# Respiratory Depression of Shredded Cabbage Using Xenon at Atmospheric Pressure

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## ABSTRACT

In the current studies, we investigated whether dissolving a nonpolar gas in cell sap can prolong the shelf life of horticultural products. Specifically, we examined the effect of Xe, which increases the viscosity of aqueous solutions, on cabbage that has been shredded to promote access of the gas to the cell sap. The respiration rate of shredded cabbage stored in a modified atmosphere composed of 79 kPa Xe and 22 kPa $O_2$ was ca. 6% slower than in air. Because postharvest respiration is one of the most important issues in the deterioration of horticultural products, this suggests that storage in Xe can be used to maintain product quality.

**Keywords:** respiration, cabbage, xenon, storage, modified atmosphere, vegetable

## 1. INTRODUCTION

Even after the supply of the nutrients and water have been cut off as a result of harvesting, horticultural products continue to lose water by transpiration, and they continue to have biological activities that consume carbohydrates, fats, and organic acids. This biological activity is the main cause of postharvest discoloration. Because the nutritional components are utilized by respiratory reactions that produce adenosine triphosphate (ATP), the respiration rate is directly related to the biological activity and the rate of product deterioration. Therefore, reducing the respiratory rate is expected to preserve quality.

Refrigeration, the most popular technique for storage (Thompson, 1998), slows the deterioration by reducing the rate of chemical and enzymatic reactions. Deterioration may also be slowed by adding a nonpolar gas. When a nonpolar gas dissolves in water, hydrophobic hydrations are formed, and the water becomes more structured. Computer simulations indicate that this is due to an increase in the number of hydrogen-bonded water molecules (Tanaka and Nakanishi, 1991). The amount of hydrogen bonds is one of the most important factors governing the motion of water molecules, and the viscosity increases as the water has more hydrogen bonding. This reduces the rate of substrate diffusion, thereby decreasing the enzymatic reaction rate. Thus, it is expected that biochemical reactions can be suppressed with nonpolar gases.

Oshita *et al.* (1996) reported that the shelf life of carnations can be extended by storing them under 500 kPa of Xe in a pressure-resistant container, one of the most water-soluble nonpolar gases. We also reported that the relaxation time of protons in intracellular water of broccoli measured by NMR was decreased under 600 kPa of Xe (Oshita *et al.*, 1999). However, these methods will be difficult to commercialize because it requires a large amount of nonpolar gas as well as pressure-resistant containers. In the current study, we evaluated the effectiveness of Xe at atmospheric pressure to improve the shelf life of a horticultural product, cabbage. For these studies, we increased the surface area of the product by shredding to promote access of the storage atmosphere to the cell sap.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Head cabbage (*Brassica oleracea* L., cv. Ranpou) harvested on the previous day, was cut into quarters to remove the core, and shredded into 2 × 2 mm squares with a CQ-36R cooking cutter (Toshiba Co., Tokyo). The shredded cabbage was washed twice in a stainless steel pan with tap water. The water adhering to the surface of cabbage was removed by centrifugation (189 ×g for 1 min) using a H-110A centrifugal separator (Kokusan Co., Tokyo).

