

## Production of Volatile Sulfur Compounds in Polyethylene Film Packaged Broccoli Held at Different Temperatures

Kazuhiro DAN<sup>1\*</sup>, Yoichi YAMATO<sup>1</sup>, Masayasu NAGATA and Ichiji YAMASHITA

Department of Physiology and Quality Science, National Institute of Vegetables and Tea Science (Ano, Mie 514–2392, Japan)

### Abstract

The production of volatile sulfur compounds in modified atmosphere of packaged broccoli was investigated. Broccoli heads were sealed in 0.03-mm-thick low density polyethylene film and held at 10, 20 or 30°C for 72 h. Nonpackaged heads were also stored at the same temperatures. In the package held at 30°C, O<sub>2</sub> and CO<sub>2</sub> concentrations were less than 1% and more than 19%, respectively, and methanethiol was detected within 6 h. In the package held at 20°C, O<sub>2</sub> and CO<sub>2</sub> concentrations were less than 0.4% and more than 17%, respectively, and methanethiol was detected within 18 h. The percentage of electrolyte leakage from packaged broccoli held at 20°C and 30°C increased to a greater extent than that of nonpackaged broccoli during storage. However, storing the packaged broccoli at 10°C created a favorable modified atmosphere condition of around 3% O<sub>2</sub> and 10% CO<sub>2</sub> inside the package. In this condition volatile sulfur compounds were not detected and no significant change was observed in percentage of electrolyte leakage. At all temperatures, total chlorophyll contents were higher in packaged broccoli than in nonpackaged broccoli. These results indicate that, volatile sulfur compounds such as methanethiol were formed when the atmosphere inside the package had extremely low O<sub>2</sub> and high CO<sub>2</sub> even if broccoli was green and the visual quality had not deteriorated.

**Discipline:** Postharvest technology

**Additional key words:** anaerobic condition, dimethyl disulfide, methanethiol, modified atmosphere packaging

### Introduction

Broccoli is a highly perishable vegetable and its quality greatly depends on the storage condition. Modifying the storage atmosphere by lowering O<sub>2</sub> and increasing CO<sub>2</sub> prolonged storage life and maintained a high quality of broccoli<sup>7</sup>. Packaging with polymeric films modifies the atmosphere condition (low O<sub>2</sub> and high CO<sub>2</sub>), which extends the shelf life of broccoli<sup>4,10</sup>. The composition of the atmosphere in the package depends on the respiration rate of the broccoli and the gas diffusion characteristics of the film. Generally, modified atmosphere packaging is combined with low temperature during storage. However, it is difficult to maintain an optimum temperature during transportation. Increasing the

package temperature causes O<sub>2</sub> concentration to decrease and CO<sub>2</sub> concentration to increase because of increased respiration. This results in the development of off-odors if the gas permeability of the film is insufficient. Forney et al.<sup>3</sup> reported that volatile sulfur compounds such as methanethiol and dimethyl disulfide were main contributors to the off-odors of broccoli stored under anaerobic condition. However, volatile sulfur compounds evolved by broccoli under anaerobic packaging conditions have not been well studied, especially in very early stages of the storage. An understanding of these volatiles is needed to facilitate the development of strategies to prevent off-odor formation in packaged broccoli. The objective of this research was to investigate the biogenesis of volatile sulfur compounds in broccoli stored under polyethylene film packaging conditions at different tempera-

Present address:

<sup>1</sup> Department of Vegetable and Flower Research, National Agricultural Research Center for Kyushu Okinawa Region (Kurume, Fukuoka 839–8503, Japan)

\*Corresponding author: fax +81-942-43-7014; e-mail [kdan@affrc.go.jp](mailto:kdan@affrc.go.jp)

Received 27 December 2004; accepted 11 March 2005.

tures for the development of a better storage method of packaged broccoli.

## Materials and methods

### 1. Plant material and treatment

Broccoli (*Brassica oleracea* L. var. *italica* cv. Hait-su) was grown in the experiment field using standard cultural practices. Freshly harvested heads were placed in 0.03-mm-thick low density polyethylene bags (30 cm × 30 cm), sealed with a heat sealer, and held at 10, 20 or 30°C for 72 h. Nonpackaged heads were also stored at the same temperatures. Relative humidity was maintained near 100% by humidifiers in the storage room.

