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Suppression of Undesirable Sulfurous Aromas of Cruciferous Vegetables with Caraway Sulfhydryl Oxidase

Ki-Hwan Shim and R.C. Lindsay*

Department of Food Science and Technology, Gyeongsang National University, Korea
**Department of Food Science, University of Wisconsin, Madison, U.S.A.*

Abstract

Aromas of sulfur-containing volatiles from two vegetable varieties of *Cruciferae Brassica oleracea* and the suppression of undesirable sulfurous aromas of cruciferous vegetables by sulfhydryl oxidase of caraway seeds were examined. Aroma components were separated by gas chromatography equipped with a dual flame photometric detector. The volatile sulfides produced from cabbage and broccoli varied, in the relative quantities and rates of production, according to the amount of caraway seeds added and incubation time. The amount of methanethiol and dimethyl disulfide in the cabbage and broccoli with caraway seeds was far less than those in the cabbage and broccoli. Removal of methanethiol and dimethyl disulfide was proportional to the amount of caraway seeds added, and was remarkable with 2.5% aqueous slurries of caraway seeds added.

Key words: sulfhydryl oxidase, caraway seeds, sulfur-containing volatiles

Introduction

The cruciferous vegetables are characterized by a pungent sulfuraceous flavor, which arises due to slicing, crushing, chopping, and cooking, etc. The characteristic flavors of cruciferous vegetables have been attributed to volatile sulfur-containing compounds such as hydrogen sulfide, carbonyl sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide, allyl isothiocynate, and allyl cyanide. These have been reported to be abundant components of cruciferous vegetable volatiles[1-3]. The pungent nature is both due to enzymic and non-enzymic reactions[4,5]. The isothiocyanates are released from glucosinolates enzymically while other volatiles such as sulfides, thiazole, etc. by non-enzymic reactions. The presence of pungent flavor in food does not always ensure a desirable aroma. These can also directly and indirectly influence the health-associated attributes of foods[6,7]. A better understanding of the development of cruciferous vegetable flavor is necessary to enable the control and manipulation of cruciferous vegetable flavor. It is believed that the elimination of cruciferous vegetable aroma was caused by volatile suppression rather than a masking effect of the spice. In 1967, sulfhydryl oxidase activity was reported in raw milk, which was observed to catalyze the oxidation of cysteine, gluthathione and cysteine-containing proteins[8]. This enzyme was first purified in bovine milk and found to be a metallo-glycoprotein; containing iron and 11-15% carbohydrate[9]. In recent years, biochemical studies of enzymes which catalyze thiol: protein-disulfide interchange have been reported[10]. Some of these enzymes catalyze the oxidation of sulfhydryl groups in protein using small peptide disulfides as electron acceptors, while others catalyze the reduction of disulfides in proteins in the presence of small thiols[10,11]. Subsequent purification and characterization of these enzymes have been the object of continuing investigations[12-14], and the general range of substrate activity for these enzymes suggests its potential for industrial utilization[15,16]. In this paper, we describe the suppression of undesirable sulfurous aromas of cruciferous vegetables with caraway seeds via sulfhydryl oxidase activity which catalyzes the oxidation of thiol groups.

Corresponding author: Ki-Hwan Shim, Department of Food Science and Technology, Gyeongsang National University, #900 Kaja-Dong, Chinju 660-701, Korea

Materials and Methods

Materials

Cabbage (*Brassica oleracea capitata* L.), broccoli (*Brassica oleracea var. italica*) and caraway (*Carum carvi* L.) seeds used in these studies were purchased

from the local market. Methanethiol was obtained from Eastman Kodal Co. (Rochester, NY). Carbonyl sulfide was obtained from Matheson Gas Products (East Rutherfold, NJ). Dimethyl sulfide, dimethyl disulfide, carbon disulfide, sodium sulfide and phosphoric acid were obtained from Aldrich Chemical Co., INC. (Milwaukee, WI). Hydrogen sulfide was synthesized from sodium sulfide and phosphoric acid.

