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

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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 30ºC for 72 h. Nonpackaged heads were also stored at the same temperatures. In the package held at 30ºC, O_2 and CO_2 concentrations were less than 1% and more than 19%, respectively, and methanethiol was detected within 6 h. In the package held at 20ºC, O_2 and CO_2 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 20ºC and 30ºC increased to a greater extent than that of nonpackaged broccoli during storage. However, storing the packaged broccoli at 10ºC created a favorable modified atmosphere condition of around 3% O_2 and 10% CO_2 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_2 and high CO_2 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_2 and increasing CO_2 prolonged storage life and maintained a high quality of broccoli[7]. Packaging with polymeric films modifies the atmosphere condition (low O_2 and high CO_2), which extends the shelf life of broccoli[4,10]. 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_2 concentration to decrease and CO_2 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.[3] 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-

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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. Haitsu) 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 30ºC 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[6].

3. O_2 and CO_2 analysis

O_2 and CO_2 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 80ºC and the temperatures of the injector and detector were 100ºC and 120ºC, 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 50ºC for 10 min, increased to 150ºC at 3ºC /min, and held at 150ºC for 10 min. Injector and detector temperatures were 150ºC and 180ºC, 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 25ºC. 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-phthaldialdehyde (OPA) by HPLC analysis, according to the method of Ziegler and Sticher[11].

Results and discussion

The O_2 concentration in the package at 30ºC dropped to below 1% and the CO_2 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_2 con-

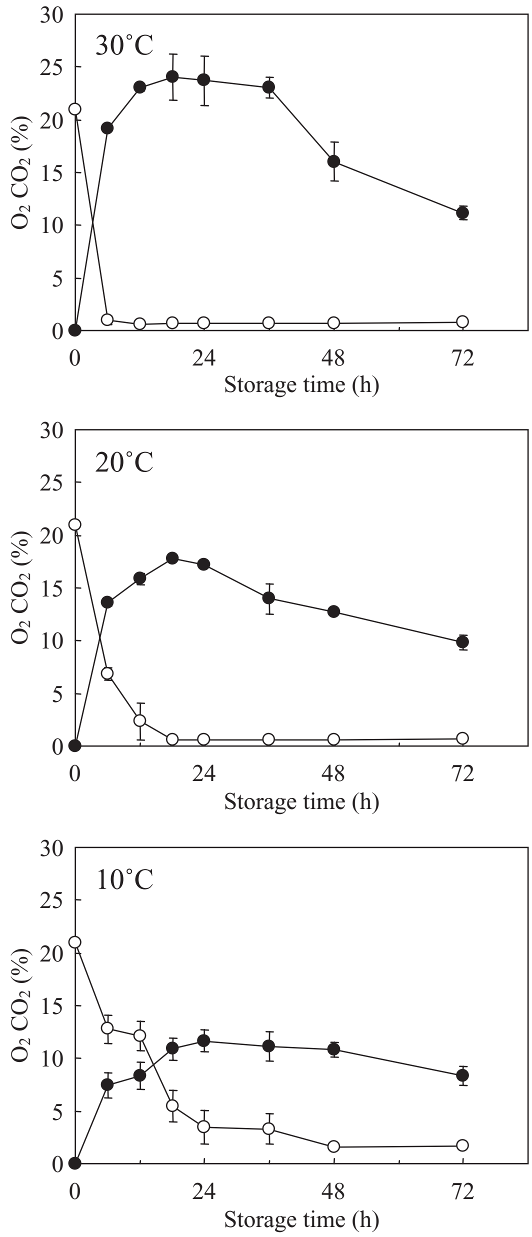

Fig. 1. Changes in oxygen and carbon dioxide concentration of atmosphere in the package of broccoli during storage
○ : O_2, ● : CO_2.
Values are means ± SE for n = 4.