### 2. Chlorophyll analysis

Total chlorophyll content was determined by the method of Mackinney<sup>6</sup>.

### 3. O<sub>2</sub> and CO<sub>2</sub> analysis

O<sub>2</sub> and CO<sub>2</sub> concentrations in the package were analyzed with a gas chromatograph with a 1.8 m WG-100 column (GL Science) and a thermal conductivity detector (TCD). The column temperature was 80°C and the temperatures of the injector and detector were 100°C and 120°C, respectively.

### 4. Methanethiol and dimethyl disulfide analysis

Methanethiol and dimethyl disulfide in the headspace of the package was measured with a gas chromatograph with a 60 m × 0.53 mm (i.d.) DB-WAX column (J&W Scientific) and a flame photometric detector (FPD). The column temperature was 50°C for 10 min, increased to 150°C at 3°C/min, and held at 150°C for 10 min. Injector and detector temperatures were 150°C and 180°C, respectively.

### 5. Electrolyte leakage

Flower buds were cut lengthwise into two divisions with a razor. Flower buds were shaken for 1 h in a flask containing deionized water at 25°C. Electrical conductivity of the sample medium was measured using a conductivity meter. Total electrolyte leakage was obtained by boiling samples for 5 min. Results were expressed as percentage of total electrolytes.

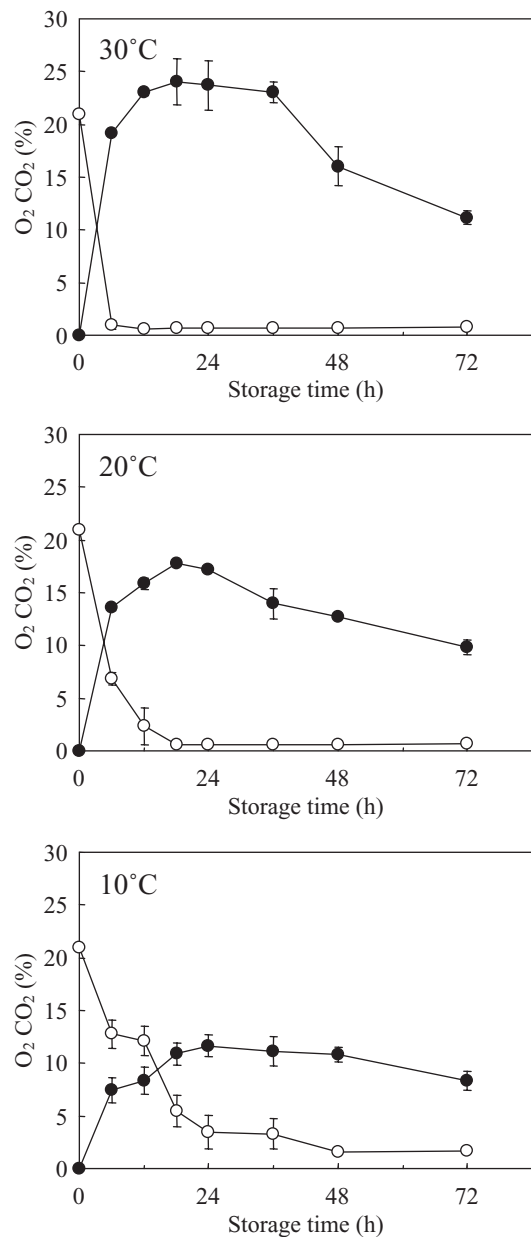
### 6. Analysis of S-methyl-L-cysteine sulfoxide (SMCSO)

Flower buds were homogenized with 20 volumes of 50% (v/v) aqueous methanol. After centrifugation, SMCSO in the supernatant was analyzed as the pre-column derivative of o-phthalaldehyde (OPA) by HPLC

analysis, according to the method of Ziegler and Sticher<sup>11</sup>.

## Results and discussion

The O<sub>2</sub> concentration in the package at 30°C dropped to below 1% and the CO<sub>2</sub> concentration increased to over 19% within the first 6 h (Fig. 1). At the same time, a small amount of methanethiol was detected in the headspace of the package (Table 1). The O<sub>2</sub> con-



**Fig. 1. Changes in oxygen and carbon dioxide concentration of atmosphere in the package of broccoli during storage**

○ : O<sub>2</sub>, ● : CO<sub>2</sub>.

