

Relaxation Time of Protons in Intracellular Water of Broccoli

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ABSTRACT

Viscosity of intracellular water was shown to be related to physiological change. The structured water, i.e., the water having a large population of hydrogen-bonded water molecules, formed by dissolution of xenon (Xe) gas is expected to increase the water viscosity, thus reducing physiological changes in food products. Proton T_1 of distilled water showed 3.37 s at 298 K, and it decreased by dissolution of xenon to 2.80 s under 0.6 MPa of xenon pressure. The change in proton T_1 induced by xenon suggested the change in viscosity of water, because it is known that T_1 is inversely in proportion to viscosity of water. Broccoli branchlets were stored at 293 K for 25 days under atmospheric air as the control. Proton T_1 of water in broccoli determined by one-term exponential equation exhibited the trend of prolongation until Day 8. Thereafter, the trend changed and became shorter. A 2-term exponential equation gave the best-fit relaxation times for proton T_2 , that is, a long relaxation time T_{2a} and a short relaxation time T_{2b} . During the period of Day 2 to Day 9, both T_{2a} and T_{2b} showed longer values than the initial values with no significant change in fraction of the two components, i.e., the component of T_{2a} and that of T_{2b} . This explains the change in the mobility of water molecules. In successive periods, T_{2a} and T_{2b} were almost constant, however, the fraction of the two components changed. This suggested water leakage, which implies the degradation of the cell membrane. In fact, a little dehydration from the sample was observed on Day 9 and distinct surface browning occurred on Day 11. For xenon-treated broccoli, it was stored under xenon-mixed air with xenon partial pressure of 0.45 MPa at the same temperature as the control. The initial value of T_1 determined by one-term exponential fit was nearly the same as that of the control. Also, the xenon-treated broccoli showed elongation of T_1 similar to that of the control except it was always shorter until Day 9. After this period, T_1 did not decrease and it was kept almost constant until Day 25. For T_{2a} and T_{2b} , there was the trend of elongation with a fraction change from Day 1 to 2. From Day 2 to 25, however, there was little difference not only in relaxation time but also in the fractions of both components. These observations would indicate that the mobility of water in both components did not change so much. Neither water leakage nor visible browning occurred which is probably attributed to the formation of structured water induced by dissolution of xenon.

Keywords: Relaxation time, Broccoli, Structured water, Xenon, Viscosity

INTRODUCTION

In recent years, NMR technique became an important method in the investigation of the state of water in food and agricultural products. The state of water in starches and in some vegetables was studied by Lechert et al. (1980) through NMR measurement. NMR study was reported to predict peanut maturity by Tollner et al. (1998), and the maturity evaluation of fruits by Chen et al. (1993, 1996). Both the spin-lattice relaxation time T_1 and spin-spin relaxation time T_2 of water in fruits were reported to correlate with the maturity of the fruit. This implies that relaxation times of water are an index of the physiological state of agricultural products.

The physiological state of agricultural products changes with metabolism which is based on enzyme reactions. Hence, by reducing the enzyme reactions the freshness of agricultural products can be prolonged. A mechanism of such suppression can be understood through the physical state of water. The rate of enzyme reaction is regulated by the diffusion of substrate that is represented by the self-diffusion coefficient D expressed by Stokes-Einstein equation, as follows:

$$D = kT/6\pi \mathbf{h} r \quad (1)$$

where k is Boltzmann constant, T is absolute temperature, \mathbf{h} is viscosity of the solvent (in this case, intracellular water,) and r is radius of the solute (in this case, substitute). Eqn (1) shows that both a lower value of temperature and a larger value of viscosity result in a smaller value of diffusion coefficient. The contribution of \mathbf{h} to the diffusion coefficient is thought to be greater than that of temperature due to the following reason. When temperature decreases from 303 K to 283 K, \mathbf{h} increases from 0.801 mPa s to 1.31 mPa s (Jonas et al., 1976). In other words, T decreases to 93 % and $1/\mathbf{h}$ decreases to 61 % of their initial value. So, a reduction of D to 57 % will lead to the suppression of the enzyme reaction rate. In this manner, the increase in \mathbf{h} will contribute to reduce the enzyme reaction rate more effectively than the decrease in temperature.