### 2.2 Storage Method

The prepared shredded cabbage was stored under a modified atmosphere created by replacing $N_2$ in the air by Xe. A schematic diagram of the apparatus used for the experiments is shown in Figure 1. Shredded cabbage (0.2 kg) was enclosed in a stainless steel container (effective inner volume = 2.124 *l*) in a room controlled at 20°C. The air initially included in the container was flushed out through the clearance between the lid and the main body of the container by introducing $O_2$, and the internal space was filled with $O_2$ to over 101.325 kPa by closing the lid and turning off the $O_2$. The pressure of $O_2$ in the container was maintained at 22 kPa using a 16233U pressure regulator (Kojima Instruments Inc., Kyoto, Japan) and a vacuum pump. Finally, the modified atmosphere for the experiments was generated by introducing a mixture of Xe and 22 kPa $O_2$ into the container until the pressure gauge indicated atmospheric pressure. In the control conditions, shredded cabbage sampled from the same head was stored in a separate stainless steel container containing air. The atmosphere in the container with the shredded cabbage was removed by vacuum until the pressure was 22 kPa, and the pressure was brought up to atmospheric pressure by introducing air into the container to match the conditions of the cabbage prior to storage. Run 1 was defined as storage for 8 h under control or experimental conditions. Run 2 was defined as the same storage experiment using a sample from a separate head of cabbage. Runs 3 and 4 were defined as duplicate runs where shredded cabbage (0.35 kg) was stored for about 60 h using the same conditions as in Runs 1 and 2 but at a temperature of 5°C. Shredded cabbage used for the same Run number was prepared from the same head.

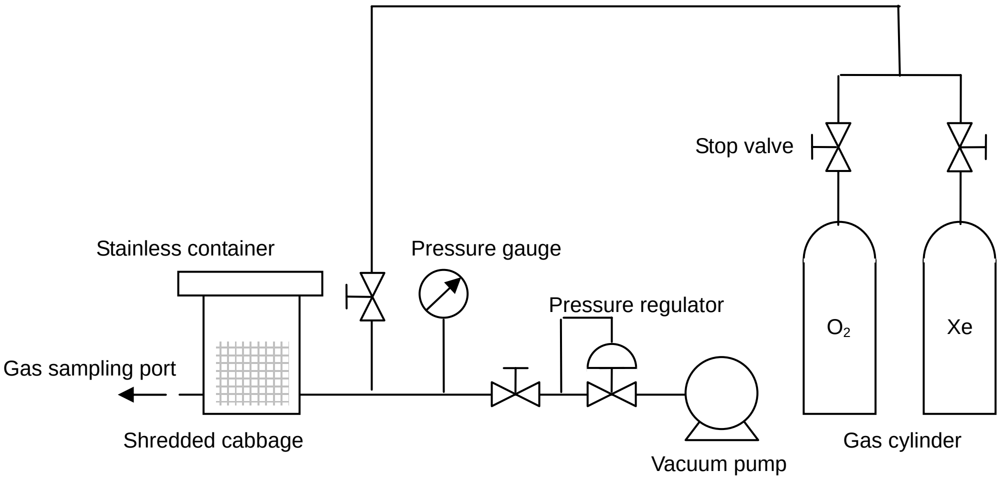

Figure 1. Schematic diagram of the apparatus used for storing shredded cabbage under the modified atmosphere (79 kPa Xe + 22 kPa $O_2$).

## 2.3 Gas Analysis

A 200-μ*l* sample of gas was taken from each container through a silicon rubber septum using a gas-tight syringe. The $O_2$, $N_2$, Xe, and $CO_2$ concentrations were measured over time in the samples using a GC-14A gas chromatograph (Shimadzu Co., Kyoto, Japan) equipped with a thermal conductivity detector and under the following conditions: column temperature, 50°C; bridge current, 80 mA; carrier gas (He) flow rate, 30 m*l* $min^{-1}$; column and detector temperatures, 100°C. A Molecular Sieve 5A-packed column (GL Sciences Inc., Tokyo) was used for the analysis of $O_2$, $N_2$ and Xe, and a Gaskuropack-54 packed column (GL Sciences Inc., Tokyo) was used for the analysis of $CO_2$.