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	30ºC	–	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
	20ºC	–	nd	nd	0.2 ± 0.02	0.3 ± 0.03	0.4 ± 0.02	9.3 ± 3.7	38.4 ± 4.1
	10ºC	–	nd	nd	nd	nd	nd	nd	nd
Dimethyl disulfide	30ºC	–	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
	20ºC	–	nd	nd	nd	nd	nd	0.02 ± 0.008	0.13 ± 0.02
	10ºC	–	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_2 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_2 concentration in the package at 20ºC reduced to below 0.4% and remained at the same level until the end of the experiment, while the CO_2 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[2]. Methanethiol was one of the first compounds formed under anaerobic condition and is primarily responsible for the off-odors[3]. O_2 concentrations that induce the production of off-odors appear to be influenced by the concentration of CO_2 present. Lipton and Harris[5] reported that off-odors developed when O_2 was less than 0.25% and CO_2 was absent; however, in the presence of 10% CO_2, off-odors developed even in 1% O_2. In this study, O_2 and CO_2 concentrations in the package stored at 20ºC and 30ºC were less than 0.5% and more than 10%, respectively; volatile sulfur compounds also developed under these anaerobic conditions. At 10ºC, the highest CO_2 level (12.5%) was detected after 24 h of storage and the lowest O_2 level (2.7%) was detected after 48 h of storage; thereafter the levels of gases changed gradually (Fig. 1). O_2 and CO_2 concentrations within the package equilibrated to 3% and 10%, respectively. Volatile sulfur compounds were not detected in the headspace of the package (Table 1). Barth et al.[1] reported that storage of broccoli under 9% CO_2 and 3% O_2 resulted in better maintenance of quality. In this experiment, storing the packaged broccoli at 10ºC 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[8,9]. 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[8]. 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 20ºC and 30ºC, 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 10ºC, 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 (20ºC and 30ºC) and suitably packaged (10ºC) broccoli was electrolyte leakage. In this study, the increase in the electrolyte leakage was parallel to the production of volatile

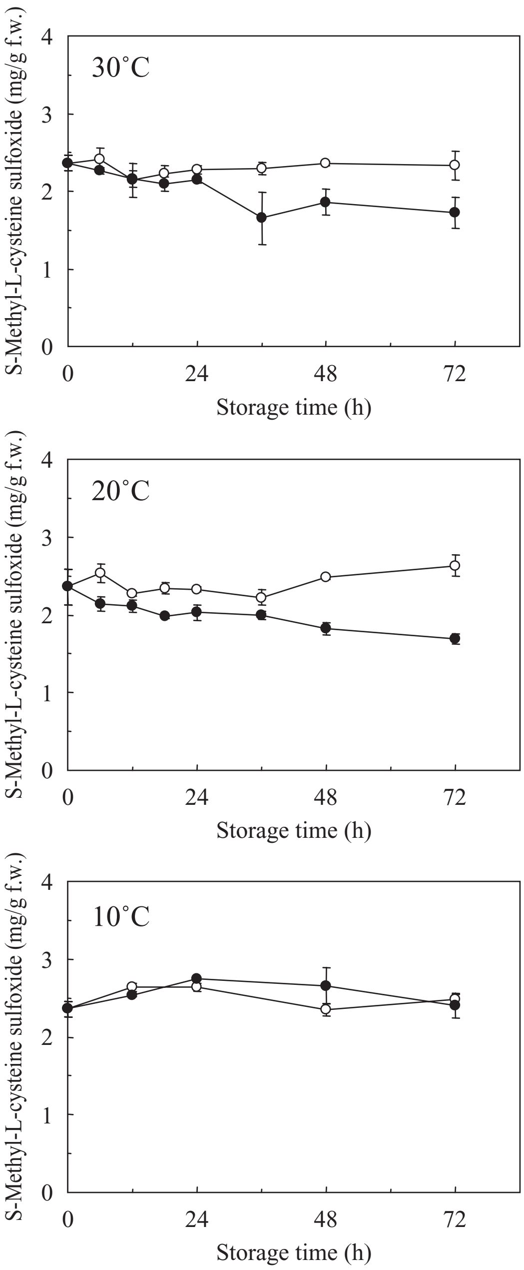

Fig. 2. Changes in S-methyl-L-cysteine sulfoxide (SMCSO) content in packaged and nonpackaged broccoli during storage

○ : Nonpackaged, ● : Packaged.

Values are means ± SE for n = 4.

