Effects of Pre-Storage Duration and Storage Temperatures on the Formation of Volatile Sulfur Compounds in Broccoli under Anaerobic Conditions

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Summary

Fresh broccoli florets, which were sealed in glass bottles after air was replaced with 100% N₂, were held at 10 °C, 20 °C, or 30 °C for 48 hours. High temperature promoted the production of methanethiol and dimethyl disulfide from anaerobically treated florets, whereas then the fresh broccoli heads were pre-stored in air at 1 °C for 7, 14, and 21 days, and the florets were held in 100% N₂ at 20 °C for 48 hours, the production of methanethiol and dimethyl disulfide declined. The production of methanethiol and dimethyl disulfide under an anaerobic condition was significantly greater in flower buds than in stems, and likewise, their precursor, *S*-methyl-L-cysteine sulfoxide, was about 4 times higher in flower buds. The activity of *C*-*S* lyase, which catalyzes the conversion of *S*-methyl-L-cysteine sulfoxide to methanethiol and dimethyl disulfide, was significantly higher in flower buds than in stems.

Key Words: Anaerobic, Broccoli, Dimethyl Disulfide, Methanethiol.

Introduction

Broccoli is a highly perishable vegetable and its quality and shelf-life greatly depend on the storage conditions. Controlled atmosphere (CA) storage of broccoli under suitable O₂ and CO₂ conditions has had some success in extending the shelf-life of broccoli (Makhlouf et al., 1989). However, fresh broccoli held in CA storage containing extremely low O₂ and/or extremely high CO₂ produce an objectionable off-odor, although the original appearance is retained (Izumi et al., 1996; Yamashita et al., 1993). The production of this objectionable odor appears to be critical factor of marketing losses.

In a previous study, the volatile sulfur compounds, methanethiol and dimethyl disulfide, were found to be the main contributors to the off-odor of broccoli stored under anaerobic conditions (Dan et al., 1997 a). However, in broccoli, the factors which influence the production of the these compounds, under anaerobic conditions have not been studied; an understanding of their production under CA storage and modified atmosphere storage needs to be researched.

The objectives of the present study are to investigate the effects of pre-storage duration and storage temperatures on the production of volatile sulfur compounds by broccoli under anaerobic conditions. The difference between flower buds and stems in the production of volatile sulfur compounds and related enzyme activities were also studied.

Materials and Methods

Plant materials and treatments

Broccoli (*Brassica oleracea* L. var. *italica* cv. Haitsu) were grown in the experimental field of Natl. Res. Inst. Veg., Ornam. Plants & Tea in standard cultural practices.

To study the effects of temperature, freshly harvested heads (ca. 400 g) were cut into florets. A 20-g sample of florets was sealed in a 300-ml glass bottle after replacing the air with 100% N₂. The sealed bottles were held at 10 °C, 20 °C, and 30 °C for 48 hours. During this time, headspace was periodically sampled and the gas analyzed for methanethiol and dimethyl disulfide. *C*-*S* lyase was subsequently extracted from fresh florets and the crude extract was assayed for *C*-*S* lyase activity by measuring pyruvate formation in the standard assay mixture at 10 °C, 20 °C, and 30 °C.

To study the effects of pre-storage low temperature on quality, fresh heads were pre-stored in air at 1 °C for 7, 14, and 21 days; 20 g of florets were sealed in a 300-ml glass bottle with 100% N₂. The sealed bottles were held at 20 °C for 48 hours and the headspace was periodically sampled and the gas analyzed for methanethiol and dimethyl disulfide. The florets were analyzed for *S*-methyl-L-cysteine sulfoxide (SMCSO) content and *C*-*S* lyase activity.

To ascertain the levels of volatiles fresh florets were divided into flower buds and stems (pedicels) which were analyzed for SMCSO and *C*-*S* lyase. A 20-g sample of flower buds or stems was sealed in a 300-ml glass

bottle with 100% N₂, and held at 20 °C for 48 hours after which the headspace was periodically sampled and the gas analyzed for methanethiol and dimethyl disulfide.

Analysis of methanethiol and dimethyl disulfide

Methanethiol and dimethyl disulfide in the headspace of the sealed bottles were measured with a gas chromatograph (GC-17A, Shimadzu) equipped with a flame photometric detector (FPD). The analytical conditions were described previously (Dan et al., 1997 b).

Analysis of C-S lyase activity

One g of tissue was homogenized with a chilled mortar and pestle in 3 ml of cold phosphate buffer (pH 7.0), containing 3% (w/v) polyvinylpyrrolidone. The homogenate was filtered through 4 layers of gauze and the filtrate centrifuged at 10,000×g for 20 min at 4 °C. The supernatant was assayed for C-S lyase according to the method described by Hamamoto and Mazelis (1986) by measuring the pyruvate formed colorimetrically (Friedemann and Haugen, 1943).