### 2.4 Analysis of the Respiration Rate

The respiration rate of the shredded cabbage was determined from the slope of the linear regression line for the change in $O_2$ or $CO_2$ partial pressure *vs.* time according to the closed method (Fonseca *et al.*, 2002). The calculation (equation 1) assumes that that the respiration rate was constant in the aerobic atmosphere in the container:

$$r_i = \frac{|s_i|(v_{total} - v_{prod})}{RTM} \tag{1}$$

where, $r$ is the respiration rate in mmol kg$^{-1}$ h$^{-1}$, $s$ is the slope of the linear regression line for change in $O_2$ or $CO_2$ partial pressure *vs.* time in kPa h$^{-1}$, $v_{total}$ is the volume of the stainless steel container in $l$, $v_{prod}$ is the volume of the shredded cabbage in $l$ (equivalent to the mass of the shredded cabbage in kg, assuming a density of 1.0), $R$ is the universal gas constant $8.314 \times 10^{-3}$ $l$ kPa K$^{-1}$ mmol$^{-1}$, $T$ is the absolute temperature in K, and $M$ the mass of shredded cabbage in kg. The subscript $i$ denotes the type of gas (*i.e., $i = O$* for $O_2$ and $i = C$ for $CO_2$). Therefore, $r_O$ is $O_2$ uptake rate and $r_C$ is the $CO_2$ production rate, respectively. Statistical significance of the differences of respiration rates between experimental and control conditions was determined by analysis of covariance. The respiratory quotient (RQ) was calculated as $r_C/r_O$.

The temperature coefficient of the respiration rate ($Q_{10}$) was calculated according to equation 2 (McLaughlin and O'Beirne, 1999):

$$Q_{10} = \left( \frac{r_{i-2}}{r_{i-1}} \right)^{\frac{10}{T_2 - T_1}} \tag{2}$$

where, the subscripts 1 and 2 represent the individual temperatures. The $Q_{10}$ values for $O_2$ uptake and $CO_2$ production rates in the experimental and control experiments at 20 and 5°C were calculated using the averages of two measurements.

## 3. RESULTS AND DISCUSSION

Variations in $N_2$ and Xe partial pressures in the containers are shown in Table 1. We confirmed that there was little variability in the experimental data and that the averages did not differ significantly from the initial values.

Table 1. Partial pressure of $N_2$ or Xe gas during storage of shredded cabbage

| Temperature (°C) | Atmosphere | Run | $N_2$ (kPa) [1] | Xe (kPa) [1] | n [2] |
|---|---|---|---|---|---|
| 20 | Air | 1 | 79.0 ± 0.8 | – | 5 |
| | 79 kPa Xe + 22 kPa $O_2$ | 1 | 0.9 ± 0.0 | 76.7 ± 0.6 | 5 |
| | Air | 2 | 80.0 ± 0.3 | – | 5 |
| | 79kPa Xe + 22kPa $O_2$ | 2 | 0.9 ± 0.1 | 78.2 ± 0.3 | 5 |
| 5 | Air | 3 | 81.7 ± 0.8 | – | 8 |
| | 79 kPa Xe + 22 kPa $O_2$ | 3 | 4.3 ± 0.3 | 77.2 ± 0.3 | 8 |
| | Air | 4 | 82.9 ± 0.6 | – | 7 |
| | 79 kPa Xe + 22 kPa $O_2$ | 4 | 5.7 ± 0.8 | 74.9 ± 0.7 | 7 |

[1] Values represent the means ± SE

[2] Number of replicate measurements

The changes in $O_2$ and $CO_2$ partial pressures at 20 and 5°C are shown in Figures 2 and 3. Differences in initial $O_2$ partial pressures between the experimental and control experiments in Runs 1−3 were caused by the independent regulation of the atmospheres in the different containers. The initial $CO_2$ partial pressure, which was always near 0 kPa, was independent of the experiments or the containers. The changes in $O_2$ and $CO_2$ partial pressures over time during storage at 20°C were approximated by straight lines for the duration of the experiment, and at 5°C, the changes were linear for about 50 h. The correlation coefficients for the partial pressure changes ranged from 0.987 to 1.000 and were significant at a 99% level. The fact that the slopes of the plots did not change indicates that the respiration rates did not change substantially during the storage period. Fonseca *et al.* (2002) described that the respiration rate of a horticultural product undergoes an initial gradual decrease at relatively high $O_2$ levels followed by a rapid decline as the $O_2$ level approaches zero. McLaughlin and O'Beirne (1999) reported that the change in $O_2$ uptake rate of a dry coleslaw mix composed of 80% shredded cabbage and 20% shredded carrot was small at $O_2$ partial pressures between 2 and 10 kPa. In the current study, the linear approximation was carried out at partial pressures over 1 kPa $O_2$. Based on the linear regression results shown in Figures 2 and 3, it appeared that there was little variation in the respiration rate of the shredded cabbage and that it was not affected by the partial pressure of $CO_2$.

