Volatile Compounds Produced by Broccoli under Anaerobic Conditions

Charles F. Forney,*,† James P. Mattheis,‡ and Rodney K. Austin†

Horticultural Crops Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 2021 South Peach Avenue, Fresno, California 93727, and Tree Fruit Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 1104 North Western Avenue, Wenatchee, Washington 98801

Severe off-odors are produced by broccoli (*Brassica oleracea* L., Italica Group) when it is held under anaerobic conditions, which can develop in modified atmosphere packages. The compounds responsible for these off-odors, which render the broccoli unmarketable, were collected using headspace sampling and identified using GC-FPD and GC-MS. Broccoli florets were held in sealed jars at 15 or 20 °C in which the O_2 concentration dropped to around 0.5%. Volatile compounds detected were identified as ethanol, methanethiol, hydrogen sulfide, ethyl acetate, dimethyl disulfide, dimethyl sulfide, acetaldehyde, methyl acetate, and acetone. Methanethiol was one of the first compounds formed under anaerobic conditions and appears to be primarily responsible for the objectionable odor.

INTRODUCTION

Broccoli (*Brassica oleracea* L., Italica Group), held in atmospheres of low O_2 and/or high CO_2 concentrations, has a longer shelf life and higher quality following storage than that held in air. Concentrations of 0.5–2% O_2 and/or 10% CO_2 reduced rates of respiration and inhibited yellowing of broccoli held at 5 or 7.5 °C (Lebermann et al., 1968; Lipton and Harris, 1974). Modified atmosphere packaging has been used to develop these beneficial atmospheres in which the atmosphere composition is dependent on the permeability of the package and the respiration rate of the broccoli (Elkashif et al., 1983; Rij and Ross, 1987; Wang and Hruschka, 1977). When respiration rates increase due to increased holding temperatures, O_2 concentrations may decrease to levels that result in the development of objectionable off-odors if the permeability of the package to O_2 is insufficient (Ballantyne et al., 1988; Forney and Rij, 1991; Kasmire et al., 1974). 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.

Volatile components of cooked broccoli (Buttery et al., 1976) and other closely related members of the *Brassica* genus have been reported (Dateo et al., 1957; Itoh et al., 1985; MacLeod and MacLeod, 1970). However, volatile compounds evolving from fresh broccoli under either aerobic or anaerobic conditions have not been well characterized. Lieberman and Spurr (1955) tentatively identified methanethiol, acetaldehyde, and ethyl acetate as volatiles evolved from broccoli held under a nitrogen atmosphere. However, the chemical nature of the odor produced by broccoli held under anaerobic conditions has not been further characterized. A better understanding of these volatiles is needed to facilitate the development of strategies to prevent off-odor formation in packaged broccoli.

In this study, volatile compounds that evolved from broccoli held under extreme modified atmospheres were characterized and compounds responsible for the objectionable odors were identified.

† Horticultural Crops Research Laboratory.
‡ Tree Fruit Research Laboratory.

EXPERIMENTAL PROCEDURES

Plant Material. Broccoli was purchased locally in the market. Individual florets measuring from 15 to 30 mm in diameter and 40 mm long were cut from each head. Florets were weighed, and 50 g was placed into each of three pint-sized canning jars and sealed with a lid containing a septum. Jars containing the broccoli were held at 15 °C for 7 days. During this time, headspace was periodically analyzed for O_2, CO_2, and sulfur-containing volatiles. Samples for GC–MS analysis were collected from sealed 4-L glass jars containing 375 g of florets held for 4 days at 20 °C.

Headspace Analysis. Samples (0.5 mL) of the atmosphere inside the sealed pint-sized jars were taken 1, 2, 4, 6, 24, 30, 48, 72, and 168 h after the jars were sealed; the samples were analyzed for O_2 and CO_2 composition using a gas chromatograph with a thermal conductivity detector. Samples were injected into a 1.9 m × 6.4 mm (o.d.) CTR I column (Alltech Associates Inc.) with a helium carrier flow of 50 mL/min. The oven temperature was 50 °C, and the injector and detector were held at 70 °C.