Analysis of SMCSO

Samples were rapidly diced and immediately homogenized with a Physcotron homogenizer (Nition Medical Supply) at the highest speed for 1 min 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 the HPLC analysis, according to the method of Ziegler and Sticher (1989). OPA derivative of SMCSO was separated at 30 °C on a Shim-pack CLC-ODS column (150 mm×4.6 mm, Shimadzu) connected to a fluorescence detector (RF-10A, Shimadzu). The excitation and emission wavelengths were 230 and 420 nm, respectively. The mobile phase was composed of 19% acetonitrile in 50 mM potassium phosphate buffer (pH 7.0) with a flow rate of 0.7 ml/min.

Results and Discussion

Effect of temperature on formations of methanethiol and dimethyl disulfide

Volatile sulfur compounds, methanethiol and dimethyl disulfide, are among the major contributors to the undesirable odor of broccoli stored under anaerobic conditions (Dan et al., 1997 a; Forney et al., 1991; Hansen et al., 1992). Forney et al. (1991) reported that the methanethiol was one of the first compounds formed under anaerobic conditions and appeared to be a primary contributor to the objectionable odor.

Among the fresh florets which were held at 10 °C, 20 °C, and 30 °C under an anaerobic condition for 48 hours, in those kept at 30 °C, the concentration of methanethiol increased rapidly, reaching a maximum of 171 ppm at 24 hours after treatment; the level then de-

creased gradually (Fig. 1). The concentration of dimethyl disulfide increased linearly, surpassing 65 ppm at the end of the treatment. At 20 °C, the concentration of methanethiol increased gradually, reaching a maximum of 99 ppm at 48 hours after treatment (Fig. 1); the concentration of dimethyl disulfide remained relatively low until 12 hours after treatment, and then increased gradually. At 10 °C, the concentration of methanethiol remained low until 12 hours after treatment, then increased slightly, reaching a maximum of 43 ppm at 48 hours (Fig. 1). The concentration of dimethyl disulfide remained low until the end of the treatment.

SMCSO, a nonproteinaceous, sulfur-containing amino acid, which has been reported in *Brassica* vegetables including broccoli (Marks et al., 1992; Morris and Thompson, 1956). And SMCSO is believed to be hydrolyzed by C-S lyase in *Brassica* to generate methyl methanethiosulfinate (Marks et al., 1992). It was hypothesized that methyl methanethiosulfinate eventually decomposes into volatile sulfur compounds, such as methanethiol and dimethyl disulfide (Chin and Lind-

Fig. 1. Effect of storage temperatures on formations of methanethiol and dimethyl disulfide from broccoli florets under an anaerobic condition. Fresh florets were sealed in glass bottles with 100% N₂. The bottles were held at 10 °C, 20 °C and 30 °C for 48 hr. Values are means±SE for n=3.

say, 1994). A crude extract from fresh florets, used for C-S lyase assay (Fig. 2) revealed that its activity is greatly affected by temperature. The enzyme reaction was stimulated by an increase of reaction temperature.

It is well known that temperature is the most important environmental factor in the postharvest life of fresh vegetables because, within the physiological temperature range, the velocity of a biological reaction, increases two- to three-fold for every 10 °C rise in temperature (Kader, 1987). Therefore, it is understandable that the formation of methanethiol and dimethyl disulfide from anaerobically treated broccoli was affected by the temperature.

Effect of low temperature pre-storage duration on formations of methanethiol and dimethyl disulfide

After heads were pre-stored in air at 1 °C for 7, 14, and 21 days, the florets, which were held under an anaerobic condition at 20 °C for 48 hours, formed methanethiol and dimethyl disulfide (Fig. 3). The concentrations of methanethiol and dimethyl disulfide in the sample pre-stored for 7 days were lower than those of fresh broccoli (0 day) throughout the experiments. The production of methanethiol after 14 and 21 days of pre-storage was high compared with fresh broccoli (0 day). Thereafter, the evolutions of methanethiol were reduced in florets pre-stored for 14 or 21 days. The concentration of methanethiol after a 21-day pre-storage was significantly lower than that of fresh broccoli (0 day) at the end of the treatment. The concentrations of dimethyl disulfide were lower in florets pre-stored for 14 or 21 days than in that of fresh broccoli. It seemed that the production of methanethiol and dimethyl di-

Fig. 2. Effect of temperature on C-S lyase activity. A crude extract from fresh florets was used for C-S lyase assay. The enzyme activity was determined by measuring pyruvate formation in the standard assay mixture at 10 °C, 20 °C or 30 °C and expressed as percentage of the value at 30 °C.

Fig. 3. Effect of pre-storage duration on formations of methanethiol and dimethyl disulfide from broccoli florets under an anaerobic condition. Heads were pre-stored in air at 1 °C for 7, 14 and 21 days. Then, the florets were sealed in glass bottles with 100% N₂. The bottles were held at 20 °C for 48 hr. Values are means $\pm$ SE for n=3.