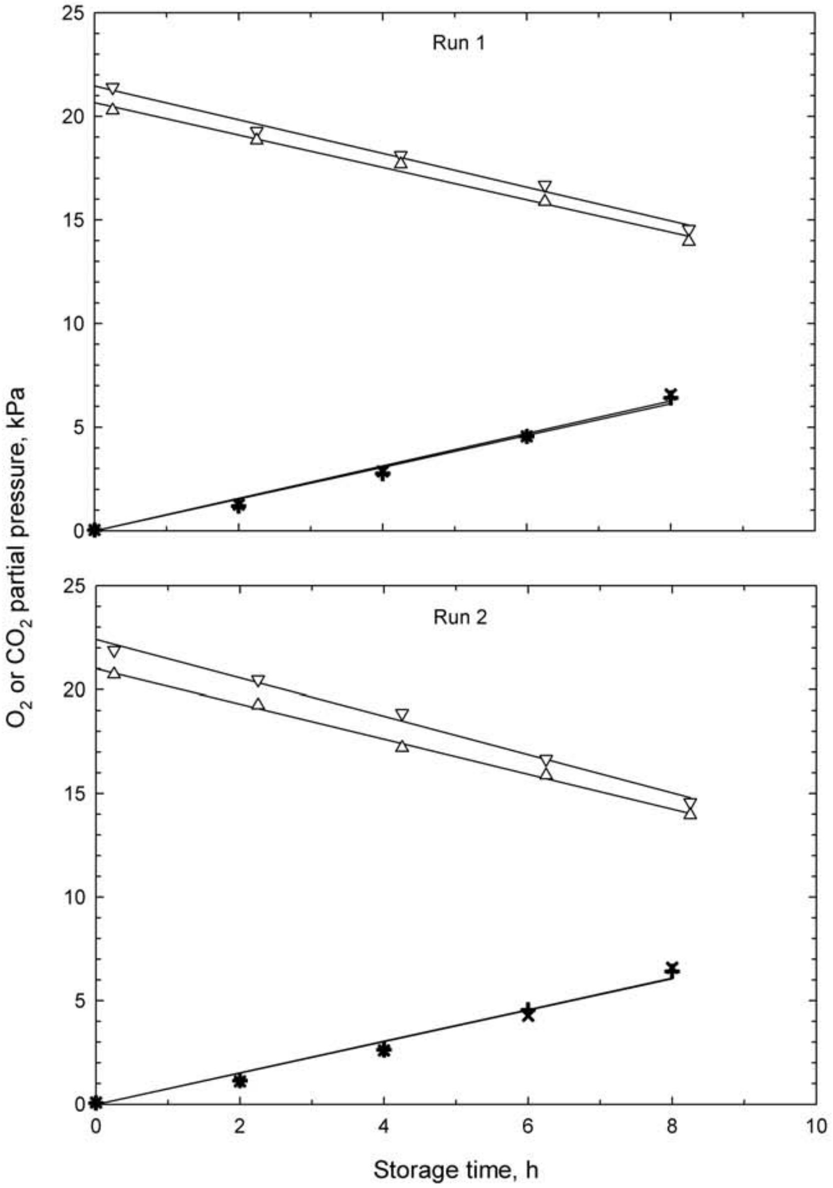

Figure 2. Changes in $O_2$ (▽) and $CO_2$ (✕) partial pressures in the air and in $O_2$ (△) and $CO_2$ (+) in the modified atmosphere (79 kPa Xe +22 kPa $O_2$) over time in stainless steel containers containing shredded cabbage at 20°C. Solid lines indicate the linear regression lines. The experiment was replicated twice, and Run 1 and 2 denote the first and second experiments, respectively.

