppressed the browning for 15 days. Metabolic activity was suppressed in the xenon treated samples. Therefore, xenon gas could be used as an alternative storage method to increase the shelf life and maintaining the quality of eggplants.

Acknowledgments

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