As a way to make \mathbf{h} greater, hydrophobic hydration can be utilized. Hydrophobic hydration with non-polar gases have been studied by many researchers (Shinoda and Fujihira, 1968; Parrish and Prausnitz, 1972; Franks, 1973; Garg et al., 1977; Davidson and Ripmeester, 1978). The dissolution of xenon (Xe), one of the non-polar gases, 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 “structured”, and the structured water is the term used to describe the water with a large population of hydrogen-bonded water molecules. The viscosity of water has a close relationship with intermolecular potential energy

including hydrogen-bond energy. Therefore, the increase in hydrogen-bonded water molecules could be interpreted as the increase in viscosity of water. On the other hand, the viscosity of water determines the diffusion rate of substrate and also regulates the enzyme reaction rate because of the relationship expressed in Eqn (1). Hence, when the intracellular water of agricultural products becomes structured, metabolic activities are thought to be suppressed. In order to estimate the increase in viscosity of structured water, NMR measurement can be employed. Proton spin-lattice relaxation time T_1 is reported as approximately related to self-diffusion coefficient D and viscosity \boldsymbol{h} (Simpson and Carr, 1958; Hindman, 1974), and the relationship is given as follows:

$$T_1 \propto D \propto T / \boldsymbol{h} \propto \exp(-E / RT) \quad (2)$$

with a common activation energy E being satisfied from 273 to 313 K (Simpson and Carr, 1958). From Eqn (2), the increase in viscosity \boldsymbol{h} can be estimated through the decrease in spin-lattice relaxation time T_1 .

In this study, xenon gas was applied in order to increase the value of \boldsymbol{h} by utilizing the hydrophobic hydration between xenon and water molecules. Xenon gas was first applied to distilled water to form structured water, and then, applied to broccoli up to a given partial pressure as described in the following section.

The objectives of this study were (1) to confirm the formation of structured water through the change in spin-lattice relaxation time T_1 ; and (2) to examine a change in both spin-lattice relaxation time T_1 and spin-spin relaxation time T_2 of water in broccoli to estimate the physiological state of untreated broccoli (control) comparing with the xenon-treated broccoli.

MATERIALS AND METHODS

Xenon Selection

Among non-polar gases that can form structured water, inert gases are most suitable since they can be expected not to cause biochemical changes in cell because of their low chemical reactivity. Table 1 shows solubilities of inert gases into water under the partial pressure of 101 kPa at 298 K (Lide, 1993). Xenon was selected to form structured water because it shows the greatest solubility. In other words, xenon has the greatest ability for the formation of structured water as compared to the other inert gases.

Relaxation Time

To detect the physical state of water, spin-lattice relaxation time T_1 and spin-spin relaxation time T_2 were measured with a JEOL pulsed NMR spectrometer (JNM-MU25 manufactured by JEOL LTD.) operating at 25 MHz corresponding to a magnetic field strength of 0.58 T. For the T_1 measurement, the saturation recovery

technique was employed and a repeated $90^\circ\text{-}\tau\text{-}90^\circ$ pulse sequence was used, where τ is the time interval between the two pulses. For T_2 , the CPMG (Carr-Purcell-Meiboom-Gill) technique with $90^\circ\text{-}\tau\text{-}180^\circ\text{-}2\tau\text{-}180^\circ\text{-}2\tau$ pulse sequence was used. Pressure-tight NMR tubes having an outside diameter of 10 mm were used. The temperature was controlled with the temperature control unit of the spectrometer and had an accuracy of about ± 0.5 K.

Sample Preparation

For normal water sample, the paramagnetic dissolved oxygen was removed by flushing triple distilled water several times with nitrogen gas. The nitrogen gas was then evacuated and the sample was sealed in equilibrium with its own vapor. For xenon solutions, xenon gas was applied to pressure-tight NMR tube containing oxygen-free water up to given partial pressure. Measurements were conducted at least 12 hours after the application of xenon gas to make sure that the equilibrium state was reached.

