JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Formation of Methanethiol and Dimethyl Disulfide in Crushed Tissues of Broccoli Florets and Their Inhibition by Freeze−Thawing

ARTEMIO Z. TULIO, JR.,† HIROYUKI YAMANAKA,*,‡ YOSHINORI UEDA,† AND YOSHIHIRO IMAHORI†

Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 599-8531, Japan, and Hagoromo-Gakuen Junior College, Hamadera Minami-machi, Sakai, Osaka 592-8344, Japan

The formation of methanethiol and dimethyl disulfide in crushed, homogenized, and frozen−thawed tissues of broccoli florets was investigated. These volatile sulfur compounds were produced in crushed florets, but their formation was inhibited in frozen−thawed tissues. Only dimethyl disulfide was formed in homogenized tissues. High pH treatment triggered the release of dimethyl disulfide in frozen−thawed tissues and also enhanced the action of cysteine sulfoxide lyase in all disrupted tissues. Methyl methanethiosulfinate and methyl methanethiosulfonate were not detected in crushed florets; thus, the favored mechanism for the formation of methanethiol and dimethyl disulfide is the chemical disproportionation of methanesulfenic acid. In contrast, the formation of dimethyl disulfide in frozen−thawed and homogenized tissues occurs from the chemical disproportionation of methyl methanethiosulfinate that was detected in these tissues. The inhibition of dimethyl disulfide production during freeze−thawing must be caused by a sudden drop in the pH of the tissue, adherence of dimethyl disulfide on the tissue surfaces, and weakening of the cysteine sulfoxide lyase activity under acidic conditions.

**KEYWORDS: Broccoli florets; methanethiol; dimethyl disulfide; cysteine sulfoxide lyase; freeze−thawing; volatile sulfur compounds**

## INTRODUCTION

Broccoli (*Brassica oleracea* L.) and other cruciferous vegetables, such as cauliflower, cabbage, and Brussels sprouts, are characterized by sulfurous aroma compounds. These are derived mainly from the breakdown of the nonvolatile substrate *S*-methyl-L-cysteine sulfoxide (SMCSO) into pyruvate, ammonia, and methanesulfenic acid (MSI) after the action of cysteine sulfoxide lyase (C-S lyase) on tissue disruption (*1*).

Methanethiol (MT) and dimethyl disulfide (DMDS) are generally considered as the major contributors to objectionable odor among the volatile sulfur compounds already identified in broccoli (*2*−*6*). Control of these objectionable odors is critical to the consumption and marketing potential of broccoli in light of the recent studies about its medical importance. Recently, it has been reported that some organosulfur compounds produced in *Brassica* vegetables have potential anticarcinogenic properties (*7*−*12*). Some of these secondary metabolites are indol-3-methyl-glucosinolates (*9*) and sulforaphane (*10*).

Off-odor formations had been demonstrated in several ways, such as storage of broccoli under anaerobic conditions (*2*−*6*), hot-water dip treatment (*13, 14*), and mechanical disruption of tissues (*15*). However, other methods of tissue disruptions such as manual crushing, sudden mechanical homogenization, and freeze−thawing that could also induce the formations of the two main volatile sulfur compounds in broccoli have not yet been elucidated. This information is necessary to provide further understanding of the mechanisms of formation and aid in the regulation of the production of objectionable odors in *Brasicca* vegetables. Thus, in this study, the formation of MT and DMDS in crushed and homogenized tissues and their inhibition in frozen−thawed tissues were investigated in broccoli florets.

## MATERIALS AND METHODS

**Chemicals.** Methanethiol (1 $\mu$g/$\mu$L benzene solution) and dimethyl disulfide (0.1 $\mu$g/$\mu$L benzene solution) standard solutions, 2,4-dinitrophenyl hydrazine, ninhydrin, polyclar SB-100, and all organic solvents used in the extraction were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Authentic methyl methanethiosulfonate and *S*-methyl-L-cysteine sulfoxide were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI) and Research Organics, Inc. (Cleveland, OH), respectively. Bio-Rad protein assay (dye reagent concentrate) was obtained from Bio-Rad Laboratories (Hercules, CA). Pyridoxal 5-phosphate and bovine serum albumin were supplied by Sigma Chemical Co. (St. Louis, MO).

* To whom correspondence should be addressed. E-mail yamanaka@hagoromo.ac.jp.
† Osaka Prefecture University.
‡ Hagoromo-Gakuen Junior College.

10.1021/jf010673g CCC: $22.00 
Published on Web 02/16/2002

**Plant Material.** Fresh broccoli (*B. oleracea* L.) heads weighing about 300−400 g were purchased from a local supermarket in Osaka, Japan. Individual florets measuring 2−3 mm long were cut from each broccoli head. One broccoli head was used in the preparation of 10 g each of control, crushed, homogenized, and frozen−thawed tissues. Three broccoli heads were used for treatments without buffer, and another three broccoli heads for treatments with buffer.