Values are means ± SE for n = 4.

**Table 1. Concentrations of methanethiol and dimethyl disulfide in the package of broccoli storage**

	Storage temperature	Concentration (ppm)							
		Storage time							
		0 h	6 h	12 h	18 h	24 h	36 h	48 h	72 h
Methanethiol	30°C	–	0.2 ± 0.05	9.4 ± 3.2	206.0 ± 40.3	218.8 ± 8.6	309.7 ± 23.8	215.5 ± 17.3	58.6 ± 21.1
	20°C	–	nd	nd	0.2 ± 0.02	0.3 ± 0.03	0.4 ± 0.02	9.3 ± 3.7	38.4 ± 4.1
	10°C	–	nd	nd	nd	nd	nd	nd	nd
Dimethyl disulfide	30°C	–	nd	0.01 ± 0.003	0.8 ± 0.2	7.2 ± 1.3	11.6 ± 3.8	25.4 ± 5.6	12.5 ± 3.5
	20°C	–	nd	nd	nd	nd	nd	0.02 ± 0.008	0.13 ± 0.02
	10°C	–	nd	nd	nd	nd	nd	nd	nd

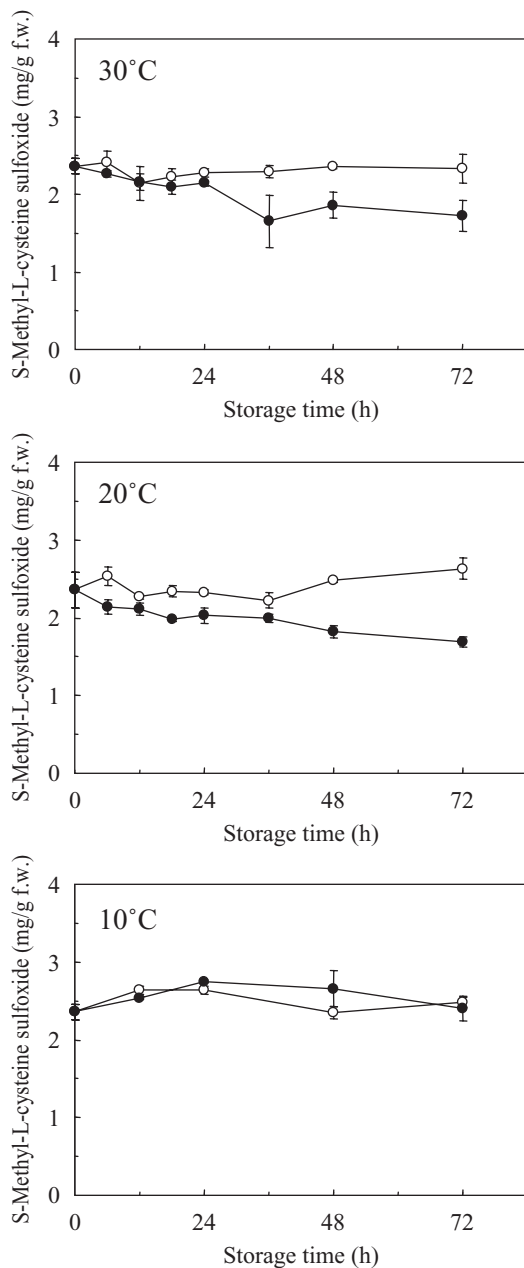
Data represent mean ± SE (n = 4).

nd: not detected.