For the measurement of water in broccoli (*Brassica oleracea L*, Italica group), the product was harvested in a local farm and transported to the laboratory in the same day. Two entire branchlets were cut from the main head axis and then florets were trimmed off from entire branchlets. The remaining branchlets were cut in the size of about 4 x 4 x 18 mm. Each piece of branchlet was then placed in a pressure-tight NMR tube. Immediately after the sample preparation, initial measurements of T_1 and T_2 were conducted for both samples. Thereafter, xenon gas was superimposed to one NMR tube up to 0.45 MPa to use as the xenon-treated sample. The other NMR tube with the remaining sample was used as the control. Both samples were placed in a constant temperature room of 293 K. All data were recorded at this constant temperature and the measurements were conducted three times and averaged.

RESULTS AND DISCUSSION

Structured Water and T_1

The spin-lattice relaxation time T_1 of protons in oxygen-free water was measured under atmospheric pressure at 5 different temperatures ranging from 278 to 298 K. Average values of 3 measurements at each temperature were plotted in Figure 1 (open circle). The results reported by Simpson and Carr (1958), shown by solid line in the Figure, were used to predict the experimental data. Thus, Figure 1 shows that T_1 agreed well with the predicted curve over the temperature range. This fact supported the validity of both preparation procedure of water sample and NMR spectrometer system including the application of the measuring technique.

Figure 2 shows T_1 of protons in xenon solutions at 298 K. The open circle represents the averaged value

of 3 measurements. T_1 became shorter as xenon partial pressure increased, i.e., T_1 decreased from 3.37 s without xenon to 2.80 s at 0.6 MPa. This indicates that formation of structured water occurred, since a higher partial pressure of xenon results in a greater amount of xenon dissolved in water. Moreover, the kinetic processes of water estimated by both spin-lattice relaxation time and viscosity are related to the same activation energy as expressed in Eqn (2). Therefore, the decrease in T_1 caused by structured water indicated the increase in viscosity.

Change in T_1 of Water in Broccoli

Proton spin-lattice relaxation time T_1 of water in both xenon-treated and the control broccoli at 293 K was determined by the analysis of a graphical adjustment to reduce the curvature of the signal intensity vs pulse interval plots. The recovery curve with one-term exponential equation was found to give the smallest variance among multi-terms exponential equations. An example of the analysis of T_1 of the control broccoli on Day 2 is shown in Figure 3. Figure 4 shows the change in T_1 of xenon-treated broccoli (solid circle) and that of the control broccoli (open rectangle). Each symbol represents the averaged value of 3 measurements. For the control broccoli, T_1 became gradually longer from the initial value of 820 ms to 1220 ms on Day 8. Thereafter, T_1 became shorter and a little dehydration from the sample surface was observed on Day 9 by visual inspection. T_1 showed 1130 ms on Day 9 and it decreased to 811 ms on Day 25. Distinct surface browning occurred on Day 11.

For xenon-treated broccoli, the initial value of T_1 of 864 ms was almost the same as the control. However, T_1 on Day 2 and later showed nearly constant value, i.e., T_1 was in the range from 984 ms to 1070 ms. This is noticeable difference from the behavior observed for the control. Also, browning was not recognized on the surface of xenon-treated broccoli. This implies the contribution of structured water, causing greater viscosity than the normal water, to the suppression of enzymatic browning in the treated sample.

Examination of T_2

For spin-spin relaxation time T_2 , a 2-term exponential equation gave the smallest variance among one- and multi-terms exponential equations for both xenon-treated and the control broccoli, and it allowed calculation of a best-fit relaxation time with two components. Figure 5 shows an example of the analysis with a 2-term equation for the determination of T_2 of the control broccoli on Day 2. Table 2 shows the results of 2-term exponential analysis that gives two components with different relaxation times, that is, a long relaxation time T_{2a} and a short relaxation time T_{2b} for the control broccoli. The fraction in Table 2 indicates the ratio of both long and short components of water that were determined by NMR signal intensity of each component. The corresponding results for the xenon-treated broccoli are shown in Table 3.

