STORAGE OF BROCCOLI BY MAKING THE WATER STRUCTURED

-Suppression of metabolism-

S. OSHITA, Y. SEO , Y. KAWAGOE, M. A. RAHMAN
Graduate School of Agricultural and Life Sciences, THE UNIVERSITY OF TOKYO
Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, JAPAN

ABSTRACT

The effect of structured water by dissolution of xenon was examined from the view point of the suppression of both browning and respiratory metabolism of broccoli. The structured water is formed due to hydrophobic interaction when xenon gas dissolves into water. NMR measurements were carried out to determine proton spin-spin relaxation time, T2, for water. There was a difference in proton T2 between distilled water and structured water. This can be interpreted as the change of water structure. For the broccoli cut in half stored for 16 days at 279 K, the section color did not change appreciably for the sample whose water was structured by dissolution of xenon whose initial partial pressure was 0.39 MPa. In contrast to this, the browning of section surface was observed for the sample stored under the condition of nitrogen gas at the same partial pressure as xenon and for the sample stored under atmospheric condition. These results led to the conclusion that the suppression of browning by oxidation was due to structured water but not to applied pressure. Adding to this, the water structured by xenon has resulted in suppression of respiratory metabolism of broccoli.

Key Word: Broccoli, Metabolism, NMR, Storage, Structured water

INTRODUCTION

Vegetables are living organism and they continue to respire after harvest. The rate of respiration is indicative of the rapidity with which compositional changes are taking place within vegetables, and hence indicates the potential shelf-life of vegetables. Respiratory activity has a close relationship with the freshness which is affected by such things as temperature, humidity, the composition of the atmosphere which surrounds them. Among these factors, temperature affects greatly on shelf-life of broccoli, with a storage life of 3 to 4 weeks in air at 273 K and 2 to 3 days in air at 293 K "King and Morris (1994)". Temperature is thought to be a factor which governs the rate of enzyme reaction. And metabolism is based on intracellular chemical reactions which are, for the most part, enzyme mediated. Therefore, low temperature causes suppression of enzyme reactions which leads to extended shelf-life of vegetables. On the other hand, the rate of enzyme reaction is regulated by the diffusion rate of substrate which depends inversely on the viscosity of water. The viscosity of water is expected to increase when the water becomes "structured". Structured water means the water with a large number of hydrogen-bonded water molecules. Hence, a way for the suppression of metabolism is thought to make intracellular water structured. Structured water can be formed by hydrophobic hydration caused by dissolution of nonpolar gas. In this study, the formation of structured water was examined and the effect of structured water on the suppression of metabolism of broccoli was investigated.

CONCEPT OF STRUCTURED WATER

When nonpolar gas dissolves into water, a hydrophobic hydration is formed and the water becomes structured. This can be interpreted as the increase in the number of hydrogen-bonded water molecules on the basis of computer simulations, "Tanaka and Nakanishi (1991)". Because the number of hydrogen-bonded water molecules is one of factors governing the motion of water molecules, the viscosity of water will increase when water becomes structured. The rate determining factor of enzyme reaction is the diffusion rate of substrate which is regulated by the viscosity of intracellular water. Therefore, if the viscosity of intracellular water increases, enzyme reaction rate decreases. This leads to the suppression of biochemical reactions in cell. In this way, biochemical reactions are expected to be suppressed when intracellular water changes into structured water.

MATERIALS AND METHODS

NMR measurement of structured water

Among many nonpolar gases, inert gases are most suitable since they can be expected not to cause biochemical changes in cells because of their low chemical reactivity. Xenon was selected as the inert gas in this study because of the greater degree of structured water formation with xenon compared to other inert gases.