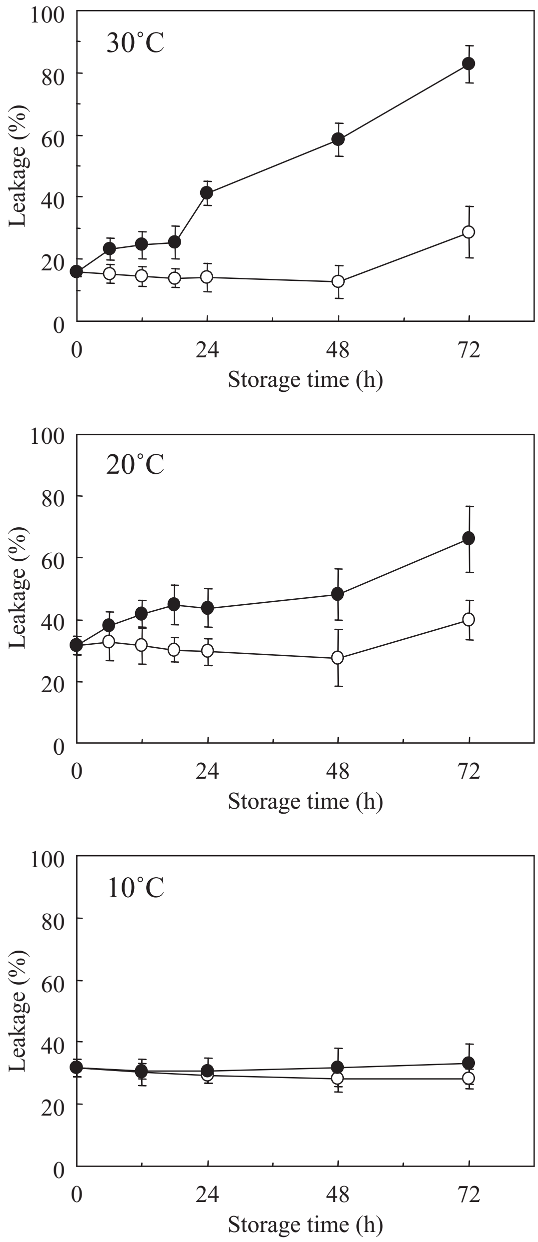

Fig. 3. Changes in percentages of electrolyte leakage in packaged and nonpackaged broccoli during storage

○ : 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 yellowing 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_2 and elevated

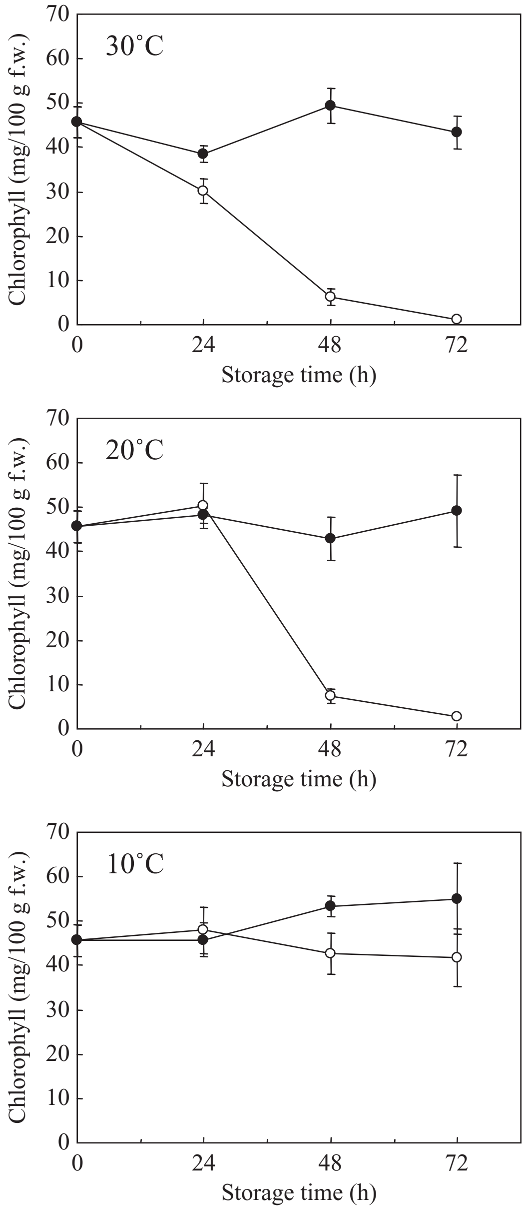

Fig. 4. Changes in chlorophyll content in packaged and nonpackaged broccoli during storage
○ : Nonpackaged, ● : Packaged.
Values are means ± SE for n = 4.

CO_2 conditions[5]. 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_2 and high CO_2 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.

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