**Tissue Preparation.** *Crushed Tissues.* Broccoli florets were crushed manually by using mortar and pestle. After crushing, these tissues were used with or without the addition of 20 mL of 0.1 M phosphate buffer solution (pH 8.0).

*Homogenized Tissues.* Broccoli tissues were homogenized in 20 mL of distilled water or 0.1 M sodium phosphate buffer solution (pH 8.0) by using a Vortex homogenizer (IKA Laboratory Technology Japan Ltd.) at the highest speed for 1−2 min.

*Frozen Tissues.* Broccoli florets were frozen at −80 °C for 24 h and thawed. After thawing, these tissues were used in the study with or without 20 mL of 0.1 M phosphate buffer solution (pH 8.0). Furthermore, buffer at various pH values (pH 5.0, 6.0, 7.0, 8.0, and 9.0) was also added in the frozen−thawed tissues.

*Fresh Tissues.* Fresh, nondisrupted tissues with or without 20 mL of 0.1 M phosphate buffer solution (pH 8.0) were used as control.

Each treated tissue from three broccoli heads was then placed individually in a 100-mL Erlenmeyer flask. All of the flasks were sealed with silicon stoppers and held at 30 °C in a water bath for 2 h before headspace gas analysis with gas chromatography-flame photometric detector (GC−FPD).

About 100 $\mu$L of standard DMDS vapor was added in the flasks containing control and frozen-thawed broccoli tissues. The flasks were sealed with silicon stoppers and held for only 30 min at 30 °C in a water bath before headspace gas was analyzed by using GC−FPD.

**GC−FPD.** MT and DMDS that accumulated in the headspace of the flasks containing various tissue treatments were analyzed by using GC−FPD. Two milliliters of headspace samples were withdrawn from each flask at various holding times at 30 °C with a 5-mL gastight syringe. Headspace gas samples were then injected into a Hitachi model 163 gas chromatograph (Hitachi Co. Ltd., Tokyo, Japan). The gas chromatograph was equipped with a flame photometric detector and a D-2500 chromato integrator. The volatile sulfur compounds were separated on two glass columns connected in series: first, a 10 cm × 3 mm (i.d.) column packed with Porapak QN (Millipore Corp., Milford, MA); second, a 3 m × 3 mm (i.d.) column packed with Polyphenyl ether 5-rings [*m*-bis(*m*-phenoxyphenoxy)benzene] (5% coating on Diasolid S) (Nihon Kuromato Kogyo, Tokyo, Japan). Flow rates for oxygen, hydrogen, and nitrogen were 25, 70, and 80 mL/min, respectively. The column temperature was maintained at 70 °C. The injector and detector ports were both kept at 180 °C. Peaks were identified by comparing the retention times with those of MT and DMDS standard solutions.

**C-S Lyase Activity.** *Crude Extract.* Five grams each of fresh and frozen−thawed tissues were homogenized in 45 mL of cold 0.1 M sodium phosphate buffer solution (pH 7.0) containing 3% (w/v) polyclar SB-100 by using a Vortex homogenizer at the highest speed for 1−2 min. The homogenate was filtered through four layers of gauze and centrifuged at 10 000*g* for 20 min at 4 °C. The supernatant was used as crude enzyme solution.

*C-S Lyase Assay.* C-S lyase activity was assayed according to the modified procedure described by Hamamoto and Mazelis (*16*). The standard reaction mixture contained 0.6 mL of 0.1 M sodium phosphate buffer (pH 8.5), 0.1 mL of 0.25 mM pyridoxal 5-phosphate, 0.1 mL of 0.04 M SMCSO, and 0.2 mL of crude enzyme solution with a total volume of 1.0 mL. Buffers at various pH values (pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0) were also used. The reaction mixture was incubated at 30 °C in a water bath for 10 min. The reaction was terminated by the addition of 2 mL of 10% trichloroacetic acid and centrifuged at 3000*g* for 10 min. An aliquot of the supernatant was assayed for pyruvate colorimetrically by the total keto acid method of Friedemann and Haugen (*17*).

*Protein Determination.* Protein content of fresh and frozen-thawed tissues was estimated according to the dye-binding method of Bradford (*18*) with bovine serum albumin as the standard.