Sulfur-containing volatile compounds that accumulated in the headspace of the pint-sized jars were analyzed periodically for 7 days using a Tracor GC with a flame photometric detector (FPD) used in the sulfur mode. Headspace samples (1 mL) were injected into a 1.9 m × 2 mm (i.d.) Teflon column packed with Chromosorb 108. Column temperature was 80 °C for 5 min, increased to 180 °C at 5 °C/min, and held at 180 °C for 10 min with a nitrogen carrier flow of 35 mL/min. Injector and detector temperatures were 130 and 235 °C, respectively. Peaks were identified by comparing retention times with that of known standards. To identify peaks responsible for the off-odor, the detector flame was turned off and effluent from the column was evaluated using olfaction.

Samples analyzed on the GC–MS system were collected by passing 5 mL of headspace through a trap containing 50 mg of Tenax. Trapped volatile compounds were introduced into the GC–MS system via thermal desorption using a 200 °C hot air gun and cryofocusing (Farwell et al., 1979).

Volatile compounds in the headspace were analyzed using a Hewlett-Packard 5890A-5971A GC–MS system equipped with a 60 m × 0.25 mm (i.d.) DB-Wax column (J&W Scientific, 0.25-μm film thickness). The initial oven temperature was held at 35 °C for 5 min, increased to 50 °C at 2 °C/min, increased to 200 °C at 5 °C/min, and held at 200 °C for 5 min with a linear helium carrier flow of 30 cm/s at 35 °C. Mass spectra were obtained by electron ionization at 70 eV. Transfer line and ion source temperatures were 280 and 180 °C, respectively. Spectra were recorded on a Hewlett-Packard 59970C Chemstation. Identification was made by matching against the Wiley/NBS library and by GC retention time against standards. Estimates of concentrations of headspace compounds were made via selected ion monitoring using response factors determined from authentic standards.

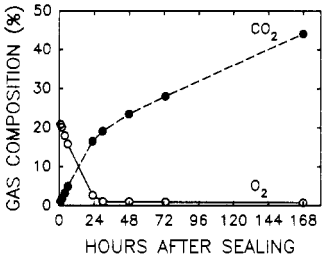

Figure 1. Oxygen (O_2) and carbon dioxide (CO_2) composition of atmospheres inside jars containing 50 g of broccoli held at 15 °C over time.

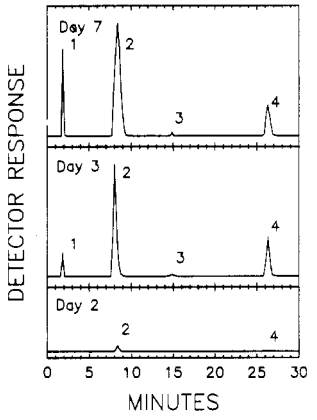

Figure 2. GC-FPD analysis of volatile sulfur compounds in the headspace of jars containing broccoli 2, 3, and 7 days after sealing at 15 °C. 1, Hydrogen sulfide; 2, methanethiol; 3, dimethyl sulfide; 4, dimethyl disulfide.

RESULTS

The oxygen concentration dropped rapidly in jars of broccoli during the first 24 h following sealing (Figure 1). Over the same time period CO_2 concentration increased at an equal but opposite rate. During this time aerobic respiration appeared to predominate and the ratio of CO_2 produced and O_2 consumed was about 1. After the first 24 h, oxygen concentration reached 0.5% and remained there through day 7 while CO_2 concentration continued to increase, reaching over 40% after 7 days. During this time anaerobic respiration predominated as seen by the lack of O_2 depletion.

Two days after the broccoli was sealed in the jars or about 1 day after anaerobic metabolism began, an objectionable odor could be detected through olfactory evaluation of the headspace. At this same time small amounts of volatile, sulfur-containing compounds were detected by GC-FPD analysis of headspace from the sealed jars (Figure 2). These compounds had the same retention times as methanethiol and dimethyl disulfide. The concentration of these two compounds increased greatly 3 days following sealing and two additional compounds were detected, hydrogen sulfide and dimethyl sulfide. By 7 days following sealing, concentrations of these four compounds continued to increase and a small unidentified peak was observed with a retention time of about 12.4 min.