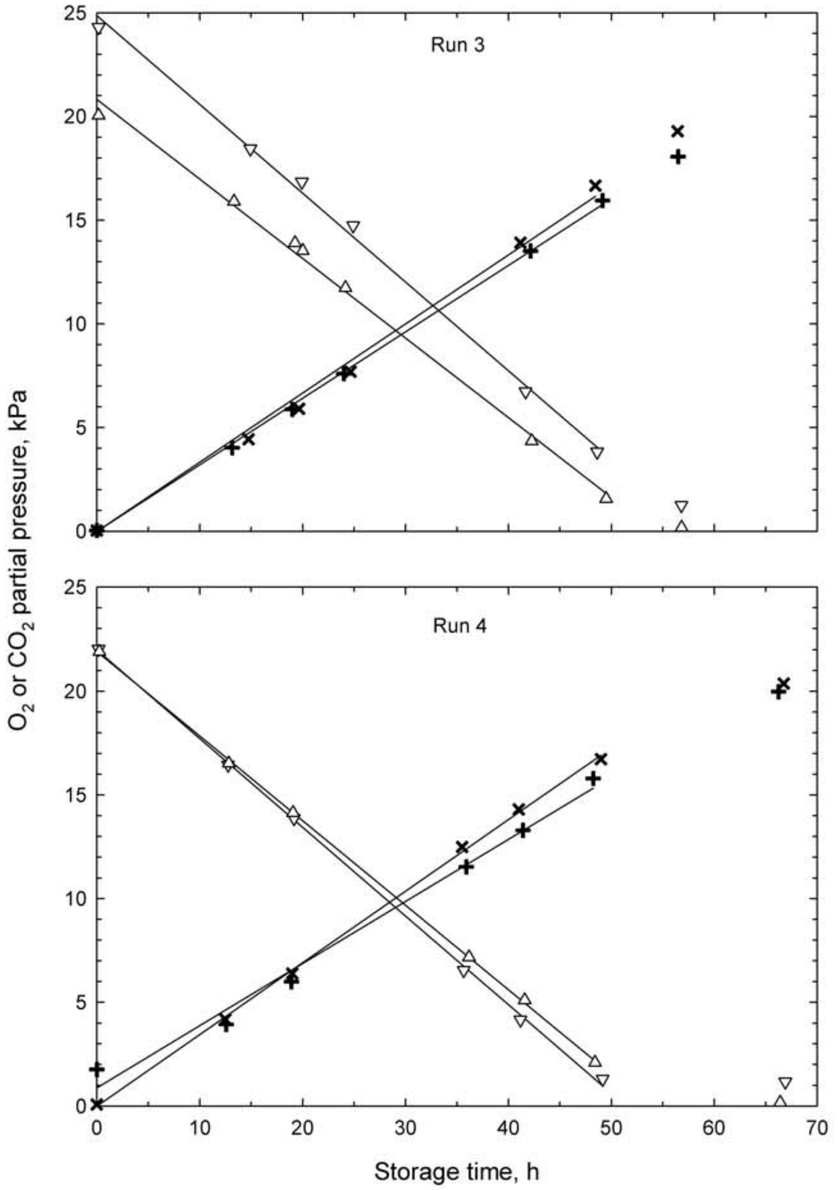

Figure 3. Changes in $O_2$ (▽) and $CO_2$ (✕) partial pressures in the air and in $O_2$ (△) and $CO_2$ (✚) in the modified atmosphere (79 kPa Xe + 22 kPa $O_2$) over time in stainless steel containers containing shredded cabbage at 5°C. Solid lines indicate the linear regression lines. The storage experiment was replicated twice, and Run 3 and 4 denote the first and second experiments, respectively.