Pressure NMR tubes having an outside diameter of 10 mm were used. Xenon gas was applied to pressure NMR tube containing distilled water up to given partial pressure. Partial pressures of xenon within NMR tubes, equilibrated at 293 K, were 0.88 MPa and 0.27 MPa. As the amount of xenon dissolving in water corresponds to xenon partial pressure, xenon solution at higher partial pressure signifies the water with greater degree of structured. In this way, xenon solutions with different structured levels were prepared. By the use of the pulse spacing dependence of the CPMG sequence, NMR measurements were carried out with a

JEOL pulsed NMR spectrometer (JNM-MU25) operating at 25 MHz to determine proton spin-spin relaxation time, T2, for xenon solutions at 293 K. Xenon gas was then released from NMR pressure tubes to obtain distilled water. The proton spin-spin relaxation time of distilled water in each NMR tube was also measured at 293 K in a same way as xenon solution.

Examination of browning

Broccoli (Green sprouting broccoli) were obtained from a local supermarket in Tokyo. At a room temperature about 293 K, broccoli were cut along their stems in halves and each of three pieces was placed in an individual pressure container. The pressure container, shown in Photo 1, has a volume of 2.124 L with an inside diameter of 130 x 10^{-3} m and a height of 160 x 10^{-3} m. One container was filled with atmospheric air as a control (control treatment). The second one was filled with atmospheric air on which xenon gas was superimposed up to 0.39 MPa partial pressure in order to make intracellular water of broccoli structured (xenon treatment). As the solubility of xenon in water is very small, the partial pressure of xenon was made higher to make the amount of xenon dissolving in water in broccoli greater. The third one was filled with atmospheric air on which nitrogen gas was superimposed up to 0.39 MPa partial pressure same as xenon (nitrogen treatment). Because the solubility of nitrogen in water is about one-tenth of that of xenon, nitrogen will not contribute to the formation of structured water. The nitrogen gas was, therefore, used only to examine the effect of pressure on browning of the section surface. Three containers with each piece of half broccoli were placed in a constant temperature chamber at 279 K for 16 days. After 16 days storage, color differences between section surfaces of broccoli were measured by the colorimeter (NIPPON DENSHOKU, Z-1001DP).

Examination of respiratory activity

Broccoli (Medium Green Sprouting) were harvested in a farmers field. As it was reported that broccoli shows high respiratory rate immediately after harvest "Wang (1977), Bastrash et al. (1993), King and Morris (1994)", broccoli were placed in a chamber kept at 278 K for 1 day to avoid extraordinary respiration. As a control, a whole broccoli of 267 x 10^{-3} kg in mass was placed in a pressure container with air at atmospheric pressure (control treatment). To examine the effect of structured water on respiratory activity, another one of whole broccoli whose mass was 257 x 10^{-3} kg was placed in a pressure container filled with air, on which xenon gas was superimposed up to 0.24 MPa partial pressure (xenon treatment). They were stored in a constant temperature chamber at 275 K for 6 days. The total pressure and CO_2 concentration of mixed gas in each pressure container were measured every day. Gas chromatography (Shimadzu GC-14) were used. The partial pressure of CO_2 was calculated from volumetric concentration of

CO_2 by the use of equation (1). Then, mass of CO_2 in a pressure container per unit mass of sample was calculated from equation (2).

$$P_{CO_2} = P_t\, C / 100 \qquad (1)$$

$$M_{CO_2} = 44000\, P_{CO_2}\, V_f / (R\, T\, M_s) \qquad (2)$$

where,

P_{CO_2} :partial pressure of CO_2, Pa
P_t :total pressure of mixed gas, Pa
C :volumetric concentration of CO_2, %
M_{CO_2} :mass of CO_2, mgCO_2 / kg of sample
V_f :volume of free space in container, m^{-3}
R :gas constant, 8.3143 J K^{-1} mol^{-1}
T :absolute temperature, K
M_s :mass of sample, kg