**SMCSO Content.** Five grams each of fresh and frozen−thawed tissues were extracted with 20 mL of 99% ethyl alcohol by using reflux tubes for 15 min. The crude extract was filtered and washed twice with 80% cold ethyl alcohol. The filtrate was made up to 100 mL volume with 80% cold ethyl alcohol. The extract (10 mL) was evaporated at 40 °C and dissolved in 10 mL of 0.1 N HCl. The aliquot was passed through a 0.45-$\mu$m cellulose nitrate membrane filter. Of this aliquot, 100 $\mu$L was used to analyze the SMCSO with an amino acid analyzer (Kyowa Seimitsu K-101 model, Japan) under the following conditions: a 4.6 m × 150 mm (i.d.) column packed with 62210 F ($Na^+$) (Kyowa Seimitsu, Japan) at 50 °C and elution buffers of 0.2 N sodium citrate (pH 3.18 and 4.25) and 2.0 N sodium citrate (pH 4.45) at a flow rate of 0.5 mL/min. The ninhydrin solution had the same flow rate as the elution buffers.

**Methyl Methanethiosulfinate (MMTSI) and Methyl Methanethiosulfonate (MMTSO) Extraction.** Crushed, homogenized, and frozen−thawed tissues including control were used in the preparation of the extracts of MMTSI and MMTSO with the modified procedure of Nakamura et al. (*19*). MMTSI and MMTSO extracts of crushed and frozen−thawed tissues in various buffer solutions (pH 6.5, 7.0, 8.0, and 9.0) were also prepared. Fifteen milliliters of methanol was added to each treatment before homogenation with 20 mL of acetone for 1−2 min. The homogenate was extracted three times with 20-mL aliquots of acetone, and this acetone extract was washed three times with 10 mL of cyclohexane. The aqueous solution was then extracted three times with 20-mL aliquots of benzene. The resulting cyclohexane-benzene extract was evaporated to dryness before dissolving in 2 mL of acetone.

**Gas Chromatography−Mass Spectrometry (GC-MS).** MMTSI and MMTSO were analyzed by injecting 2 $\mu$L of the acetone extract in a Shimadzu gas chromatograph-mass spectrometer system (GCMS-QP5000). The volatiles were separated on a 30 m × 0.25 mm (i.d.) DB-1 column with a film thickness of 0.25 $\mu$m (J&W Scientific, Folsom, CA). The initial oven temperature was held at 50 °C, increased to 180 °C at 3 °C/min, and finally held at 180 °C for 10 min. The injector and detector temperatures were 250 °C. Helium at a flow rate of 1.7 mL/min was used as the carrier gas. Mass spectra were obtained by electron ionization at 70 eV. The interface temperature was 230 °C. The mass spectra were recorded on a Fujitsu FMV-5100D6 processor. Tentative identification of MMTSI and MMTSO was made by matching their mass spectra and retention times against the standard MS spectra database, NIST Class 5000 Library (Shimadzu Corp., Kyoto, Japan). Identification of MMTSO was verified by comparison of mass spectra and retention times with the authentic compound.

## RESULTS AND DISCUSSION

MT and DMDS are the main volatile sulfur compounds associated with the off-flavor development of *Brassica* vegetables. **Figure 1** shows the production of these two volatile compounds in crushed, homogenized, and frozen−thawed tissues of broccoli florets after incubation at 30 °C in a water bath. MT and DMDS were detected in the headspace of the flask containing the crushed tissues in considerable and slight amounts, respectively (**Figure 1A**). After 2 h at 30 °C, the concentration of MT formed in crushed tissues reached 28.1 nmol/g fresh weight, whereas DMDS was only 3.0 nmol/g fresh weight. These figures jumped by 2- and 6-fold, respectively, when the pH of the crushed tissues was adjusted with pH 8.0 buffer solution (**Figure 1B**). The formation of these volatile compounds in crushed tissues of broccoli florets was not induced by anaerobic conditions, because enough $O_2$ concentration still remained in the flask after 2 h of incubation (data not shown).

In homogenized tissues, rapid disruption of the fresh tissues of broccoli florets produced significant quantities of DMDS but only trace amounts of MT (**Figure 1A**). The amount of DMDS formed in tissues homogenized with distilled water was 8.8 nmol/g fresh weight after 2 h at 30 °C. Changes in the tissue pH with pH 8.0 buffer solution increased the amount of DMDS by almost 3-fold, but the amount of MT detected remained