Fig. 4. Changes in S-methyl-L-cysteine sulfoxide (SMCSO) content and C-S lyase activity in broccoli florets during pre-storage. Heads were pre-stored in air at 1 °C for 7, 14 and 21 days. One unit of enzyme activity produces 1 μ mol pyruvate/min at 30 °C. Values are means $\pm$ SE for n=3.

sulfide from anaerobically treated florets declined as the low temperature treatment was prolonged.

Changes in SMCSO content and C-S lyase activity in broccoli during pre-storage are shown in Fig. 4. The SMCSO contents in the florets were maintained on a same level during pre-storage, whereas C-S lyase activities in the florets decreased slightly when the pre-storage period was extended. This indicates that SMCSO and C-S lyase did not appear to contribute to the decrease of volatile sulfur compounds productions during low temperature pre-storage treatment. The decrease of

volatile sulfur compounds production seems to be due to a change of some unknown factor, which participates in the formations of methanethiol and dimethyl disulfide and except for SMCSO content and C-S lyase activity, with an increase in the pre-storage period. The visual appearance, such as green color and tightness of head, deteriorated as the low temperature pre-storage period was lengthened (data not shown).

Effect of plant parts on formations of methanethiol and dimethyl disulfide

Production of methanethiol and dimethyl disulfide from broccoli flower buds under an anaerobic condition increased rapidly (Fig. 5), reaching over 115 ppm at 48 hours after treatment, whereas dimethyl disulfide was detected 12 hours after treatment; it then increased gradually, reaching 40 ppm at 48 hours after treatment. On the other hand, methanethiol in stems remained low until 36 hours after treatment, and then it increased slightly. Dimethyl disulfide in stems also remained low during the experiment.

The SMCSO contents and C-S lyase activities in fresh flower buds and fresh stems are shown in Table 1. The SMCSO content in the flower buds was about 4 times higher than that in the stems. Furthermore, the C-S lyase activity in flower buds was about 6 times higher than that in the stems. In this study, the concentration of SMCSO in broccoli was nearly the same as previously reported (Morris and Thompson, 1956).

Methanethiol and dimethyl disulfide are derived from the common precursor, methyl methanethiosulfinate, which is a product of the C-S lyase-catalyzed degradation of SMCSO. The selection or breeding for cultivars with low SMCSO content or C-S lyase activity may provide broccoli with fewer undesirable volatile sulfur compounds under an extremely low O₂ and/or extremely high CO₂ condition.

We conclude that the production of methanethiol and dimethyl disulfide depends on temperature, low temperature remarkably reduced the C-S lyase activity and the production of methanethiol and dimethyl disulfide from anaerobically treated broccoli. Low temperature pre-storage reduced methanethiol and dimethyl disulfide formations under an anaerobic condition, even though SMCSO content and C-S lyase activity did not decrease. Methanethiol and dimethyl disulfide were produced mainly by flower buds under anaerobic conditions because they have more SMCSO and a higher C-S lyase activity than have stems.

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Fig. 5. Methanethiol and dimethyl disulfide productions from broccoli flower buds or stems under an anaerobic condition. Fresh flower buds or stems were sealed in glass bottles with 100% N₂. The bottles were held at 20 °C for 48 hr. Values are means ± SE for n=3.

Table 1. Concentration of S-methyl-L-cysteine sulfoxide and activity of C-S lyase in fresh broccoli flower buds and stems.

Plant part	S-methyl-L-cysteine sulfoxide (mg/g f.w.)	C-S lyase activity (units/g f.w.) ²
Flower buds	2.12 ± 0.004	7.7 ± 0.57
Stems	0.51 ± 0.086	1.2 ± 0.06

² One unit of enzyme activity produces 1 μ mol pyruvate/min at 30 °C. Values are means ± SE for n = 3.

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予備貯蔵の期間および貯蔵温度が嫌気条件下におけるブロッコリーからの含硫揮発性成分の発生に及ぼす影響

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摘 要

新鮮なブロッコリー小花をガラス瓶に入れ、瓶内の雰囲気 を 100% N₂ で置換後密封し 10℃, 20℃ および 30℃ で 48 時間保存した。貯蔵温度が高いほどメタンチオールおよびジメチルジスルフィドは多量に発生した。新鮮なブロッコリーを 1℃ の空気下に 7, 14 および 21 日間予備貯蔵し、その後ブロッコリー小花をガラス瓶に入れ、100% N₂ で置換後密封し 20℃ で 48 時間保存した。予備貯蔵の期間が長いものほど嫌気条件下に置かれた後のメタンチオールおよびジメチル

ルジスルフィドの発生量は少なくなった。花茎に比べ、花蕾からのメタンチオールおよびジメチルジスルフィドの発生は顕著であった。メタンチオールおよびジメチルジスルフィドの前駆物質である S-メチル-L-システインスルホキシド含量は花茎に比べ花蕾では約 4 倍多かった。また、S-メチル-L-システインスルホキシドからメタンチオールやジメチルジスルフィドへの代謝に関与する C-S リアーゼ活性は花茎に比べ花蕾で顕著に高かった。