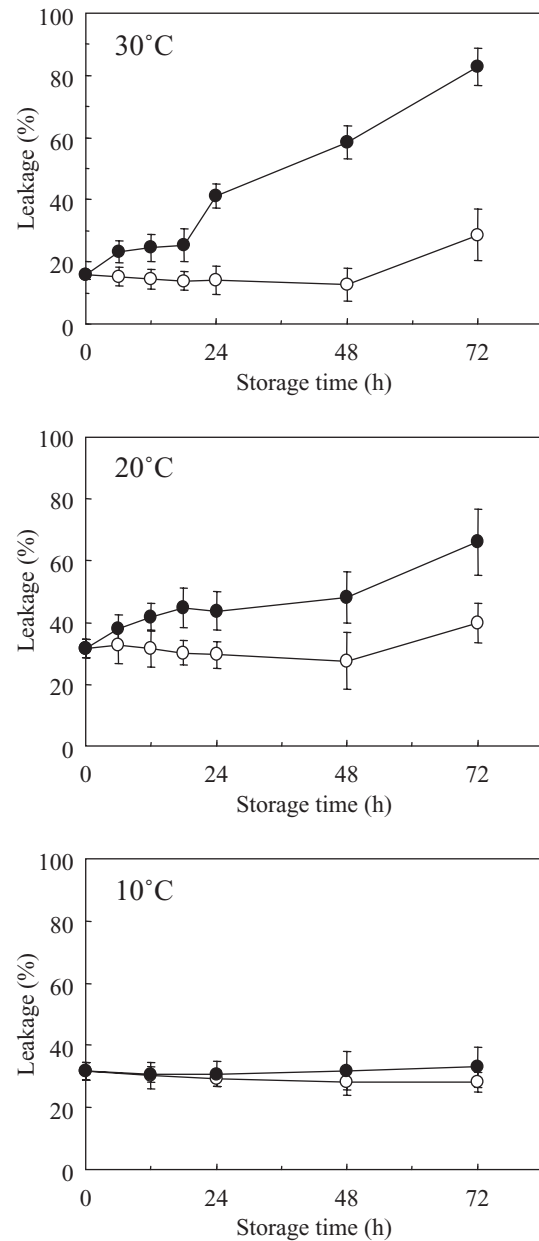
centration remained at the same level until the end of the experiment, whereas the CO<sub>2</sub> concentration increased to a maximum after 18 h and subsequently declined (Fig. 1). The concentrations of methanethiol increased gradually, reaching to a maximum of 309.7 ppm 36 h after packaging (Table 1). Dimethyl disulfide was detected in the headspace of the package within 12 h and increased gradually, reaching to a maximum of 25.4 ppm 48 h after packaging (Table 1). The O<sub>2</sub> concentration in the package at 20°C reduced to below 0.4% and remained at the same level until the end of the experiment, while the CO<sub>2</sub> concentration increased to over 17% within the first 18 h and then decreased gradually (Fig. 1). A small amount of methanethiol was detected in the headspace of the package within 18 h and increased greatly, reaching to a maximum of 38.4 ppm 72 h after packaging (Table 1). Dimethyl disulfide was detected after 48 h of storage (Table 1). Volatile sulfur compounds, such as methanethiol and dimethyl disulfide, are the main contributors to the undesirable odor of broccoli stored under anaerobic condition<sup>2</sup>. Methanethiol was one of the first compounds formed under anaerobic condition and is primarily responsible for the off-odors<sup>3</sup>. O<sub>2</sub> concentrations that induce the production of off-odors appear to be influenced by the concentration of CO<sub>2</sub> present. Lipton and Harris<sup>5</sup> reported that off-odors developed when O<sub>2</sub> was less than 0.25% and CO<sub>2</sub> was absent; however, in the presence of 10% CO<sub>2</sub>, off-odors developed even in 1% O<sub>2</sub>. In this study, O<sub>2</sub> and CO<sub>2</sub> concentrations in the package stored at 20°C and 30°C were less than 0.5% and more than 10%, respectively; volatile sulfur compounds also developed under these anaerobic conditions. At 10°C, the highest CO<sub>2</sub> level (12.5%) was detected after 24 h of storage and the lowest O<sub>2</sub> level (2.7%) was detected after 48 h of storage; thereafter the levels of gases changed gradually (Fig. 1). O<sub>2</sub> and CO<sub>2</sub> concentrations within the package equilibrated to 3% and 10%, respec-

tively. Volatile sulfur compounds were not detected in the headspace of the package (Table 1). Barth et al.<sup>1</sup> reported that storage of broccoli under 9% CO<sub>2</sub> and 3% O<sub>2</sub> resulted in better maintenance of quality. In this experiment, storing the packaged broccoli at 10°C must have favorably modified the atmosphere condition inside the package.