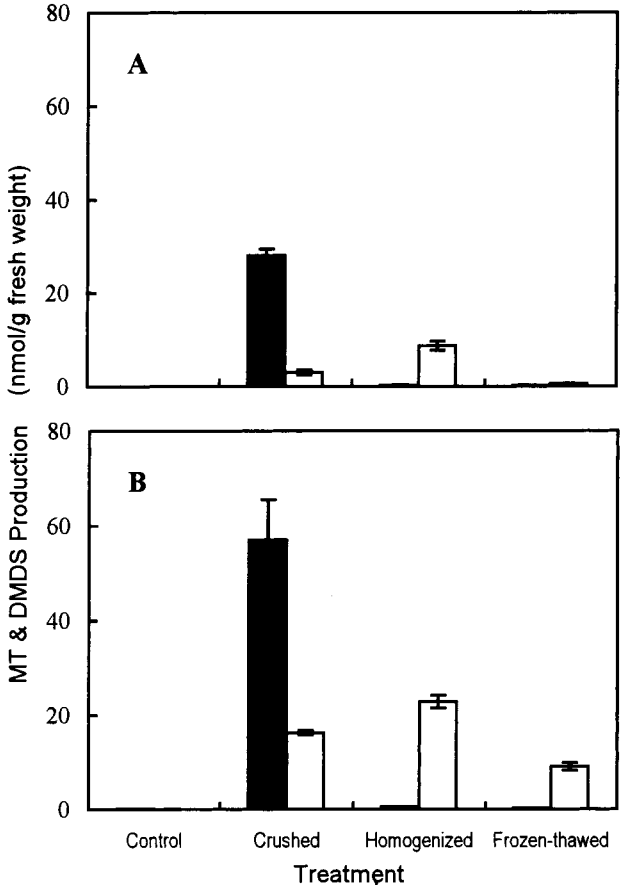


**Figure 1.** Production of MT (■) and DMDS (□) in various disrupted tissues of broccoli florets (A) without and (B) with 0.1 M sodium phosphate buffer (pH 8.0) after 2 h at 30 °C. Bars are mean ± SD ($n = 3$).

insignificant (**Figure 1B**). Dan et al. (*13*) reported trace amounts of MT in the headspace of a capped test tube containing the mechanically disrupted fresh broccoli tissue solution.

Freeze−thawing the tissue of broccoli florets, however, inhibited the emission of the two main volatile sulfur compounds to a great extent (**Figure 1A**). The amount of MT and DMDS formed was only 0.2 and 0.6 nmol/g fresh weight, respectively. Addition of the buffer solution (pH 8.0) in frozen−thawed tissues did not induce the formation of MT, although the amount of DMDS rose to 9.1 nmol/g fresh weight (**Figure 1B**).

In crushed tissues, MT preceded the formation of DMDS (**Figure 2**). It has been previously reported that MT was one of the first compounds that appeared in the headspace of broccoli florets while DMDS increased slowly (*2*−*6*). Chin and Lindsay (*20*) also reported that MT was detected near the maximum concentrations in the headspace of cabbage right after tissue disruption, but DMDS was detected after a long time. The ratio of MT to DMDS was 58:1 after 10 min at 30 °C. The ratio reached its equilibrium of 1.7:1 after 90 min in the water bath (30 °C), whereas the individual concentrations of these compounds leveled off. They remained unchanged and reached their peak after 5 h before the production of both compounds started to decrease gradually.

**Table 1** shows the activity of C-S lyase at pH 8.5 and SMCSO content of fresh and frozen−thawed tissues of broccoli florets. It illustrates that C-S lyase and SMCSO were present in both tissues. The activity of C-S lyase in frozen−thawed tissues had no marked difference from that in the fresh florets. Similarly, no notable difference occurred between the SMCSO contents of fresh and frozen−thawed tissues (**Table 1**). These

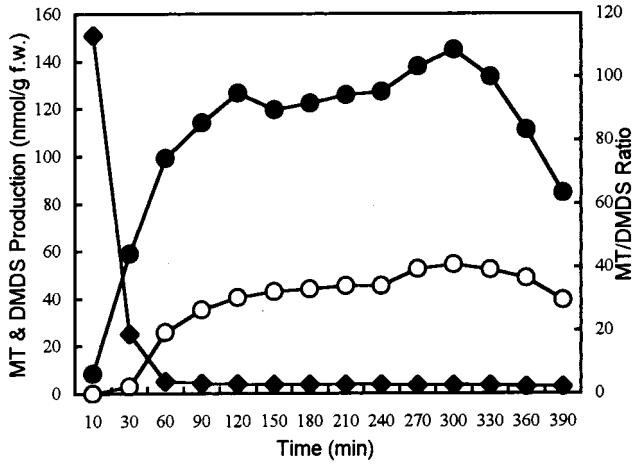


**Figure 2.** Time course of the production of MT (●) and DMDS (○) and MT/DMDS ratio (◆) in crushed tissues of broccoli florets at 30 °C. f.w., fresh weight.