The regression lines in Runs 3 and 4 were generated between the time of initial storage and about 50 h after. The calculated RQ values for the test and control conditions after about 50 h from the start of storage were 1.51 and 1.04, respectively, in Run 3 and 2.17 and 27.3, respectively, for Run 4. According to Kader *et al.* (1989), the range in RQ values under aerobic conditions is between 0.7 and 1.3. Thus, the RQ values that we calculated suggest that fermentation was induced in the product in all conditions except for Run 3 under control conditions.

Fermentation produces ethanol, which is one of the most important off-odors generated in the absence of $O_2$ consumption. Therefore, to use only atmospheric data collected under aerobic conditions, we decided to remove the partial pressure data measured after about 50 h from the regression analyses for Runs 3 and 4. Table 2 shows the $O_2$ uptake, $CO_2$ production rates, and RQ values calculated using equation 1 for the various storage temperatures and atmospheres. Because the RQ values were between 0.7 and 1.3, it appears that the aerobic respiration remained in the linear range. In fact, the RQ values obtained in this study were close to the average value for shredded cabbage (0.853) reported by Makino *et al.* (1997).

Table 2. Effects of storage temperature and atmosphere on the $r_O$, $r_C$, and RQ of shredded cabbage

| Temperature (°C) | Atmosphere | Run | $r_O$ (mmol kg$^{-1}$ h$^{-1}$) | $r_C$ (mmol kg$^{-1}$ h$^{-1}$) | RQ |
|---|---|---|---|---|---|
| 20 | Air | 1 | 3.21 (100) $^a$ | 3.09 (100) $^a$ | 0.96 |
| | 79 kPa Xe + 22 kPa $O_2$ | 1 | 3.09 (96) $^b$ | 3.03 (98) $^a$ | 0.98 |
| | Air | 2 | 3.64 (100) $^a$ | 2.99 (100) $^a$ | 0.82 |
| | 79 kPa Xe + 22 kPa $O_2$ | 2 | 3.34 (92) $^b$ | 3.01 (101) $^a$ | 0.90 |
| 5 | Air | 3 | 0.94 (100) $^a$ | 0.73 (100) $^a$ | 0.78 |
| | 79 kPa Xe + 22 kPa $O_2$ | 3 | 0.84 (90) $^b$ | 0.70 (96) $^b$ | 0.84 |
| | Air | 4 | 0.94 (100) $^a$ | 0.76 (100) $^a$ | 0.81 |
| | 79 kPa Xe + 22kPa $O_2$ | 4 | 0.89 (95) $^b$ | 0.66 (87) $^b$ | 0.73 |

The numbers in parentheses indicate the respiration rate in the modified atmosphere (79 kPa Xe + 22 kPa $O_2$) divided by the rate in the air of the same run × 100. Respiration rates with different superscripts in the same run were significantly different over the 95% level.

In Runs 1 and 2, 8 h after the initiation of storage, the $O_2$ partial pressures remained around 14 kPa and the $CO_2$ partial pressures were reduced to below 7 kPa. The $O_2$ partial pressures decreased to 1−2 kPa, and 50 h from the time of initial storage, the $CO_2$ partial pressures in Runs 3 and 4 increased to about 17 kPa. Dilley (1978) reported that the $O_2$ partial pressure range needed to maintain aerobic respiration in cabbage is 1 to 10 kPa. Therefore, respiration at 50 h in Runs 3 and 4 may have been fermentation. This suggests that fermentation had already commenced at 50 h even though the plots were linear until this time.

As shown in Table 2, the $O_2$ uptake rates in the presence of 79 kPa Xe were lower than those in the control experiments. No difference in $CO_2$ production rates was found between the experimental and control conditions at 20°C. In contrast, at 5°C, both the $O_2$ uptake and $CO_2$ production rates in the experimental conditions were lower than in the control conditions. Further, the $O_2$ partial pressures for control conditions were 0–4 kPa higher than those for the test conditions (Figures 2 and 3). Because the $O_2$ uptake and $CO_2$ production rates can be assumed to be constant and despite the fact that the $O_2$ partial pressure varied by approximately 7 kPa at 20°C and 20 kPa at 5°C, the differences between the respiration rates for test and control conditions were not due to differences in the initial $O_2$ partial pressure but rather differences in the atmosphere.

