Broccoli Storage under Low-Oxygen Atmosphere: Identification of Higher Boiling Volatiles

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Volatiles were isolated from broccoli stored under controlled atmospheres containing different levels of N_2, O_2, and CO_2. The volatiles were analyzed by capillary GLC and MS. The presence of the previously identified methanethiol, ethanol, ethyl acetate, and dimethyl disulfide in low oxygen stored broccoli was confirmed. Additional compounds, identified in the present work, increasing under these low oxygen conditions included 3-hydroxybutan-2-one, methyl thiocyanate, hexanal, (*E*)-2-hexenal, 3-methylbutanol, dimethyl trisulfide, and 21 other compounds. Major compounds include methanethiol, ethanol, ethyl acetate, 3-hydroxybutan-2-one, and methyl thiocyanate. The combination of threshold and concentration data indicated that the major contributors to the odor of the objectionable samples included methanethiol, dimethyl trisulfide, and β-ionone.

INTRODUCTION

Controlled-atmosphere storage of broccoli, by reducing the oxygen content and increasing the CO_2 content, has had some success in extending the life of broccoli (Lipton and Harris, 1974; Berrang et al., 1990). However, occasionally under certain conditions of storage, at oxygen levels ca. <0.5%, strong off-odors and off-flavors develop (Lipton and Harris, 1974). A recent study by Forney et al. (1991) identified ethanol, methanethiol, hydrogen sulfide, ethyl acetate, dimethyl disulfide, acetaldehyde, methyl acetate, and acetone in broccoli stored under a controlled atmosphere containing 0.5% oxygen. They considered the methanethiol primarily responsible for the off-odor. The present study identified higher boiling volatiles in order that some better understanding might be obtained on their contribution to off-odor in controlled-atmosphere stored broccoli.

EXPERIMENTAL PROCEDURES

Materials. Freshly harvested broccoli (*Brassica oleracea* var. Italica cv. Marathon) was obtained from Mann Packing Co., Salinas Valley, CA, during the months of February-April 1991. The broccoli heads were obtained on the same day of harvest at Davis, being transported in package-iced cardboard boxes and stored at 0 °C until the start of the experiment, usually the next day. All experiments were carried out with miniflorets cut from the upper 2.5 cm of the broccoli head. They were surface sterilized by washing with distilled water containing 50-100 ppm of NaOCl.

Controlled-Atmosphere Treatment. Samples (500-650 g) were placed in 3.8-L glass jars which were closed with a neoprene rubber stopper containing the inlet and outlet $^1/_4$-in. o.d. polyethylene tubes used for introducing the flow of controlled-atmosphere gases. In most later experiments the stopper was covered with aluminum foil to minimize the effect of volatiles from the neoprene. In some earlier experiments, however, the stoppers were used uncovered. A blank run, made with the glass jar, uncovered rubber stopper, and polyethylene tubing showed a negligibly small amount of background volatiles, which were analyzed by GLC-MS and taken into account.

The humidified gas mixtures were established by controlling air, nitrogen, and carbon dioxide flows through different size capillary tubes and verified by gas chromatography. The first group of experiments was carried out by holding the containers at 7.5 °C for 12 days with a gas flow of 5.1 L h^{-1} kg^{-1} and holding the treated broccoli in air for ca. 8-10 h before isolation of the volatiles. A second group of experiments was carried out using 10 °C for 7 days with a gas flow of 15.6 L h^{-1} kg^{-1}. A third group used the same conditions as the second but included flushing the containers with air for 2 more days without change of flow or temperature before the broccoli was analyzed. The gas mixtures used were as follows: (1) 100% N_2; (2) air; (3) 20% CO_2 in N_2; (4) 0.5% O_2 in N_2; (5) 0.5% O_2, 20% CO_2 in N_2.

All experiments were carried out in the dark.