Preparation and quantitative analyses of headspace samples

Samples for analysis of sulfur-containing volatiles were prepared by blending 35g of cabbage or broccoli (ca. 2 cm long) with 70 m*l* distilled water in a Waring blendor (30s), with or without 2.5% ground caraway seeds ground for cabbage or broccoli, to which was added internal standard (17.5 μl of 1% carbon disulfide). And samples for investigation on removal of thiols were prepared by 100 m*l* of 0.1-2.5% aqueous slurries of caraway seeds, ground and blended with water (10:1, w/w) at high speed for 3 min., with 50 μl of 1% methanethiol and dimethyl disulfide. Samples were placed in 125 m*l* Erlenmeyer flask with a glass arm and stopcock, and then set in an incubator at 30 °C (Fig. 1). A gas sampling syringe was inserted through the rubber septum and pumped in and out 9 times and a 3 m*l* sample was withdrawn for immediate injection into gas chromatography and gas chromatography-mass spectrometry. Relative concentrations of volatiles in cabbage and broccoli were calculated by measuring peak area and dividing by the peak area of the internal standard without caraway seeds, depending on the position of the compounds on the chromatogram, and the ratio of removal of methanethiol and dimethyl disulfide was calculated by calibration curve of standard compounds.

Gas chromatography and gas chromatography-mass spectrometry

A Tracor MT-220 gas chromatograph (Micro Tek Instruments Corp. Austin, TX) equipped with dual channel electrometers and a Melpar flame photometric detector (FPD) combined with a flame ionization detector (FID) was used. The effluent stream from the column was detected simultaneously by the FPD and FID. An omniscribe dual pen strip chart recorder (Model B-5218-5, Houston Instruments, Austin, TX) recorded output from each the FPD and FID detectors. A 2 m × 4 mm i.d. glass column packed with 40/60 mesh Carbopack BHT-100 (Supelco, INC., Bellefonte, PA) was used. The column temperature was programmed from 60 to 130 °C at a rate of 25 °C/min. Injection port and detector temperature were 140 °C. High purity nitrogen carrier gas at a flow rate of 40 m*l*/min was passed through an Alltech gas purifier (Deerfield, IL) before entering the column. For the FID/FPD flame, hydrogen of 126 m*l*/min, oxygen of 11 m*l*/min and air of 53 m*l*/min were used. A Finnigan 4021 GC-MS system equipped with a PPINICI (pulsed positive ion, negative ion chemical ionization) analyzer and an INCOS 2300 data system (Finnigan instruments, Sunnyvale, CA) was used to indentify the headspace volatile components. Ion source operating temperatures were maintained at 250 °C (EI) and ionization voltage was 70 eV during scanning. Capillary column analyses were made with splitless injection (activated 0.5 min after injection) on a Supelcowax 10 (60 m × 0.32 mm i.d.) fused silica capillary column using helium carrier gas (head pressure 10 psi, split 50 m*l*/min, sweep 5 m*l*/min) and a program rate of 4 °C/min from 40 to 150 °C.