To associate these compounds with the off-odor that developed in the jars of broccoli, the flame of the FPD detector was turned off and the odor of the carrier stream entering the detector was evaluated subjectively. The methanethiol peak was found to have the strongest odor and appears to be the primary compound responsible for the objectionable off-odor. Olfactory detection of the methanethiol peak was possible 3 days after the broccoli was sealed. Seven days following sealing, the hydrogen sulfide and the dimethyl disulfide peaks had a detectable odor in addition to the methanethiol peak.

Table I. Volatile Compounds Identified from Broccoli Headspace Using GC-MS[a]

volatile compd	RRt,[b] min	major peaks in the EI spectra,[c] m/z	$\mu L\ L^{-1}$
methanethiol	0.79	47, 48, 45, 46, 44	58.215
acetaldehyde	0.97	44, 29, 43, 42, 48	0.138
dimethyl sulfide	1.46	62, 47, 45, 46, 61	0.768
acetone	2.55	43, 58, 42, 44	0.002
methyl acetate	2.83	43, 74, 42, 59	0.002
ethyl acetate	4.45	43, 61, 45, 70, 29	24.074
ethanol	6.74	31, 45, 29, 27, 43	338.420
dimethyl disulfide	12.92	94, 79, 45, 46, 47	11.640

[a] Samples were collected onto Tenax traps after 375 g of florets were held for 4 days at 20 °C in a sealed 4-L glass jar. Values are averages of three samples. [b] RRt, retention time relative to CO_2 (consistent with that of authentic samples). [c] In order of decreasing abundance (mass spectra consistent with published spectra, Wiley/NBS library).

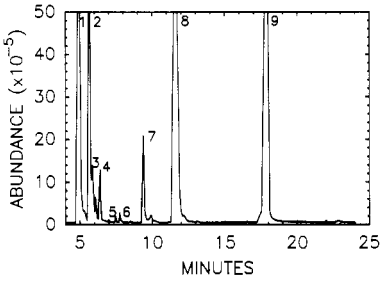

Figure 3. Total ion chromatogram of the GC-MS analysis of volatile compounds in the headspace of 4-L jars containing 375 g of broccoli 4 days after sealing at 20 °C. Volatile compounds from 5 mL of headspace were collected on a Tenax trap and cryofocused prior to injection. 1, Carbon dioxide; 2, methanethiol; 3, acetaldehyde; 4, dimethyl sulfide; 5, acetone; 6, methyl acetate; 7, ethyl acetate; 8, ethanol; 9, dimethyl disulfide.

Volatile compounds collected on Tenax traps and analyzed via GC–MS confirmed the presence of methanethiol, dimethyl sulfide, and dimethyl disulfide (Table I; Figure 3). Hydrogen sulfide, however, was not detected because it was not retained by the Tenax trap. In addition to the sulfur compounds detected, ethanol, ethyl acetate, acetaldehyde, methyl acetate, and acetone were present in significant quantities. None of these compounds were found in detectable amounts in the atmospheres of broccoli packages that contained atmospheres with greater than 1.5% O_2 (data not shown).

DISCUSSION

Methanethiol appears to be the primary contributor to the objectionable odor that broccoli develops when held in anaerobic conditions. The odor of methanethiol has been described as an "intensely putrid, fecal-like aroma" (Lindsay and Rippe, 1986) or that of rotten cabbage (Windholz et al., 1983). The detection threshold for methanethiol is 0.02 ppb (Lindsay and Rippe, 1986), and it is very volatile, having a boiling point of 6 °C (Windholz et al., 1983). This high volatility may explain why the off-odor induced by anaerobic conditions in broccoli tended to dissipate upon aeration (Kasmire et al., 1974); Lipton and Harris, 1974).