**Table 1.** C-S Lyase Activity at pH 8.5 and SMCSO Content of Fresh and Frozen–Thawed Tissues of Broccoli Florets[a]

| treatment | C-S lyase activity[b] ($\mu$g·min$^{-1}$·mg$^{-1}$ protein) | SMCSO ($\mu$g/100 g f.w.)[c] |
|---|---|---|
| fresh tissues | 1.79 ± 0.19 | 340.2 ± 17.5 |
| frozen–thawed tissues | 1.41 ± 0.13 | 305.9 ± 10.3 |

[a] Values are mean ± SD ($n = 3$). [b] Computed in terms of pyruvate. [c] f.w., fresh weight.

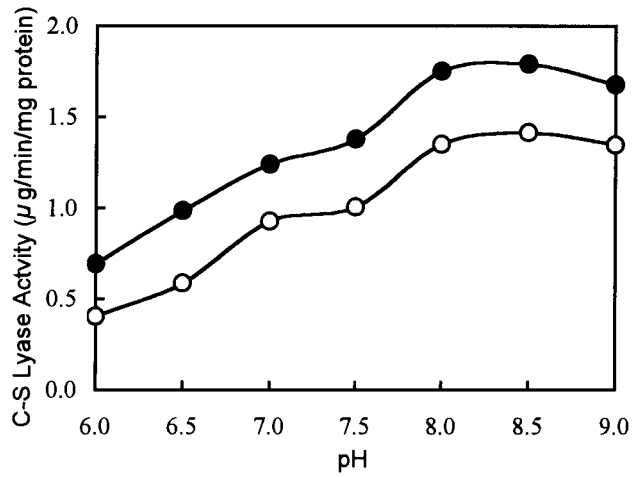


**Figure 3.** Effect of pH on the C-S lyase activity in crushed (○) and frozen–thawed (●) tissues of broccoli florets.

results show that freezing the tissues of the broccoli florets had no marked effect on both precursor and enzyme activity. SMCSO content was diminished slightly and the C-S lyase was not inactivated but only retarded by freeze−thawing. In contrast, Schwimmer and Guadagni (*21*) observed that C-S lyase activity was completely inactivated by freezing in commercially frozen onion extracts.

The activities of C-S lyase from frozen−thawed tissues of broccoli florets with various pH values were lower than those in the fresh tissues (**Figure 3**). However, these activities increased notably as the pH of both tissues was increased. In addition, in frozen−thawed tissues, as in fresh tissues, the activity of this enzyme had reached its optimum at pH 8.0−9.0. Hamamoto and Mazelis (*16*) reported that the optimum pH for C-S lyase activity in fresh broccoli is between 8.0 and 8.5,

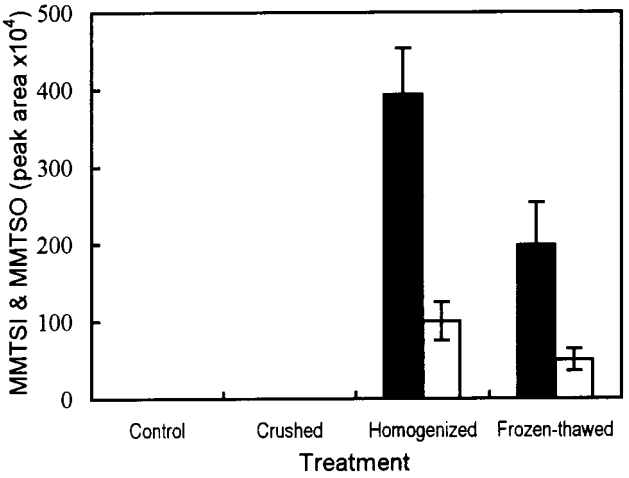


**Figure 4.** Production of MMTSI (■) and MMTSO (□) in various disrupted tissues of broccoli florets after 2 h at 30 °C. Bars are mean ± SD ($n$ = 3).

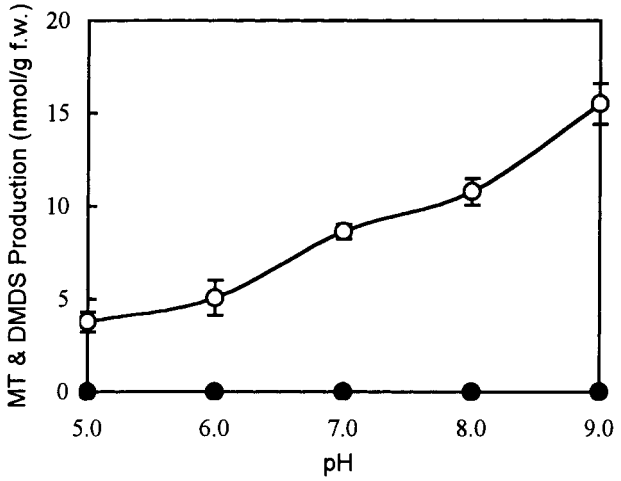


**Figure 6.** Effect of pH on the production of MT (●) and DMDS (○) in frozen–thawed tissues of broccoli florets after 2 h at 30 °C. Values are mean ± SD ($n$ = 3). f.w., fresh weight.