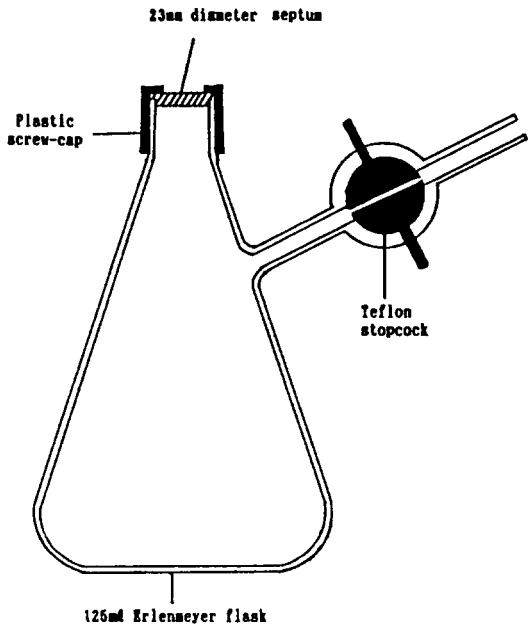


Fig. 1. Cross-sectional view of evacuation falsk

Results and Discussion

The volatiles of cabbage and broccoli were collected by the headspace method using 125 m*l* Eylenmeyer flask with a glass arm and stopcock, and determined by gas chromatography. Gas chromatograms of volatile sulfur-containing compounds are shown in Fig. 2. Five volatiles were detected and their identities confirmed by comparison with the retention times of known volatile compounds and by mass spectrometry. The volatile compounds were hydrogen sulfide, carbonyl sulfide, methanethiol, dimethyl sulfide and dimethyl disulfide. The amounts of the volatiles in cabbage and broccoli were contained dimethyl disulfide and carbonyl sulfide more than others, and those volatiles were higher in broccoli than in cabbage.

Table 1 shows the results of gas chromatographic analyses of sulfur-containing volatiles, low boiling compounds in the cabbage and broccoli with or without caraway seeds.

A considerable difference was observed among the four samples in the amount of the volatile sulfur-containing compounds. The main difference between volatile sulfides produced from cabbage and broccoli were the relative quantities and rates of production of hydrogen sulfide, carbonyl sulfide, methanethiol and dimethyl disulfide according to caraway seeds added and their incubation time. The most striking difference was in the concentration of dimethyl disulfide according to incubation time in the broccoli. The amount of dimethyl disulfide incubated for 24 hrs contained 22.399 ppm, approximately 1,100 times as much as in the incubation for 30 min. Methanethiol and dimethyl disulfide were also found in significant concentration in the four samples, but hydrogen sulfide and carbonyl sulfide were notably absent or insignificant. The amount of methanethiol and dimethyl disulfide in the cabbage and broccoli with caraway seeds was far less than those in the cabbage and broccoli. Dimethyl sulfide is a well established aroma compound in the cruciferous vegetables, but not detected in this current study.

The flavor compounds in the cruciferous vegetables were formed through enzymic processes in disrupted tissues and through cooking. The fresh flavors of the disrupted tissue were caused mainly

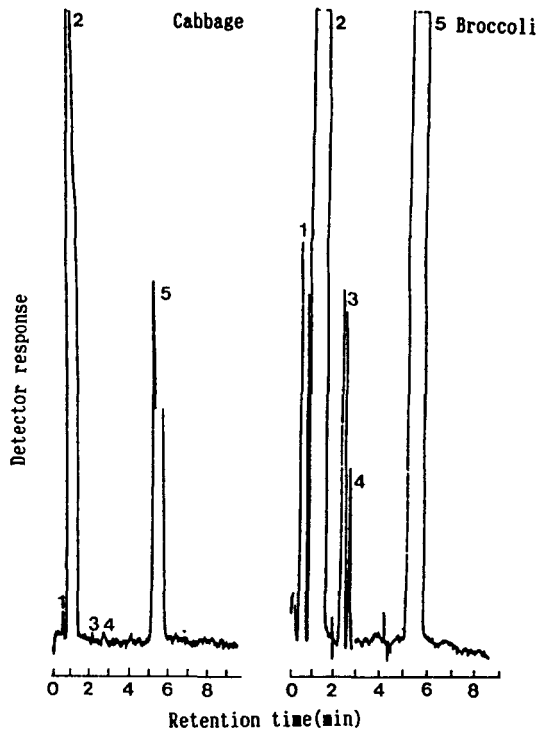


Fig. 2. Gas chromarograms of volatile sulfur-containing compounds in cabbage and broccoli

The compounds identified are: 1 = hydrogen sulfide; 2 = carbonyl sulfide; 3 = methanethiol; 4 = dimethyl sulfide; 5 = dimethyl disulfide.