Isolation of Volatiles. This was carried out ca. 5-6 h after the treatments were stopped. The broccoli miniflorets (500-650 g) from each of the test experiments were placed in a modified (ca. 5 L) clean Pyrex glass vacuum desicator containing a standard 34/45 conical glass joint in the lid. A suitable Pyrex glass head was fitted to this joint which allowed purified air (3 L/min flow rate) to enter the flask, pass over the broccoli pieces, and exit through a Tenax trap (Tenax GC, 60-80 mesh, 10 g, 14 cm × 2.2 cm i.d.). The isolation was carried out for 3 h at 25 °C and the trap then removed and eluted with 100 mL of freshly distilled diethyl ether (containing ca. 0.001% ethyl antioxidant 330). The ether extract was then concentrated to ca. 100 μL with a warm-water bath and Vigreux distillation column. The trap was regenerated by passing a stream of nitrogen through it for 1 h with the trap at 180-200 °C.

Capillary GLC Analysis. This was carried out using a HP 5890 gas liquid chromatograph (GLC) using a 60 m × 0.32 mm i.d. fused silica capillary column wall coated with bonded methyl silicone, DB-1. The GLC conditions were 25 min at 30 °C, raised at 4 °C/min to 200 °C, and finally 20 min at the upper limit. The injector temperature was 170 °C, and the carrier gas flow velocity was 22 cm/s. Sample size was 1 μL split 1/20.

Capillary GLC-MS Conditions. GLC-MS studies were carried out with both a 60 m long × 0.25 mm i.d. fused silica capillary coated with DB-1 and a column of the same dimensions but coated with DB-Wax. The GLC conditions were as described above. The mass spectrometer was a HP 5970 quadrupole directly coupled to the gas chromatograph described above.

For very volatile compounds GLC-MS studies were also carried out using direct injection of 5 mL of headspace gases into a 30 m long × 0.32 mm i.d. fused silica capillary coated with DB-1 and directly coupled to a modified Consolidated 21-620 mass spectrometer.

Authentic Samples. These were obtained from reliable commercial sources or synthesized according to established methods. Compounds were purified by GLC separation and their identities checked by spectral methods (MS or IR).

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Table I. Volatiles Identified in Broccoli Stored in Low-Oxygen Atmospheres

compound[a]	major MS ions[b]	KI DB-1[c]
methanethiol	47, *48*, 45, 44, 33	0430
ethanol	31, 45, *46*, 43	0440
ethyl acetate	43, 29, 45, *88*	0600
methyl thiocyanate	*73*, 45, 72, 46, 58	0665
methyl thioacetate	43, *90*, 45, 75, 59	0671
3-hydroxybutan-2-one	45, 43, *88*, 73	0674
dimethyl disulfide	*94*, 45, 79, 61, 35	0722
3-methylbutanol	55, 42, 70, 31, 57	0714
2-methylbutanol	57, 41, 31, 70, 56	0718
pentanol	42, 55, 70, 31	0740
(*E*)-2-pentenol	57, 41, 44, 67, *86*	0744
2,3-butanediol	45, 57, 31, 75, *90*	0746
(*Z*)-3-hexenal	41, 55, 69, 83, *98*	0769
hexanal	44, 56, 29, 72, 82	0772
2-(methylthio)ethanol	61, *92*, 45, 35, 47	0813
(*E*)-2-hexenal	41, 55, 69, 83, *98*	0822
(*Z*)-3-hexenol	41, 67, 55, 31, 82	0834
(*E*)-2-hexenol	57, 41, 31, 82, 67, *100*	0844
hexanol	56, 43, 31, 69, 84	0848
(*E,E*)-2,4-hexadienal	81, 41, *96*, 53, 67	0876
benzaldehyde	77, 105, 51, 39, 63	0926
dimethyl thiosulfinate[d]	47, 64, *110*, 95, 79	0930
dimethyl trisulfide	*126*, 45, 79, 64, 111, 32	0941
(*Z*)-5-octen-2-one	43, 68, 55, 97, 83, *126*	955
(*E,Z*)-2,4-heptadienal	81, 39, 53, 67, *110*, 95	979
(*E,E*)-2,4-heptadienal	81, 39, 53, 67, *110*, 95	0979
ethyl hexanoate	43, 88, 99, 60, 71, 115	0981
phenylacetaldehyde	91, *120*, 65, 39, 51, 77	1006
(*E,E*)-3,5-octadien-2-one	43, 95, 81, 53, *124*, 109	1063
guaiacol	109, *124*, 81, 53, 39	1830
methyl salicylate	120, *152*, 92, 39, 65, 53	1166
ethyl octanoate	88, 57, 43, 101, 73, 127	1180
β-cyclocitral	137, 41, 109, 81, 67, *152*	1194
β-ionone	177, 43, 122, 91, 135, 53	1462
dihydroactinodiolide	111, 137, 43, *180*, 67, 124	1480