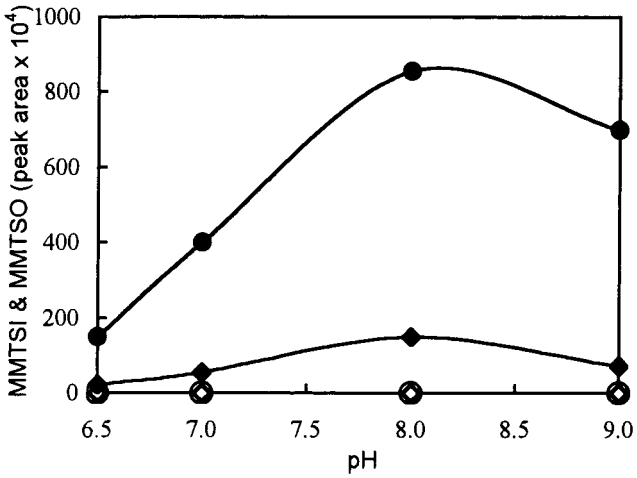


**Figure 5.** Effect of pH on the production of MMTSI and MMTSO in crushed and frozen–thawed tissues of broccoli florets after 2 h at 30 °C. MMTSI-crushed (○); MMTSI-frozen (●); MMTSO-crushed (◇); MMTSO-frozen (◆).

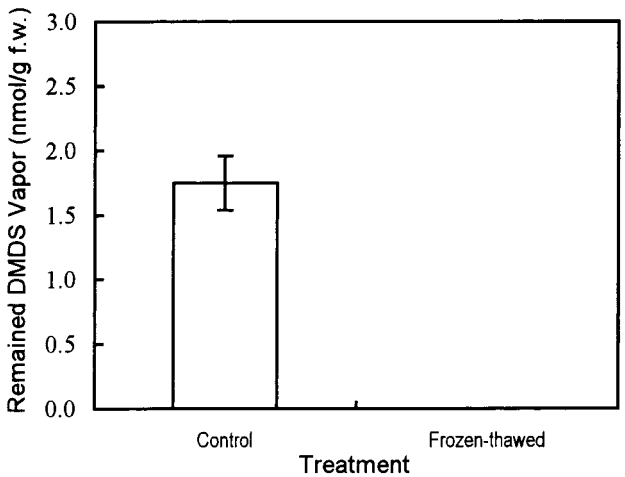


**Figure 7.** Remaining DMDS vapor after addition of standard DMDS in the flasks with control and frozen–thawed tissues of broccoli florets. Incubation time was 30 min at 30 °C. Bar is mean ± SD ($n$ = 3). f.w., fresh weight.

whereas Hall and Smith (*22*) had previously stated that, in cabbage, the optimum pH of this enzyme was in the range of 8.5−9.0. Thus, higher pH treatment in frozen−thawed tissues of broccoli florets also increased the activity of C-S lyase considerably.

**Figure 4** shows the production of MMTSI and MMTSO in crushed, homogenized, and frozen−thawed tissues of broccoli florets after incubation at 30 °C in a water bath. The secondary intermediate, MMTSI, was detected in moderate amounts in acetone extracts of frozen−thawed tissues, whereas higher concentrations were produced in homogenized tissues. However, MMTSI was not detected in the extract of crushed tissues. The peak areas of MMTSI were 199 × $10^4$ and 363 × $10^4$ for frozen−thawed and homogenized tissues, respectively. MMTSO, which is a product of the chemical disproportionation of MMTSI together with DMDS (*1*, *2*, *23*−*27*), was also detected in frozen−thawed and homogenized tissues except in crushed florets (**Figure 4**). The peak area of MMTSO in frozen−thawed tissues was 48 × $10^4$, whereas in homogenized tissues, it was 101 × $10^4$.

The effect of various pH values on the production of MMTSI and MMTSO in crushed and frozen−thawed tissues after 2 h at 30 °C is shown in **Figure 5**. The MMTSI content increased markedly whereas the MMTSO content increased slightly as the pH of the frozen−thawed tissues was increased. The notable increase in MMTSI and MMTSO contents was detected in frozen−thawed tissues treated with buffer solution ranging from pH 8.0 to 9.0. Marks et al. (*23*) demonstrated that the formation of this secondary intermediate, MMTSI, was strongly dependent on the pH of the fresh *Brassica* vegetables wherein considerable amounts were formed at basic pH. Nakamura et al. (*19*) also asserted that MMTSO formation depended on the pH value at which C-S lyase was activated rather than the SMCSO content in a tissue homogenate. Higher pH values did not contribute to the formation of MMTSI and MMTSO in crushed tissues, not even in its induction. This result means that MT and DMDS formed in the crushed tissue headspace were highly unlikely to be the byproducts of MMTSI. Furthermore, the formation of MMTSO and, consequently, MMTSI, is not only dependent on the pH of the tissues but also on the tissue treatment.