A number of microorganisms have been reported to produce methanethiol (Kadota and Ishida, 1972; Sharpe et al., 1978). However, there have been few reports of methanethiol being produced in higher plants. From the results in this study it is not clear whether methanethiol is being produced by endogenous microorganisms or by the broccoli. Schmidt et al. (1985) have reported that

pumpkin (*Cucurbita pepo*) leaf disks emitted methanethiol when fed L-methionine or *S*-methyl-L-cysteine. In intact leaves, however, methanethiol was translocated down the petiole and was not emitted.

The production of dimethyl sulfide by anaerobic broccoli was probably the result of methanethiol undergoing oxidative condensation (Lindsay and Rippe, 1986). Dimethyl disulfide, like methanethiol, has a pronounced objectionable odor; however, its detection threshold is severalfold greater at about 12 ppb (Buttery et al., 1976; Lindsay and Rippe, 1986). The lower concentrations of dimethyl disulfide compared to that of methanethiol and its higher detection threshold indicate that its contribution to the off-odor produced by broccoli is secondary to that of methanethiol. Dimethyl disulfide has been identified in other crucifers, emanating from macerated bud and leaf tissues of *Brassica napus* (Tollsten and Bergstrom, 1988) and curd tissues of cauliflower (Wallbank and Wheatley, 1976).

Oxygen concentrations that induce the production of methanethiol appear to be influenced by the concentration of CO_2 present. Lipton and Harris (1974) found that off-odors developed when O_2 concentrations were $\leq 0.25\%$ when CO_2 was absent; however, in the presence of 10% CO_2 off-odors developed at an O_2 concentration of 1%. Similarly, Makhlouf et al. (1989) found that broccoli held in 10 or 15% CO_2 and 2.5% O_2 developed off-odors after 4 weeks at 1 °C. Oxygen concentrations between 1 and 2% in modified atmosphere packages induced off-odors when CO_2 levels were 10–15% (Ballantyne et al., 1988; Forney and Rij, 1991). Additional research is needed to determine the O_2 concentrations that will induce methanethiol production in broccoli under different CO_2 concentrations and storage times and temperatures.

In addition to the volatile sulfur compounds, anaerobic conditions induced the production of ethanol, ethyl acetate, acetaldehyde, methyl acetate, and acetone. These compounds are all fairly common products of anaerobic respiration (Karaoulanis, 1983). Lieberman and Spurr (1955) also reported that anaerobic broccoli produced ethyl acetate and acetaldehyde.

ACKNOWLEDGMENT

We thank Alisa Moore and David Buchanan for their technical assistance.

LITERATURE CITED

Ballantyne, A.; Stark, R.; Selman, J. D. Modified atmosphere packaging of broccoli florets. *Int. J. Food Sci. Technol.* **1988**, *23*, 353–360.

Buttery, R. G.; Guadagni, D. G.; Ling, L. C.; Seifert, R. M.; Lipton, W. Additional volatile components of cabbage, broccoli, and cauliflower. *J. Agric. Food Chem.* **1976**, *24*, 829–832.

Dateo, G. P.; Clapp, R. C.; MacKay, D. A. M.; Hewitt, E. J.; Hasselstrom, T. Identification of the volatile sulfur components of cooked cabbage and the nature of the precursors in the fresh vegetable. *Food Res.* **1957**, *22*, 440–447.

Elkashif, M. E.; Huber, D. J.; Sherman, M. Delaying deterioration of broccoli and cucumber using polymer films. *Proc. Fla. State Hortic. Soc.* **1983**, *96*, 332–335.

Farwell, S. O.; Gluck, S. J.; Blamesberger, T. M.; Schutte, T. M.; Adams, D. F. Determination of sulfur-containing gases by deactivated cryogenic enrichment and capillary gas chromatography. *Anal. Chem.* **1979**, *51*, 609–615.

Forney, C. F.; Rij, R. E. Temperature of broccoli florets at time of packaging influences modification of package atmosphere and quality. *HortScience* **1991**, in press.