S-methyl-L-cysteine sulfoxide (SMCSO), a nonproteinaceous sulfur-containing amino acid, occurs naturally in *Brassica* vegetables including broccoli<sup>8,9</sup>. Most of the volatile sulfur compounds which are characteristic of *Brassica* vegetables after tissue rupture are due to the degradation of SMCSO by C-S lyases. However, it was reported that this reaction needs physical damage of the tissues. SMCSO is hydrolyzed by C-S lyase to generate methyl methanethiosulfinate, and eventually decomposes into volatile sulfur compounds, such as methanethiol and dimethyl disulfide<sup>8</sup>. Significant membrane degradation is reflected by a large increase in electrolyte leakage. For this reason, SMCSO content and electrolyte leakage of broccoli were measured. At 20°C and 30°C, the SMCSO content in the nonpackaged broccoli remained constant during storage, while in the anaerobically packaged broccoli it decreased gradually (Fig. 2). The percentage of electrolyte leakage from flower buds of nonpackaged broccoli increased slightly during storage, whereas that from flower buds of packaged broccoli increased faster (Fig. 3). At 10°C, however, the SMCSO contents in the packaged and nonpackaged broccoli were maintained at the same level during storage (Fig. 2). There was no significant difference in the percentage of electrolyte leakage of packaged and nonpackaged broccoli during storage (Fig. 3). The most obvious characteristic difference between anaerobically packaged (20°C and 30°C) and suitably packaged (10°C) broccoli was electrolyte leakage. In this study, the increase in the electrolyte leakage was parallel to the production of volatile



**Fig. 2. Changes in S-methyl-L-cysteine sulfoxide (SMCSO) content in packaged and nonpackaged broccoli during storage**  
 ○ : Nonpackaged, ● : Packaged.  
 Values are means ± SE for n = 4.

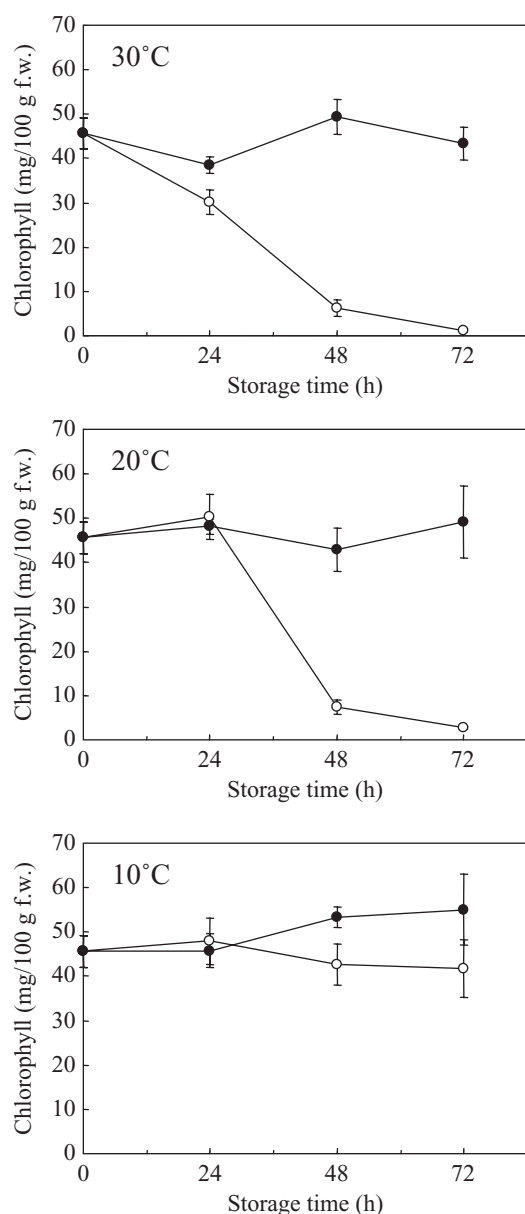


**Fig. 3. Changes in percentages of electrolyte leakage in packaged and nonpackaged broccoli during storage**  
 ○ : Nonpackaged, ● : Packaged.  
 Values are means ± SE for n = 4.

sulfur compounds in the anaerobically packaged broccoli (Table 1, Fig. 3). Presumably, the loss of membrane integrity contributes to the loss of intracellular compartmentation. This phenomenon indicates that, under an anaerobic condition, sulfur containing volatile compounds were formed by the deterioration of cellular membrane and the loss of intracellular compartmentation, thus, allowing SMCSO and C-S lyase to react.