The effect of pH on the production of MT and DMDS in frozen−thawed tissues of broccoli florets after 2 h at 30 °C was also determined (**Figure 6**). The formation of DMDS increased as the pH of the frozen−thawed tissues increased. However, the formation of MT in the same tissues was not detected and remained undetected even when the pH of the

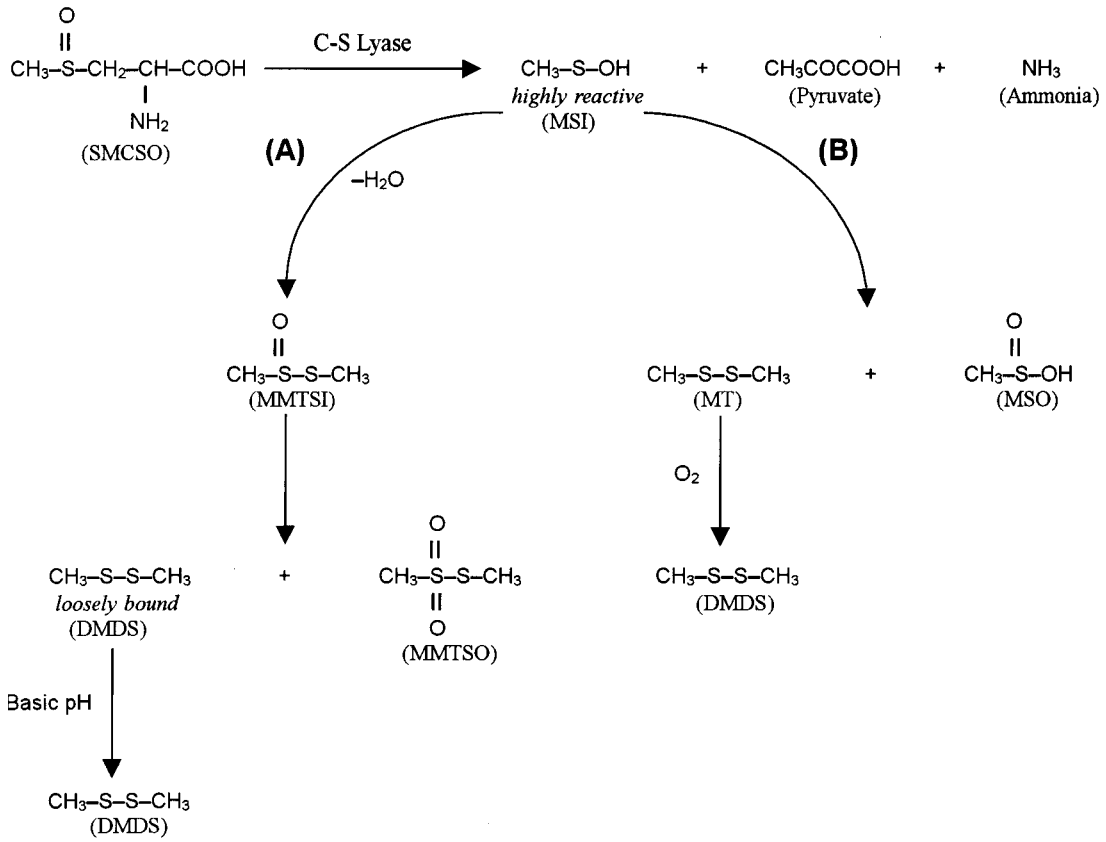


**Figure 8.** Mechanism of formation of (A) DMDS in frozen–thawed and homogenized tissues and (B) MT and DMDS in crushed tissues of broccoli florets.

tissues increased. This probably implies that DMDS is loosely bound on the surface of the frozen−thawed tissues and it has a strong affinity at acidic pH.

**Figure 7** shows the remaining DMDS vapor after addition of standard DMDS in the flasks with control and frozen−thawed tissues of broccoli florets. DMDS did not exist in the headspace of the frozen−thawed tissues compared with that in the control tissues. This suggests that the standard DMDS vapor adhered on the surface of the disrupted tissues caused by freeze−thawing. This result also agrees with the statement above that DMDS is likely to be loosely bound on the tissue surface of the frozen−thawed broccoli florets which have acidic pH conditions. The inhibition of DMDS in frozen−thawed tissues of broccoli florets must then be caused by the sudden drop in the pH of the tissue, the absorption of formed DMDS in the disrupted tissues, and the weakening of the C-S lyase activity during freeze−thawing.