For the control broccoli, both T_{2a} and T_{2b} in the period 2 are noticeably longer than those in the period 1, although there is not significant difference in the fraction of each component between the period 1 and 2 (Table 2). This marked prolongation of T_{2a} and T_{2b} would indicate the change in mobility of water molecules. Between the period 2 and 3, however, fractions of T_{2a} and T_{2b} differed significantly although T_{2a} and T_{2b} did not change too much. This is almost the opposite of the observed trend between period 1 and 2. The different trend observed in period 2 to 3 can be attributed not to the change in mobility of water but to the leakage of water from the component of the long relaxation time. In fact, water leakage from the sample was visibly noticed in period 3. This implies that permeability of the cell membrane increased due to degradation of the function of the membrane. It is interesting to note that T_1 determined by the one-term exponential equation began to decrease on the final day of period 2, Day 9, and its trend was still maintained until the final day of period 3, Day 11, as is seen in Figure 4. In period 4, both T_{2a} and T_{2b} became considerably shorter than those in period 3. Also, the fraction of the component of T_{2a} increased and that of T_{2b} decreased inversely. This might be caused by water migration from the site of T_{2b} component to that of T_{2a} component, and the increase in fraction of T_{2a} would be explained by a delicate water balance between a leakage from the site of T_{2a} component and a migration from the site of T_{2b} .

For xenon-treated broccoli, there was a comparable trend of both T_{2a} and T_{2b} elongation with a little fraction change from period 1 to 2. From period 2 to 3, i.e., Day 2 to 25, however, there was little difference not only in relaxation time but also in the fraction of both components (Table 3). These observations would indicate that the mobility of water in both components did not change so much and that neither water leakage nor visible browning occurred in the sample. There are two possibilities for no water leakage. First, an increase in evaporation energy of water associated with a greater population of hydrogen-bonded water molecules induced by xenon dissolution, and second, the maintenance of membrane function. The latter possibility cannot be checked with the data obtained in this study. However, the authors have shown that the respiratory metabolism of broccoli was suppressed by xenon-treatment (Oshita et al., 1996). In this case, the respiratory rate of xenon-treated broccoli under 0.24 MPa of xenon pressure at 275 K showed $13.8 \text{ mgCO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, while that of the control broccoli stored in normal air condition was $19.4 \text{ mgCO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ at the same temperature. The suppression of respiratory metabolism by xenon may be associated with the maintenance of the membrane function. Further study is required to discuss quantitatively the effect of the xenon gas on the membrane function.

CONCLUSIONS

Proton T_1 measurement was made on distilled water under various pressures of xenon. T_1 became shorter as xenon pressure increased, which indicated the formation of structured water having a large population of hydrogen-bonded water molecules. This supported indirectly the increase in viscosity of water.

The control broccoli branchlet was stored at 293 K in a NMR tube for 25 days. T_1 given by a one-term exponential equation exhibited the trend of prolongation until around Day 8, that is, from 820 ms to 1220 ms for T_1 . Thereafter, the trend turned inversely with shorter duration, and reached to 811 ms for T_1 on Day 25. For spin-spin relaxation time, the best-fit relaxation time was obtained by a 2-term exponential equation, and T_{2a} of the component with long relaxation time and T_{2b} of the component with short relaxation time were determined. From Day 2 to 9, both T_{2a} and T_{2b} showed longer values than the initial values with no significant change in fraction of both components. Therefore, the mobility of water molecules was estimated to change in this period. In successive period from Day 2 to 11, T_{2a} and T_{2b} were almost constant, even though the fraction of the two components changed. This implied a leakage of water suggesting the degradation of the cell membrane. In fact, a little dehydration from the sample was observed through visual inspection on Day 9 and surface browning occurred on Day 11.

Xenon-treated broccoli was also stored under xenon-mixed air with xenon partial pressure of 0.45 MPa at 293 K for 25 days. The initial value of T_1 by one-term exponential equation was nearly the same as that of the control. Also, the xenon-treated broccoli showed elongation similar to that of the control except it was always shorter until Day 9. After this period, T_1 was kept almost constant until Day 25. Neither water leakage nor visible browning occurred. This is probably attributed to the formation of structured water. For T_{2a} and T_{2b} , there was the trend of elongation without remarkable fraction change during the first 2 days associated with a change in the mobility of water molecules. From Day 2 to 25, however, there was little difference not only in relaxation time but also in the fraction of both components. These observations would indicate that the mobility of water in both long and short components' sites did not change too much. The physiological differences observed for the xenon-treated broccoli were attributed to the formation of structured water, causing greater viscosity of water.