by isothiocyanates resulting from the action of glucosinolates on thioglycoside precurosors[6,17]. The formation of hydrogen sulfide and carbonyl sulfide was as a result of hydrolysis of isothiocyanates[1]. The fresh and cooked flavors such as hydrogen sulfide, carbonyl sulfide, methanethiol and dimethyl disulfide from cabbage and broccoli were reported[18,19]. Generation of methanethiol food systems was believed to become largely from the degradation of sulfur-containing amino acids by either chemical or enzymatic reaction[20,21]. Chemical deterioration of L-methionine via the Strecker degradation had been most recently investigated in relation to food flavors[20]. Methionine in the presence of a diketone such as pyruvaldehyde, glucosamine, or ninhydrin would form methional, which then broke down to methanethiol and acrolein[22]. The formation of methional, as a result of Strecker degradation of methionine, was well known, and methionine, on dry heating, also produced methane-

Table 1. Effects of caraway sulfhydryl oxidase on volatile sulfides of cabbage and broccoli (unit: ppm)

Samples[a]	Incubation time(hrs)	Sulfur compounds			
		Hydrogen sulfide	Carbonyl sulfide	Methanethiol	Dimethyl disulfide
Cabbage	0.5	0.037	0.062	0.277	0.064
	1	0.044	0.130	0.578	0.929
	3	0.044	0.585	0.010	4.872
	6	0.026	0.999	ND[c]	7.219
	24	T[b]	0.151	11.902	19.335
Cabbage + Caraway	0.5	0.040	0.057	0.009	ND
	1	0.029	0.115	0.010	ND
	3	0.028	0.131	ND	T
	6	0.021	0.195	ND	0.020
	24	T	0.149	5.237	2.809
Broccoli	0.5	0.025	1.940	0.014	0.021
	1	0.024	2.195	T	0.216
	3	0.017	6.667	ND	3.842
	6	0.007	12.372	ND	4.061
	24	T	11.255	7.224	22.399
Broccoli + Caraway	0.5	0.028	1.235	T	ND
	1	0.026	1.718	T	ND
	3	0.018	2.167	ND	0.109
	6	0.016	5.936	ND	0.170
	24	T	2.042	1.372	8.337

[a] Sampling bottles were used 125 m*l* Erlenmeyer flask with a glass arm and stopcock, and set in incubator at 30 °C. Caraway seeds were added at a concentration of 2.5% for cabbage and broccoli.
[b] T = Present but not quantitated.
[c] ND = Not detected.

thiol and dimethyl disulfide[23]. Hydrogen sulfide and methanethiol were obtained as a result of the reaction between the naturally occuring dehydroascorbic acid and sulfurous amino acids, such as cysteine, cystine and methionine[24]. The enzymic hydrolysis of S-methyl-l-cysteine sulfoxide, found in cabbage[25], was reported to be the main precursor of dimethyl disulfide in cabbage[18]. In control of the cabbage and broccoli, the amount of hydrogen sulfide, methanethiol and dimethyl disulfide was obtained far more than in the cabbage and broccoli with caraway seeds in this study. It had been reported that in the enzyme-inhibited extracts of cabbage, only small amount of carbonyl sulfide and dimethyl disulfide were detected as sulfur-containing components of low boiling temperature, whereas in the uninhibited extracts, the amount of carbonyl sulfide and dimethyl disulfide increased[26].

The amount of methanethiol in the four samples was decreased up to around 6 hrs on incubation and then increased, whereas that of dimethyl disulfide increased rapidly in the cabbage and broccoli without caraway seeds and increased slowly in the cabbage and broccoli with caraway seeds. The amount of methanethiol and dimethyl disulfide during incubation in the cabbage and broccoli with caraway seeds was markedly less than in the cabbage and broccoli without caraway seeds (Table 1). Removal of methanethiol and dimethyl disulfide from aqueous slurries of caraway seeds via thiol oxidase activity during incubation was effective (Fig. 3 and 4). The rate of removal of methanethiol and dimethyl disulfide was proportional to the amount of caraway seeds added and was more remarkable at the concentration of 2.5% aqueous slurries of caraway seeds.