Broccoli senescence is expressed mainly by yellow-

ing and chlorophyll loss from flower buds. Therefore, chlorophyll contents in the packaged and nonpackaged broccoli were measured. At all temperatures, total chlorophyll contents were higher in packaged broccoli than in nonpackaged broccoli (Fig. 4). Packaging resulted in significantly higher retention of green color in the broccoli tissues, as indicated by total chlorophyll contents. These results confirmed previous reports of loss of chlorophyll in vegetables being retarded by reduced O<sub>2</sub> and elevated



**Fig. 4. Changes in chlorophyll content in packaged and nonpackaged broccoli during storage**

○ : Nonpackaged, ● : Packaged.  
Values are means  $\pm$  SE for n = 4.

CO<sub>2</sub> conditions<sup>5</sup>. The visual quality of broccoli was maintained in modified atmosphere conditions (packaged) and was better than broccoli stored in ambient air (nonpackaged); however, broccoli held at an anaerobic

condition developed strong off-odors. The potential production of this objectionable odor appears to be the limiting factor preventing the use of modified atmosphere packaging in the handling of fresh broccoli.

From the results of this study, it was found that methanethiol and dimethyl disulfide occurred when the atmosphere inside the package had extremely low O<sub>2</sub> and high CO<sub>2</sub> even if broccoli was green and the visual quality had not deteriorated. Under an anaerobic condition, the volatile sulfur compounds are formed by the deterioration of cellular membrane and loss of intracellular compartmentation, allowing the enzyme-substrate reaction to proceed. Proper temperature management is perhaps the most important part of postharvest handling for packaged broccoli.

## References

1. Barth, M. M. et al. (1993) Modified atmosphere packaging protects market quality in broccoli spears under ambient temperature storage. *J. Food Sci.*, **58**, 1070–1072.
2. Dan, K. et al. (1997) Formation of volatile sulfur compounds in broccoli stored under anaerobic condition. *J. Jpn. Soc. Hort. Sci.*, **65**, 867–875.
3. Forney, C. F., Mattheis, J. P. & Austin, R. K. (1991) Volatile compounds produced by broccoli under anaerobic conditions. *J. Agric. Food Chem.*, **39**, 2257–2259.
4. Kader, A. A., Zagory, D. & Kerbel, E. L. (1989) Modified atmosphere packaging of fruits and vegetables. *CRC Crit. Rev. Food Sci. Nutr.*, **28**, 1–30.
5. Lipton, W. J. & Harris, C. M. (1974) Controlled atmosphere effects on the market quality of stored broccoli (*Brassica oleracea* L., Italica group). *J. Am. Soc. Hort. Sci.*, **99**, 200–205.
6. Mackinney, G. (1941) Absorption of light by chlorophyll solutions. *J. Biol. Chem.*, **140**, 315–322.
7. Makhlof, J. et al. (1989) Long-term storage of broccoli under controlled atmosphere. *HortScience*, **24**, 637–639.
8. Marks, H. S. et al. (1992) S-Methylcysteine sulfoxide in *Brassica* vegetables and formation of methyl methanethiosulfinate from Brussels sprouts. *J. Agric. Food Chem.*, **40**, 2098–2101.
9. Morris, C. J. & Thompson, J. F. (1956) The identification of (+)S-methyl-L-cysteine sulfoxide in plants. *J. Am. Chem. Soc.*, **78**, 1605–1608.
10. Zagory, D. & Kader, A. A. (1988) Modified atmosphere packaging of fresh produce. *Food Technol.*, **42**(9), 70–77.
11. Ziegler, S. J. & Sticher, O. (1989) HPLC of S-alk(en)yl-L-cysteine derivatives in garlic including quantitative determination of (+)-S-allyl-L-cystaine sulfoxide (alliin). *Planta Medica.*, **55**, 372–378.