[a] Mass spectrum and GLC Kovats retention index consistent with that of authentic sample unless otherwise noted. [b] Major ion each 14 mass units (or a sufficient number for characterization in the case of lower MW compounds) found with most intense ions first. The molecular ions are italicized. [c] Kovats retention index found on DB-1 GLC capillary. [d] Tentative identification. Mass spectrum consistent with published data but no authentic sample available.

Odor Threshold Determinations. These were carried out on the purified authentic samples described above. The methods used were similar to that previously described by some of the authors (Buttery et al., 1971) with a panel of 16–20 judges.

RESULTS AND DISCUSSION

Table I lists the volatiles identified in the concentrates isolated from broccoli stored under low-oxygen conditions using a dynamic headspace Tenax trapping procedure similar to that used for other products (Buttery et al., 1988). Table I also includes identification from a separate study for very volatile compounds where headspace gases were directly injected onto a capillary column for GLC–MS. The major components identified from direct injection of headspace gases included methanethiol, ethyl alcohol, and ethyl acetate, confirming the findings of Forney et al. (1991). Major components identified using the Tenax trap procedure included dimethyl disulfide, acetoin, methyl thiocyanate, hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenol, (*E*)-2-hexenol, hexanol, and dimethyl trisulfide. The most unexpected compounds identified included *β*-ionone, *β*-cyclocitral, and dihydroactinodiolide, which apparently come from the carotenoid part of the broccoli. The dimethyl disulfide and trisulfide could result from normal air oxidation of methanethiol. Only very small amounts of dimethyl sulfide were detected by Forney et al. (1991), and this compound was below the level of detection in the present study, although it is a major component of cooked broccoli (Buttery et al., 1976).

Table II. Relative Amounts of Compounds Isolated from Broccoli Stored under Different Atmospheric Conditions Expressed in Terms of Concentration[a]

compound	concn found, ppb				
	air	0.5% O_2, 20% CO_2	0.5% O_2, 0% CO_2	0% O_2, 20% CO_2	100% N_2
methyl thiocyanate	<0.2	2	21	4	220
3-hydroxybutan-2-one	1	4	35	150	720
dimethyl disulfide	1	3	60	600	170
3-methylbutanol	0.3	3	25	56	86
2-methylbutanol	0.1	3	18	13	33
pentanol	0.2	<0.1	<0.1	3	33
(*E*)-2-pentenol	<0.1	<0.1	<0.1	<0.1	34
2,3-butanediol	<0.1	0.5	8	5	6
(*Z*)-3-hexenal	0.5	0.4	2	5	17
hexanal	0.1	0.06	<0.1	<0.1	92
2-(methylthio)ethanol	<0.1	0.3	<0.1	3	1
(*E*)-2-hexenal	<0.1	0.3	2	20	80
(*Z*)-3-hexenol	3	3	12	27	47
(*E*)-2-hexenol	0.2	0.2	<0.1	<0.1	30
hexanol	<0.1	0.1	<0.1	4	29
(*E,E*)-2,4-hexadienal	<0.1	<0.1	<0.1	<0.1	<0.1
benzaldehyde	0.1	0.1	0.4	1	22
dimethyl thiosulfinate	0.1	<0.1	<0.1	<0.1	4
dimethyl trisulfide	0.1	0.3	5	98	26
(*Z*)-5-octen-2-one	0.5	0.4	<0.1	0.3	3
(*E,Z*)-2,4-heptadienal	0.3	0.1	0.1	1	19
(*E,E*)-2,4-heptadienal	0.1	1.5	23	0.2	12
ethyl hexanoate	1	0.2	0.6	2	3
phenylacetaldehyde	0.1	0.5	0.2	0.3	3
(*E,E*)-3,5-octadien-2-one	0.2	<0.1	0.6	1	5
guaiacol	2	<0.1	1	18	3
methyl salicylate	0.3	<0.1	<0.1	0.2	<0.1
ethyl octanoate	<0.1	0.1	0.3	0.4	1
β-cyclocitral	0.8	0.2	0.6	0.2	7
β-ionone	0.1	2	2	3	7
dihydroactinodiolide	0.1	0.5	0.4	0.4	7