The object of our studies was to examine the stoichiometry of the reaction catalyzed. Caraway seeds sulfhydryl oxidase catalyzed the aerobic oxidation of thiol-containing compounds. The stoichiometry of the reaction[9] is

$$2RSH + O_2 \rightarrow RSSR + H_2O_2$$

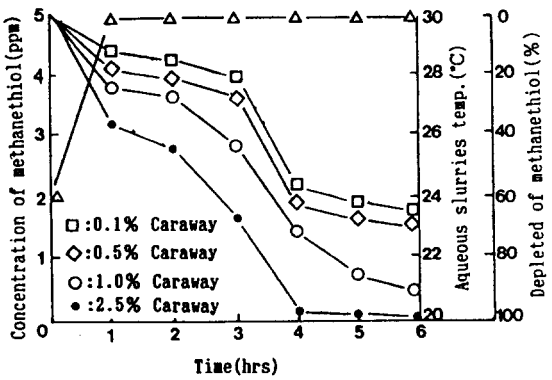


Fig. 3. Removal of methanethiol from aqueous slurries of caraway seeds via thiol oxidase activity

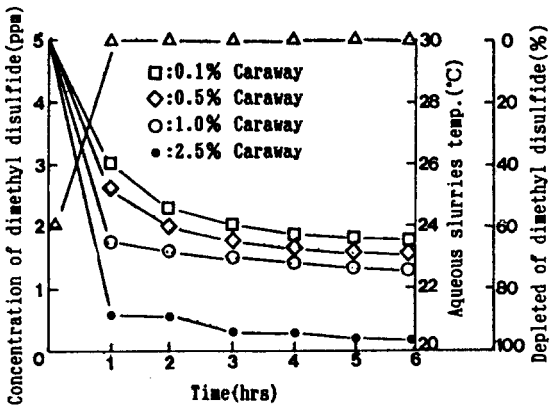
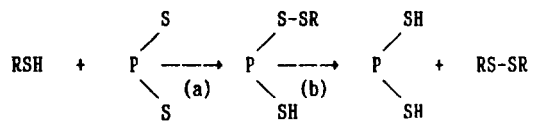


Fig. 4. Removal of dimethyl disulfide from aqueous slurries of caraway seeds via thiol oxidase activity

Sulfhydryl oxidase was found to catalyze the oxidation of sulfhydryl groups in both small compounds and proteins, using O_2 as oxidant and producing, in equimolar quantities, H_2O_2 and the corresponding disulfide[9]. The nature of the enzyme-catalyzed reaction had been characterized with respect of protein folding[27].

Depending on the nature of the thiol compounds with caraway sulfhydryl oxidase, thiol: protein-disulfide interchanges was shown that the product was mixed disulfide and protein thiol (reaction a) and further reaction with a low moleculor weight thiol led to complete reduction of the protein disulfide (reaction b).



Substantial evidence indicated the interchange of thiols and disulfides had a fundamental role in regulation of metabolism and cell function[28]. The interacellular thiol: disulfide ratio was typically maintained very high as compared to the extracellular ratio[29]. Sulfhydryl oxidase was isolated and purified from bovine skim milk[9] and had concluded that the enzyme was bound to skim milk membrane vesicles[30]. This enzyme exhibited a much broader substrate specificity than that shown by other aerobic oxidases presently characterized, catalyzing the formation of disulfide from thiols. Using molecular oxygen as an electron reduction to hydrogen peroxide, the enzyme catalyzed oxidation of cysteine and its analogues, some volatile thiols, peptide, and also thiols in proteins[16]. Furthermore, the enzyme catalytic activity of this enzyme suggested a potential for industrial utilization[16,31]. Due to their extremely low organoleptic threshold concentrations, thiols were often responsible for undesirable off-flavors in foods. It was belived that such an aroma modification could be achieved by treatment with the enzyme sulfhydryl oxidase[31]. In conclusion, caraway seeds via sulfhydryl oxidase activity were effective in the suppression of undesirable sulfurous aromas. The reduction of methanethiol and dimethyl disulfide was shown to occur in the cabbage, broccoli, and the solution of cruciferous vegetable volatiles could be achieved through the addition of caraway seeds sulfhydryl oxidase.