These experiments were conducted to investigate the influence of Xe *vs.* $N_2$ on the respiration of shredded cabbage in the atmosphere used for storage. The mol fraction solubilities of inert gases in water at 101 kPa (Gevantman, 1999) are $9.05 \times 10^{-5}$ for Xe and $1.27 \times 10^{-5}$ for $N_2$ at 20.15°C and $1.53 \times 10^{-4}$ for Xe and $1.75 \times 10^{-5}$ for $N_2$ at 5.15°C. This indicates that the solubility of Xe in water is much higher than that of $N_2$ and that the solubility increases as the temperature decreases. The greater reduction of respiration rates by Xe at the low temperature is probably due to an increased ability to promote water structure due to increased water solubility. Buchheit *et al.* (1965) reported that the growth rate of a baker's yeast correlates with the square root of the molecular mass of a nonpolar gas. This agrees with the idea that Xe causes a greater depression of metabolic activity in living cells because the square root values for the masses of Xe and $N_2$ are 11.5 and 5.3, respectively. Oshita *et al.* (1998) reported that $CO_2$ production rate of broccoli in 220 kPa Xe at 2°C was reduced to 71% of that measured in air at atmospheric pressure. The reduction of the respiration by Xe in this study may have been enhanced by shredding, which increases the access of the cell sap to the nonpolar gas, even though the pressure of the nonpolar gas was lower than in the report by Oshita *et al.* (1998).

Temperature coefficient $Q_{10}$ values for the rates of $O_2$ uptake and $CO_2$ release calculated by equation 2 were 2.39 and 2.70, respectively, for the test conditions and 2.37 and 2.56, respectively, for control conditions. Similarly, the $Q_{10}$ value reported by Makino *et al.* (1997) for the rate of $O_2$ uptake by shredded cabbage in the same temperature range is 2.44. Also, McLaughlin and O'Beirne (1999) reported that the $Q_{10}$ value for the rate of $O_2$ uptake by dry coleslaw mix between 4 and 10°C is 3.86. The value usually increases as the temperature decreases because lower temperatures reduce the rate of respiration to a greater extent. The higher $Q_{10}$ value obtained for the dry coleslaw mix may be due to the use of a lower temperature range than used in the current study or in the report by Makino *et al.* (1997). In the current study, the $Q_{10}$ values for the rate of $CO_2$ production were higher than for the rate of $O_2$ uptake, regardless of the experimental conditions.

Reduction of respiration by horticultural products using the method proposed by Oshita *et al.* (1998) is difficult to apply for practical applications because it utilizes greater than atmospheric pressures. In the current study, we demonstrated that Xe at atmospheric pressure can effectively repress the respiration. This indicates that the method employed here can be carried out in plastic pouches or flexible containers that are already used for these products. Respiration, used by living cells to produce ATP for biological activities, consumes the nutritional contents in the products. Therefore, suppression of the respiration by these products after harvest is especially important for maintaining their nutritional content and quality. Application of the method proposed in the current study to the storage of foods have to be additionally investigated, although Xe appears to be safe and nontoxic (Air Liquid, 2002).

## 4. CONCLUSIONS

Influence of modified atmosphere created by replacing $N_2$ in the air by Xe as a nonpolar gas on respiration of shredded cabbage was examined in the current study. Respiration rate of shredded cabbage stored in the modified atmosphere was lower than that in the air. This supports the depression effect of Xe on the respiration. In addition, the depression became increasingly prominent by decreasing storage temperature. This may be caused by that solubility of a gas into water increases with the decrease of temperature.

## 5. ACKNOWLEDGMENTS

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