ACKNOWLEDGMENT

The authors express their appreciation for the financial support by a Grant-in-Aid for Scientific Research (C) (No. 09660268) from the Ministry of Education, Science, Sports and Culture of Japan.

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Table 1 Solubility of inert gases in water at 298 K

Non-polar gas	Solubility under atmospheric pressure, mol Gas / mol H ₂ O
Xenon: Xe	7.890×10^{-5}
Krypton: Kr	4.512×10^{-5}
Argon: Ar	2.519×10^{-5}
Nitrogen: N ₂	1.183×10^{-5}
Neon: Ne	8.152×10^{-6}
Helium: He	6.997×10^{-6}

Table 2 Two-term exponential analysis on T₂ of the control broccoli

Period	Time, days	Long component	Short component		
		T _{2a} , ms	Fraction, [-]	T _{2b} , ms	Fraction, [-]
1	0.08	287-292	0.844-0.879	132-166	0.121-0.156
2	1.8-8.8	433-492	0.734-0.901	207-268	0.100-0.266
3	10.8	475-494	0.504-0.577	196-214	0.423-0.496
4	25.0	242-245	0.672-0.681	133-134	0.319-0.328

T_{2a}; relaxation time of long component, T_{2b}; relaxation time of short component

Table 3 Two-term exponential analysis on T₂ of xenon-treated broccoli

Period	Time, days	Long component	Short component		
		T _{2a} , ms	Fraction, [-]	T _{2b} , ms	Fraction, [-]
1	0.06	286	0.862	144	0.138
2	1.9-8.1	419-460	0.627-0.822	188-239	0.178-0.373
3	8.8-25.0	417-478	0.683-0.896	177-269	0.133-0.317

T_{2a}; relaxation time of long component, T_{2b}; relaxation time of short component