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References

1. Bailey, S.D., Bazient, M.L., Driscoll, J.L. and McCarthy, A.I.: The volatile sulfur components of cabbage. *J. Food Sci.*, **26**, 163 (1961).
2. Buttery, R.G., Guadagni, D.G., Ling, L.C., Seifert, R.M. and Lipton, W.: Additional volatile components of cabbage, broccoli and cauliflower. *J. Agric. Food Chem.*, **24**, 829 (1976)
3. Uda, Y. and Maeda, Y.: Volatile constituents occuring in autolyzed leaves of three cruciferous vegetables. *Agric. Biol. Chem.*, **50**, 205 (1986)

4. Schwimmer, S.: Alteration of the flavor of processed vegetables by enzyme preparations. *J. Food Sci.*, **28**, 460 (1963)
5. West, L.G., Badenhop, A.F. and McLaughlin, J.L.: Allyl isothiocyanate and allyl cyanide production in cell-free cabbage leaf extracts, shredded cabbage and cole slaw. *J. Agric. Food Chem.*, **25**, 1234 (1977)
6. Tookey, H.L., Van Etten, C.H. and Daxenbichler, M.E.: Glucosinolates. In *Toxic constituents of plant foodstuffs*, Liener, I.E. (ed.), Academic press, New York. p.103 (1980)
7. Van Etten, C.H., Daxenbichler, M.E., Tookey, H.L., Kwolek, W.F., Williams, P.H. and Yoder, O.C.: Glucosinolates: potential toxicants in cabbage cultivars. *J. Amer. Soc. Hort. Sci.*, **105**, 710 (1980)
8. Kiermeier, F. and Petz, E.: Uber ein sulfhydryl gluppenoxydierendes Enzym in der Milch. *Z. Lebensm. Unters. Forsch.*, **132**, 342 (1967)
9. Janolino, V.G. and Swaisgood, H.E.: Isolation and characterization of sulfhydryl oxidse from bovine milk. *J. Biol. Chem.*, **250**, 2532 (1975)
10. Freedman, R.B.: How many distinct enzymes are responsible for the several cellular processes involving thiol: protein-disulfide interchange? *FEBS LETTERS*, **97**, 201 (1979)
11. Morin, J.E., Carmichael, D.F. and Dixon, J.E.: Characterization, kinetics and comparative properties of thiol: protein disulfide oxidoreductase. *Arch. Biochem. Biophys.*, **189**, 354 (1978)
12. Janolino, V.G. and Swaisgood, H.E.: A simplified method of sulfhydryl oxidase isolation. *J. Dairy Sci.*, **62**, 64 (1979)
13. Sliwkowski, M.X., Swaisgood, H.E., Clare, D.A. and Horton, H.R.: Kinetic mechanism and specificity of bovine milk sulfhydryl oxidase. *Biochem. J.*, **220**, 51 (1984)
14. Gan, Z.R. and Wells, W.W.: Purification and properties of thioltransferase. *J. Biol. Chem.*, **261**, 996 (1986)
15. Janolino, V.G. and Swaisgood, H.E.: Effect of support pore size on activity of immobilized sulfhydryl oxidase. *J. Dairy Sci.*, **61**, 393 (1978)
16. Swaisgood, H.E.: Sulfhydryl oxidase: properties and applications. *Enzyme Microb. Technol.*, **2**, 265 (1980)
17. Clapp, R.C., Long, L., Jr., Dateo, G.P., Bissett, F.H. and Hasselstrom, T.: The volatile isothiocyanates in fresh cabbage. *J. Am. Chem. Soc.*, **81**, 6278 (1959)
18. Dateo, G.P., Clapp, R.C., Mackey, D.A.M., Hewitt, E.J. and Hasselstrom, T.: Identification of the volatile sulfur components of cooked cabbage and the nature of the precursors in the fresh vegetable. *Food Res.*, **22**, 440 (1957)
19. MacLeod, A.J. and MacLeod, G.: Flavour volatiles of some cooked vegetables. *J. Food Sci.*, **35**, 734 (1970)
20. Ballance, P.E.: Production of volatile compounds related to the flavour of foods from the Strecker degradation of DL-methionine. *J. Sci. Food Agric.*, **12**, 532 (1961)
21. Schutte, L.: Precursors of sulfur-containing flavor compounds. *CRC Crit. Rev. Food Tech.*, **4**, 457 (1985)
22. Shaw, P.E., Ammons, J.M. and Braman, R.S.: Volatile sulfur compounds in fresh orange and grapefruit juices: identification, quantitation and possible importance to juice flavor. *J. Agric. Food Chem.*, **28**, 778 (1980)
23. Guadagni, D.G., Buttery, R.G. and Turnbaugh, J.G.: Odour threshold and similarity rating of some potato chip components. *J. Sci. Food Agric.*, **23**, 1435 (1972)
24. Self, R., Rolley, H.L.J. and Joyce, A.E.: Some volatile compounds from cooked potatoes. *J. Sci. Food Agric.*, **14**, 8 (1963)
25. Synge, R.L.M. and Wood, J.C.: (+)-(S-Methyl-L-cysteine S-oxide) in cabbage. *Biochem. J.*, **64**, 252 (1956)
26. Tressl, R., Holzer, M. and Apetz, M.: Biogenesis of volatiles in fruit and vegetables. In *Proc. Int. Symp. Aroma Research*, Maarse, H. and Groenen, P.J. (ed.), Pudoc, Wageningen, p.41 (1975)
27. Janolino, V.G., Sliwkowski, M.X., Swaisgood, H.E. and Horton, H.R.: Catalytic effect of sulfhydryl oxidase on the formation of three-dimensional structure in chymotropsinogen. *A. Arch. Biochem. Biophys.*, **191**, 269 (1978)
28. Ziegler, D.M.: Role of reversible oxidation-reduction of enzyme thiols-disulfides in metabolic regulation. *Ann. Rev. Biochem.*, **54**, 305 (1985)
29. Lash, L.H. and Jones, D.P.: Purification and properties of the membranal thiol oxidase from porcine kidney. *Arch. Biophys.*, **247**, 120 (1986)
30. Sliwkowski, M.B., Swaisgood, H.E. and Horton, H.R.: Solubilization of sulfhydryl oxidase, a bovine skim milk membrane enzyme. *J. Dairy Sci.*, **65**, 1681 (1982)
31. Sliwkowski, M.X., Sliwkowski, M.B., Horton, H.R. and Swaisgood, H.E.: Resolution of sulfhydryl ox-