RESULTS AND DISCUSSION

Formation of structured water

The proton spin-spin relaxation time, T2, was measured for xenon solution and for distilled water at 293 K. The proton T2 was 1.70 s for the xenon solution at 0.88 MPa of xenon partial pressure and 1.82 s for the xenon solution at 0.27 MPa of xenon partial pressure. The proton T2 of distilled water obtained by degassing xenon from each xenon solution was 2.06 s and 1.97 s. Values of T2 determined for xenon solutions were less than those for distilled water. As T2 has a close relationship with diffusion process of water molecules, the difference in T2 can be interpreted as the change of water structure. Furthermore, xenon solution at higher partial pressure of xenon gave a shorter value of T2. This led to the fact that structured level of water depends on the xenon concentration.

Browning and color difference

Partial pressures of xenon and nitrogen in individual pressure containers were initially same as 0.39 MPa and they decreased during 16 days storage to 0.33 MPa for xenon and to 0.35 MPa for nitrogen. The decrease in partial pressure of nitrogen was greater than that calculated on the basis of solubility in water. However, the decrease in partial pressure of xenon is still greater than that of nitrogen. In other words, the quantity of xenon dissolved in broccoli was larger than that of nitrogen. This means that even if the water in nitrogen treatment broccoli became structured, the structured level of water was low as compared with the water in xenon treatment broccoli. Photo 2 shows section surfaces of broccoli before storage. There was no significant difference in section color of broccoli before storage. Section surfaces after 16 days storage were shown in

Photo 3. After 16 days storage, the section color did not change appreciably for the broccoli of xenon treatment. In contrast to this, the browning of section surface was observed for the broccoli of nitrogen treatment as well as that of control treatment. Color difference, Δ E(Lab), of section surfaces was determined relative to the section surface of xenon treatment broccoli in conformity to the Japanese Industrial Standards (JIS Z8730). Δ E(Lab) was 7.1 for the broccoli of nitrogen treatment and 18.3 for the broccoli of control treatment. As the contribution of nitrogen to the formation of structured water was very small, nitrogen worked mainly to give pressure to broccoli. On the other hand, browning was observed for the broccoli of nitrogen treatment, which indicated that applied pressure did not suppress browning. For the broccoli of xenon treatment, browning was not detected. This was thought to be the effect of structured water.

Suppression of respiration

Partial pressure of xenon in the container of xenon treatment decreased during 6 days storage at 275 K from 0.24 MPa to 0.22 MPa. The quantity of xenon dissolved in water in broccoli was approximated from the pressure drop and it was 3.0×10^{-4} molXe / mol H_2O. The weight losses of broccoli of both xenon and control treatment were around 1 %. The concentration of CO_2 was converted into partial pressure of CO_2 by using eq. (1). The converted values of partial pressure of CO_2 were shown in Table 1. The partial pressure of CO_2 in xenon treatment container was always less than that in control treatment container. Fig. 1 shows cumulative quantities of CO_2 evolved from broccoli of both xenon treatment and control treatment calculated from eq. (2). Cumulative quantity of CO_2 evolved from broccoli was also lower for xenon treatment than for control treatment. Aerobic respiration was thought to occur during the interval of the first 4 days. This was supported by the report that harmful CO_2 level for broccoli is greater than 15 % "Weichmann (1987)" and by the experimental data that partial pressure of CO_2 was less than 15 kPa, which corresponds to 15 %, over the interval of the first 4 days (Table 1). In order to compare the respiratory rate, cumulative quantity data plotted in the first 4 days were analyzed. Linear regression line was determined for each treatment, and mean value of respiratory rate was estimated from the inclination of regression line. The respiratory rate of xenon treatment broccoli was 13.8 $mgCO_2\ kg^{-1}\ h^{-1}$, and that of control treatment broccoli was 19.4 $mgCO_2\ kg^{-1}\ h^{-1}$. The respiratory rate of broccoli in air condition was reported as 20 - 33 $mgCO_2\ kg^{-1}\ h^{-1}$ "Weichmann (1987), Makhloouf et al. (1989), Lebermann et al. (1968)". The estimated respiratory rate of control treatment broccoli agreed approximately with reported values. The respiratory rate of xenon treatment broccoli was suppressed to 71 % of that of control treatment broccoli. These results indicate that respiratory activity was suppressed by xenon treatment which caused the change of water into structured.