Itoh, H.; Yano, M.; Okada, N.; Nikkuni, S. Study on volatile isothiocyanate detected in cultivar of *Brassica* vegetable. 2. Analysis of flavour of cabbage by gas-chromatography-mass spectrometry (sic). *Rep. Natl. Food Res. Inst.* **1985**, *47*, 41–48.

Kadota, H.; Ishida, Y. Production of volatile sulphur compounds by microorganisms. *Annu. Rev. Microbiol.* **1972**, *26*, 127–138.

Karaoulanis, G. The formation of enzymatic products in the fruits during growth and storage. In *Post-harvest Physiology and Crop Preservation*; Lieberman, M., Ed.; Plenum: New York, 1983; pp 231–245.

Kasmire, R. F.; Kader, A. A.; Klaustermeyer, J. A. Influence of aeration rate and atmosphere composition during simulated transit on visual quality and off-odor production by broccoli. *HortScience* **1974**, *9*, 228–229.

Lebermann, K. W.; Nelson, A. I.; Steinberg, M. P. Post-harvest changes of broccoli stored in modified atmospheres. 1. Respiration of shoots and color of flower heads. *Food Technol.* **1968**, *22*, 487–518.

Lieberman, M.; Spurr, R. A. Oxygen tension in relation to volatile production in broccoli. *Proc. Am. Soc. Hortic. Sci.* **1955**, *65*, 381–386.

Lindsay, R. C.; Rippe, J. F. Enzymic generation of methanethiol to assist in the flavor development of cheddar cheese and other foods. *ACS Symp. Ser.* **1986**, *No. 317*, 286–308.

Lipton, W. J.; Harris, C. M. Controlled atmosphere effects on the market quality of stored broccoli (*Brassica oleracea* L., Italica Group). *J. Am. Soc. Hortic. Sci.* **1974**, *99*, 200–205.

MacLeod, A. J.; MacLeod, G. Flavor volatiles of some cooked vegetables. *J. Food Sci.* **1970**, *35*, 734–738.

Makhlouf, J.; Castaigne, F.; Arul, J.; Willemot, C.; Gosselin, A. Long-term storage of broccoli under controlled atmosphere. *HortScience* **1989**, *24*, 637–639.

Rij, R. E.; Ross, S. R. Quality retention of fresh broccoli packaged in plastic films of defined CO_2 transmission rates. *Packag. Technol.* **1987**, *17* (3), 22–23.

Schmidt, A.; Rennenberg, H.; Wilson, L. G.; Filner, P. Formation of methanethiol by leaf tissue. *Phytochemistry* **1985**, *24* (6), 1181–1185.

Sharpe, M. E.; Law, B. A.; Phillips, B. A. Pitcher, B. A. Coryneform bacteria producing methanethiol from milk and cheese. In *Coryneform Bacteria*; Academic Press: London, 1978; Vol. 1, pp 289–300.

Tollsten, L.; Bergstrom, G. Headspace volatiles of whole plants and macerated plant parts of *Brassica* and *Sinapis*. *Phytochemistry* **1988**, *27* (7), 2073–2077.

Wallbank, B. E.; Wheatley, G. A. Volatile constituents from cauliflower and other crucifers. *Phytochemistry* **1976**, *15*, 763–766.

Wang, C. Y.; Hruschka, H. W. Quality maintenance in polyethylene-packaged broccoli. *Mark. Res. Rep.* **1977**, *No. 1085*.

Windholz, M., Budavari, S., Blumetti, R. F., Otterbein, E. S., Eds. *The Merck Index*, 10th ed.; Merck: Rahway, NJ, 1983.

Received for review August 9, 1991. Accepted September 18, 1991.

Registry No. Ethanol, 64-17-5; methanethiol, 74-93-1; hydrogen sulfide, 7783-06-4; ethyl acetate, 141-78-6; methyl disulfide, 624-92-0; methyl sulfide, 75-18-3; acetaldehyde, 75-07-0; methyl acetate, 79-20-9; acetone, 67-64-1.