[a] Storage conditions: Samples in first four columns were stored at 10 °C for 7 days and with the gas mixture flowing through the container at 15.6 L h^{-1} kg^{-1}. The sample in the fifth column was stored at 7.5 °C for 12 days and had a gas mixture flow rate of 5.1 L h^{-1} kg^{-1}.

Except for ethanol, methanethiol, ethyl acetate, and dimethyl sulfide [reported by Forney et al. (1991)], the compounds in Table I had not been reported as occurring in broccoli stored under low-oxygen atmosphere conditions. Some of the compounds had been reported previously in cooked broccoli [e.g., Buttery et al. (1976)]. These included the C_6 aldehydes and alcohols (common in green plants), dimethyl disulfide and trisulfide, and (*E,Z*)- and (*E,E*)-2,4-heptadienal. Alkyl isothiocyanates are well-known in brassica vegetable (Kjaer, 1958), but the presence of methyl thiocyanate is somewhat unusual. It has a mass spectrum very similar to that of methyl isothiocyanate, but they have quite different GLC retention Kovats indices (KI).

Quantitative Data. In addition to the qualitative analysis, some idea of the concentrations of the compounds was also obtained from comparison of GLC peak areas and consideration of the original weight of broccoli used. This is shown in Table II. These data represent the amounts of compound isolated from the broccoli. It would be difficult to determine whether these concentrations actually exist in the broccoli or whether there is a production of the compounds occurring during the isolation. Also, little is known about the recovery of compounds from the surface of materials such as broccoli. However, the data shown are probably of the right order of magnitude and are useful for comparison purposes. Table II allows such a comparison based on the different methods of controlled-atmosphere storage.

Although not shown in the table, separate studies showed maximum concentrations (based on wet weight of broccoli) of 130 ppm for ethanol and 9 ppm for acetaldehyde and

Table III. Changes in Concentration of Some Broccoli Volatiles with Samples Aerated for 2 Days after Low-Oxygen Atmosphere Storage

	concn, ppb					
	0.5% O_2, 0% CO_2		0.5% O_2, 20% CO_2		0% O_2, 20% CO_2	
compound	no aer	aer	no aer	aer	no aer	aer
methyl thiocyanate	21	2	2	0.3	4	13
3-hydroxybutan-2-one	35	0.5	4	0.4	150	470
dimethyl disulfide	60	160	3	3	600	160
dimethyl trisulfide	5	0.8	0.3	0.1	98	35
β-ionone	2	0.6	2	0.5	3	3

Table IV. Odor Thresholds of Some of the Identified Compounds in Water Solution and Approximate Log Odor Unit (Uo) Values for Broccoli Sample Stored under 100% Nitrogen

compound	threshold, ppb	approx log Uo
methanethiol	0.02	5.7
ethanol	100000	0.1
ethyl acetate	5000	0.3
methyl thiocyanate	10	1.3
3-hydroxybutan-2-one	800	-0.05
dimethyl disulfide	12	1.2
3-methylbutanol	250	-0.5
pentanol	4000	-2.1
(Z)-3-hexenal	0.25	1.8
hexanal	4.5	1.3
2-(methylthio)ethanol	120	-1
(E)-2-hexenal	17	0.7
(Z)-3-hexenol	70	-0.2
(E)-2-hexenol	400	-1
hexanol	500	-1
(E,E)-2,4-hexadienal	60	-2.8
benzaldehyde	350	-1.2
dimethyl trisulfide	0.01	3.4
(Z)-5-octen-2-one	30	-1
ethyl hexanoate	1	0.5
phenylacetaldehyde	4	-0.1
(E,E)-3,5-octadien-2-one	150	-1.5
guaiacol	3	0
methyl salicylate	40	-2.6
β-cyclocitral	5	0.15
β-ionone	0.007	3.0

of the order of 10 ppm each for ethyl acetate and methanethiol for storage of broccoli under 100% nitrogen for 2–9 days.