CONCLUSIONS

Effect of structured water on suppression of metabolism was examined. Formation of structured water was experimentally confirmed from the observation of change in proton spin-spin relaxation time when xenon gas dissolved in water. When xenon gas dissolved in broccoli at the given partial pressure, browning of section surface did not occur over the storage period of 16 days at 279 K. In contrast to this, browning was observed for the broccoli stored in air condition and for the broccoli stored at given pressure of nitrogen almost same as xenon pressure. Xenon treatment, which made water in broccoli structured, has resulted also in suppression of respiration. Respiratory rate, evaluated by carbon dioxide production, of the broccoli of xenon treatment was suppressed to 71 % of the broccoli stored in air condition. From these results, it was concluded that structured water made a role to suppress both enzymatic browning and respiratory metabolism of broccoli.

ACKNOWLEDGEMENTS

The authors express their appreciation for the experimental support by JEOL Ltd..

REFERENCES

Bastrash, S. et al., 1993. Optimal controlled atmosphere conditions for storage of broccoli florets, J. of Food Sci., 58(2), 338-341

King, G.A. and Morris, S.C., 1994. Physiological changes of broccoli during early postharvest senescence and through the preharvest-postharvest continuum, J. Amer. Soc. Hort. Sci., 119(2):270-275

Levermann, K. W. et al., 1968. Post-harvest changes of broccoli stored in modified atmospheres, 1. Respiration of shoots and color of flower heads, Food Technol., 22, 487-518

Makhlouf, J. et al., 1989. Long-term storage of broccoli under controlled atmosphere, HortScience, 24(4), 637-639

Tanaka, H. and Nakanishi, K., 1991: Hydrophobic hydration of inert gases: Thermodynamic properties, inherent structures, and normal-mode analysis, J. Chem. Phys., 95(5), 3717-3727

Wang, C. Y., 1977. Effect of aminoethoxy analog of rhizobitoxine and sodium benzoate on senescence of broccoli, HortScience, 12(1), 54-56

Weichmann, J., 1987. Postharvest physiology of vegetables, Marcel Dekker, 242-249

Table 1. Change in partial pressure of CO2 in container of both xenon and control treatment

Time, day	xenon, kPa	control, kPa
0.038	0.51	0.75
0.64	2.14	2.30
1.79	4.59	5.40
2.80	8.01	10.90
3.84	9.20	13.30
4.72	14.80	21.30
5.68	17.50	24.30