idase from β-glutamyltransferase in bovine milk by covalent chromatography on cysteinyl-succinamidopropyl-glass. *Biochem. J.*, **209**, 731 (1983)

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캐러웨이 Sulfhydryl Oxidase를 이용한 십자화과 채소의 함황 불쾌취 억압

심기환 · R.C. Lindsay*

경상대학교 식품공학과, *미국 WISCONSIN 주립대학교 식품과학과

십자화과의 양배추와 브로콜리에서 함황 휘발성분 함량과 캐러웨이 종자의 sulfhydryl oxidase를 이용한 십자화과 채소의 함황 불쾌취 억압을 FID와 FPD가 동시에 연결된 개스 크로마토그래피로써 조사하였다. 양배추와 브로콜리에서 생성되는 휘발성 유황화합물은 캐러웨이 종자의 첨가량과 항온처리 시간에 따라 함량 및 생성률에 차이가 있었는데, 양배추와 브로콜리에 캐러웨이 종자를 첨가한 것이 대조구보다 methanethiol과 dimethyl disulfide 생성량이 훨씬 적었다. 또한, thiol oxidase 활성을 가진 캐러웨이 종자의 액상 슬러리를 이용하여 methanethiol과 dimethyl disulfide를 제거하는데 효과가 있었으며, 이들의 제거률은 캐러웨이 종자의 첨가량에 비례하였고, 특히 2.5% 캐러웨이 종자를 함유한 액상슬러리에서 효과적이었다.