Aeration after Low-O_2 Storage. In a third group of experiments aeration was used for 2 days after the low-oxygen storage to test whether there was a loss in the off-flavor volatiles. The change in concentration for several volatiles is shown in Table III. It can be seen that even though in general there is a significant loss in most compounds, there is still as much as one-third of the dimethyl trisulfide remaining, which is still many times the concentration found in samples stored in air. The increases with some compounds may reflect the random variation commonly found in plant samples or may indicate that some deterioration processes initiated by the low-oxygen storage still continue.

Odor Properties. Odor thresholds of identified components determined in water solution are listed in Table IV. Most of these had been determined in previous studies by some of the authors [e.g. Buttery et al. (1976)]. It can be seen that, of the compounds listed, the most potent odorants are methanethiol, dimethyl trisulfide, β-ionone, (Z)-3-hexenal, ethyl hexanoate, phenylacetaldehyde, guaiacol, and β-cyclocitral. Some of the authors have used the ratio of the concentration of the compound in the food to the compound's odor threshold concentration in water (for largely aqueous foods) to give some idea of the relative importance of components to the total odor. This ratio has been called odor units (Uo) and, for mostly aqueous foods, is approximately equal to the threshold concentration of that compound in the food. If a compound's Uo value is less than 1, it would be present below its threshold and there is a low probability of the compound contributing to the odor. It seems reasonable that the larger the number of odor thresholds of a compound present, the greater the probability of its contributing to the odor. The log Uo values calculated for the broccoli components are also listed in Table IV for the sample stored at 7.5 °C under nitrogen for 12 days. The estimates used for the concentrations of methanethiol and ethyl acetate were only very rough but are probably of the correct order of magnitude. The accuracy of the Uo values is of course dependent on the accuracy of the quantitative data and the threshold data. However, as long as these values are of the right order of magnitude, the relative log values would not be affected very much; this kind of comparison should give us some idea of which compounds are the most important. It can be seen that removal of 90% of the methanethiol would still leave the broccoli a very high methanethiol Uo value, but even complete removal of methanethiol would still leave higher boiling compounds such as dimethyl trisulfide which could contribute off-odors in broccoli.

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Registry No. O_2, 7782-44-7; methanethiol, 74-93-1; ethanol, 64-17-5; ethyl acetate, 141-78-6; methyl thioacetate, 1534-08-3; 2-methylbutanol, 137-32-6; (E)-2-pentenol, 1576-96-1; 2,3-butanediol, 513-85-9; dimethyl thiosulfinate, 13882-12-7; (E,Z)-2,4-heptadienal, 4313-02-4; (E,E)-2,4-heptadienal, 4313-03-5; ethyl octanoate, 106-32-1; dihydroactinodiolide, 17092-92-1; methyl thiocyanate, 556-64-9; 3-hydroxybutan-2-one, 513-86-0; dimethyl disulfide, 624-92-0; 3-methylbutanol, 123-51-3; pentanol, 71-41-0; (Z)-3-hexenal, 6789-80-6; hexanal, 66-25-1; 2-(methylthio)-ethanol, 5271-38-5; (E)-2-hexenal, 6728-26-3; (Z)-3-hexenol, 928-96-1; (E)-2-hexenol, 928-95-0; (E,E)-2,4-hexadienal, 142-83-6; hexanol, 111-27-3; benzaldehyde, 100-52-7; dimethyl trisulfide, 3658-80-8; (Z)-5-octen-2-one, 22610-86-2; ethyl hexanoate, 123-66-0; phenylacetaldehyde, 122-78-1; (E,E)-3,5-octadien-2-one, 30086-02-3; guaiacol, 90-05-1; methyl salicylate, 119-36-8; β-cyclocitral, 432-25-7; β-ionone, 79-77-6.