Two mechanisms are presumed to be involved in the formation of MT and DMDS in crushed, frozen−thawed, and homogenized tissues of fresh broccoli florets (**Figure 8**). The first mechanism occurs by way of dehydration of the MSI, the primary byproduct of the C-S lyase action on SMCSO to form the more stable MMTSI in a weakly acidified medium (**Figure 8A**). The formation of MMTSO and DMDS in the first mechanism is rationalized by the subsequent chemical disproportionation of this secondary intermediate, MMTSI (*1*, *2*, *23*−*27*). The second one involves the chemical disproportionation of this highly reactive and unstable intermediate, MSI, to form MT and methanesulfonic acid (MSO) under aerobic conditions (**Figure 8B**). The formation of DMDS in the second mechanism involves the subsequent oxidation of MT (*1*, *2*, *28*, *29*).

The mechanism of formation of both MT and DMDS in crushed tissues keeps to the mechanism in **Figure 8B**. Furthermore, the formation of MT and DMDS in crushed tissues has a striking similarity to the formation pathway of broccoli tissues subjected to modified atmosphere packaging. Dan et al. (*2*) demonstrated it in broccoli, whereas Chin and Lindsay (*1*) verified it in cabbage. The former suggested that, under MA conditions, these volatile compounds were formed as a result of the deterioration of cellular membrane and loss of intracellular compartmentation that allowed the enzyme−substrate reaction to proceed. In crushed tissues, the formation of these compounds was enhanced by moderate injury after the gradual disruption of the fresh tissues through manual means and by the presence of air and relatively neutral pH conditions.

Chin and Lindsay (*1*) and Dan et al. (*2*) concluded that MT is formed by adhering to the pathway in **Figure 8B**, whereas DMDS might be formed in either the presence (**Figure 8B**) or absence of air (**Figure 8A**). Moreover, they both suggested that oxidation of MT is not the predominant mechanism of formation of DMDS under modified atmosphere packaging. However, because the secondary intermediate, MMTSI, and its byproduct MMTSO were not detected in crushed tissues, the oxidation of MT is the most favored mechanism for the formation of DMDS. Hence, the same mechanism (**Figure 8B**) is most likely the formation pathway of DMDS in broccoli stored under modified atmosphere packaging. Lindsay et al. (*28*) and Miller et al. (*29*) had already demonstrated the oxidation of MT to DMDS in the presence of air.

The formation of DMDS in homogenized or frozen−thawed tissues adheres to the mechanism shown in **Figure 8A**. The secondary intermediate, MMTSI, was detected in frozen tissues but MT was not. Hence, DMDS and another byproduct, MMTSO, formed on the chemical disproportionation of this secondary intermediate after the dehydration of MSI. The amount of DMDS was enhanced by the pH adjustment of the frozen−thawed tissues from acidic to basic pH by releasing the bound DMDS from the tissue surface.

We conclude that MT and DMDS were formed on the gradual disruption through manual crushing of the fresh tissues of broccoli florets, whereas rapid disruption through homogenization of the same tissues produced only DMDS. However, freeze−thawing the tissues almost completely inhibited the formation of these compounds. Off-odor formation of gradually disrupted tissues of broccoli florets had a similar course of formation with broccoli florets under modified atmosphere conditions in which MT and DMDS were both produced. The proposed mechanism of formation in crushed broccoli florets may also explain the formation of these volatile compounds under modified atmosphere packaging. Further studies on the mechanism of off-odor production in *Brassica* vegetables are warranted to completely understand their formation. Inhibition of the sulfurous volatile compounds by freeze−thawing and their recovery by adjusting the pH of the tissue from acidic to basic conditions can be used in the frozen-vegetable industry, in particular, for the cruciferous species and even species of *Allium*.

## ABBREVIATIONS USED

C-S lyase, cysteine sulfoxide lyase; DMDS, dimethyl disulfide; MMTSI, methyl methanethiosulfinate; MMTSO, methyl methanethiosulfonate; MSI, methanesulfenic acid; MSO, methanesulfonic acid; MT, methanethiol; SMCSO, *S*-methyl-L-cysteine sulfoxide.

## ACKNOWLEDGMENT

We thank Dr. Gary R. Takeoka of Western Regional Research Center, U.S. Department of Agriculture, for reviewing the manuscript.

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**Received for review May 22, 2001. Revised manuscript received November 28, 2001. Accepted November 29, 2001.**

JF010673G