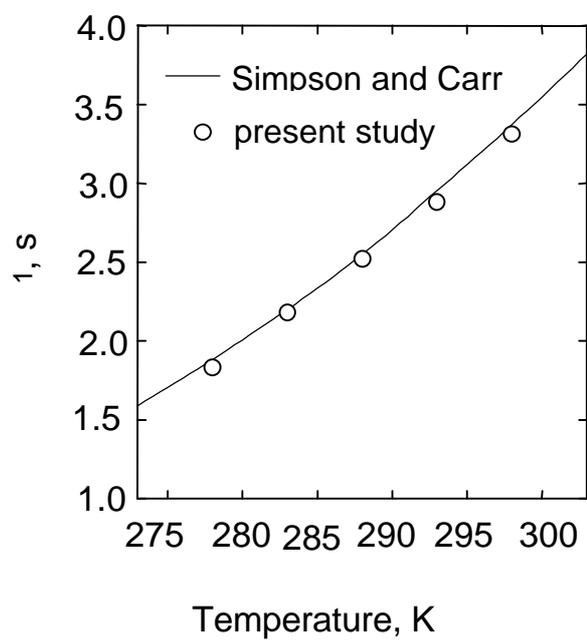


Figure 1 Variation in T_1 of protons in water with temperature

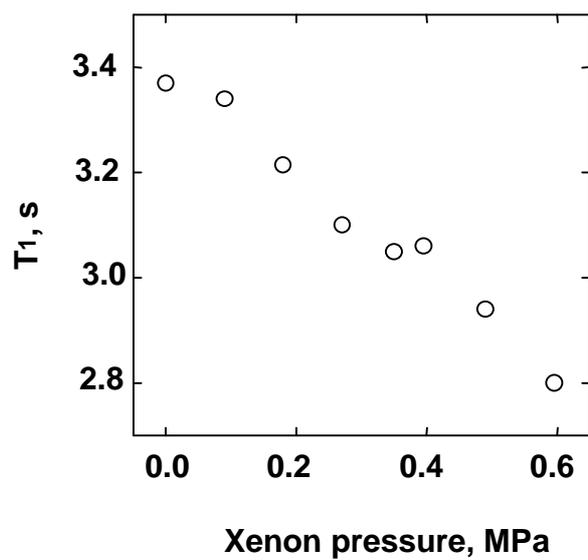


Figure 2 Variation in T_1 of protons in water with xenon pressure at 298 K

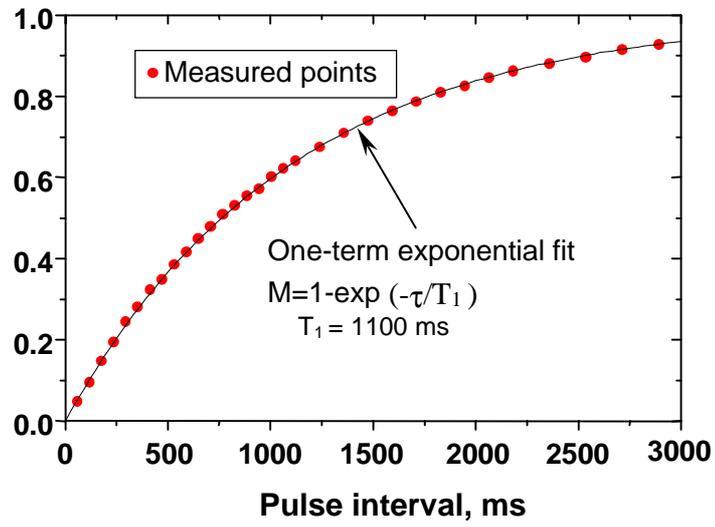


Figure 3 An example of one-term exponential analysis of T_1

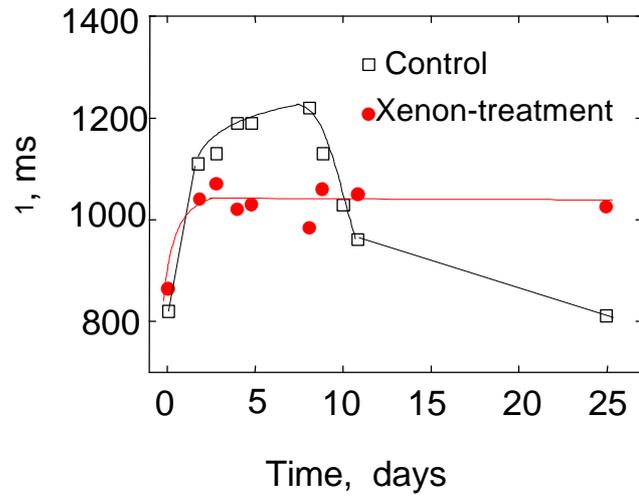


Figure 4 T₁ of water in both the control and xenon-treated broccoli at 293 K

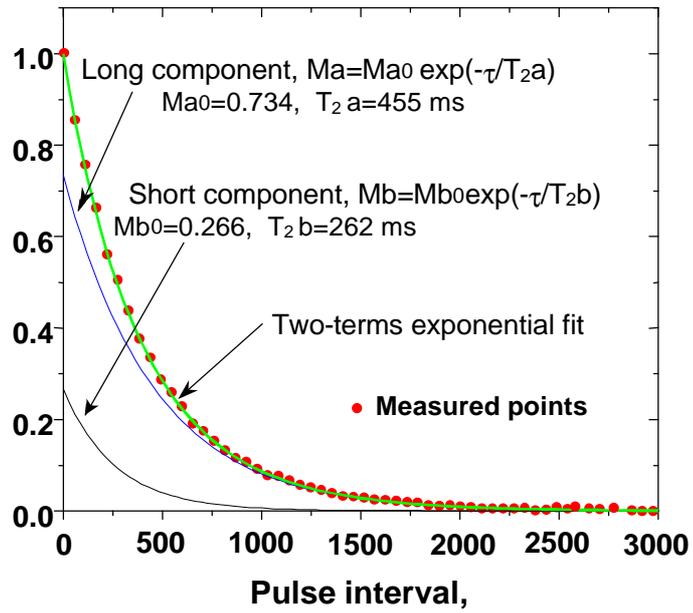


Figure 5 An example of two-terms exponential analysis of T_2