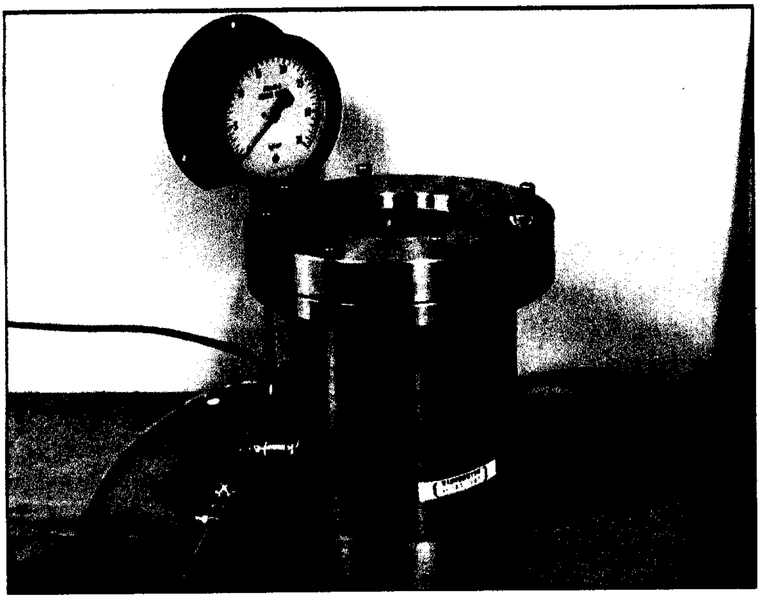

Photo 1 Pressure container used to store broccoli

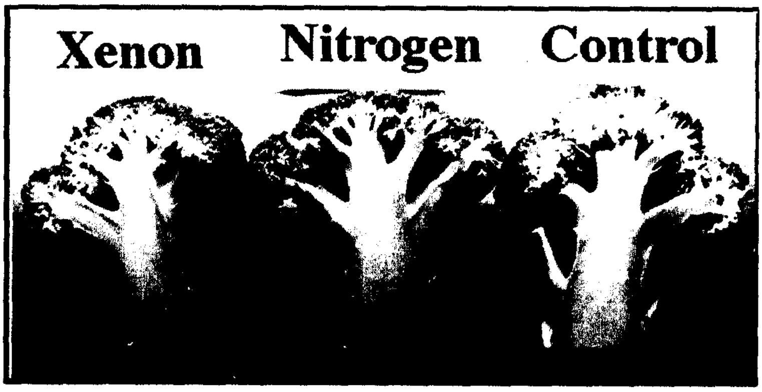

Photo 2 Appearance of section surfaces of broccoli before storage

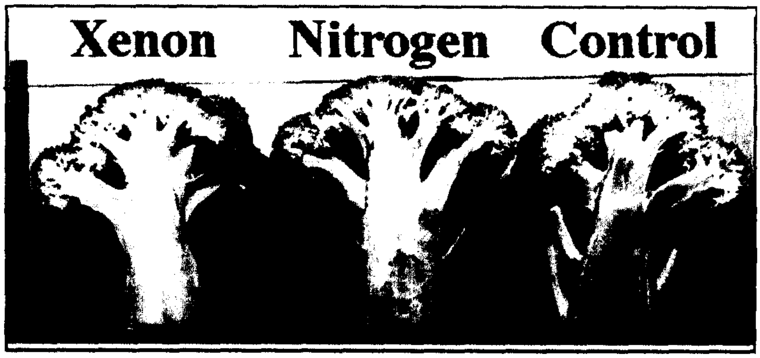

Photo 3 Appearance of section surfaces of broccoli after 16 d storage

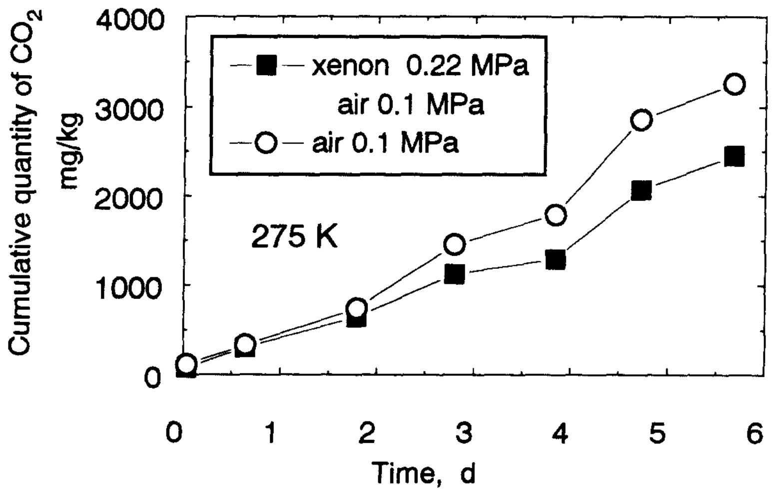

Fig. 1 Cumulative quantity of CO2 evolved from broccoli