

## A Comparative Study of the Changes in Volatile Flavor Compounds from Dried Leeks (*Allium tuberosum* R.) following $\gamma$ -Irradiation

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**Abstract** This study was performed to examine the effects of  $\gamma$ -irradiation on the volatile flavor compounds of dried leeks (*Allium tuberosum* R.). Volatile compounds of dried leeks were extracted using simultaneous steam distillation and extraction (SDE), and analyzed by gas chromatography/mass spectrometry (GC/MS). Forty-one, 51, 45, and 42 compounds were tentatively identified in control, 1, 3, and 10 kGy irradiated samples, respectively. The constituents of flavor compounds in irradiated dried leeks were similar to non-irradiated samples. However, the intensities of the peaks were clearly different between them. Sulfur-containing compounds were detected as dominant compounds in all samples and their amounts decreased after  $\gamma$ -irradiation.  $\gamma$ -Irradiation reduced the total concentration of volatile compounds from leeks by 23.19, 15.09, and 30.23% at 1, 3, and 10 kGy doses, respectively.

**Keywords:** leek,  $\gamma$ -irradiation, GC/MS, volatile sulfur-containing compounds

### Introduction

Plants belonging to the *Allium* genus, such as garlic, onion, Welsh onion, leek, etc., have been used as food and medicinal herbs in different cultures of the world since ancient times (1). These plants are known to accumulate sulfur-containing secondary compounds that are derived from cysteine (2). These flavor substances are formed by the action of alliinase (EC 4.4.1.4) on cysteine derivatives when plant material is disrupted (3). Among *Allium* plants, leek (*A. tuberosum* R.), locally called 'buchu', is an aromatic herb possessing a characteristic and long-lasting odor which resembles the flavor of onion and garlic. This plant is the major ingredient for leek *kimchi*, which has long been used as a medicinal food for the treatment of abdominal pain, diarrhea, hematemesis, snakebite, and asthma in folk remedies (4). It is rich in vitamin, fiber, and mineral compounds, and also sulfur compounds that have antibiotic properties (5, 6).

Currently, spices are irradiated worldwide on a significant scale. Spices are used primarily for flavor while the major problems associated with their storage and supply are heavy microbial contamination, insect infestation, and sprouting resulting in quality deterioration and lower market acceptability (7). Frequent contamination by microorganisms, especially by heat resistant bacteria and molds, has been causing serious problems for the food industry. Most spices grown in the tropics under prevailing production conditions contain a large number of microorganisms capable of causing spoilage or, more rarely diseases. Until recently, the widely used method for microbial decontamination of spices was fumigation with ethylene oxide. But, due to its toxic residues and

occupational health hazards, the use of this fumigant is banned in several countries including the European Union and Japan (8). Alternatively, there have been many reports that radiation treatment is a suitable method for the inactivation of microorganisms in spices, and commercial scale use of radiation processing for spices has been successful in more than 20 countries in recent years (9, 10).

Food irradiation has been recognized as an effective measure of microbial control for food preservation (11). The major advantage of food irradiation is that most food can be irradiated successfully in the final packaging. Extensive research has proven that irradiation is a safe and reliable process, and it has been approved by the Food and Drug Administration (FDA), the American Medical Association (AMA), and the World Health Organization (WHO) (10). The WHO encourages the use of the irradiation process, which is described as 'a technique for preserving and improving the safety of food' (12). The results obtained in the International Project and in national testing programs were repeatedly evaluated by the Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food (JECFI). This committee concluded in 1980 that the irradiation of any food commodity up to an overall average dose of 10 kGy presented no toxicological hazard and no special nutritional or microbiological problems (13).

Although there are some studies about substances responsible for the characteristic flavor of leek, an investigation of the complete volatile profile of irradiated dried leek has not been studied in detail. Therefore, the objective of this study was to evaluate the profile of volatile compounds from non-irradiated and irradiated (1, 3, and 10 kGy) dried leeks to assess quality with respect to their flavor constituents.

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## Materials and Methods

**Materials** Fresh leeks were obtained from a local market and freeze drying of leeks was performed in the laboratory. Dried leeks were irradiated at 1, 3, and 10 kGy using a  $^{60}\text{Co}$   $\gamma$ -irradiator (IR-79; Nordion International Co., Ltd., Ottawa, Canada, 100 kCi activity) at the Korea Atomic Energy Research Institute (KAERI) in Daejeon, Korea. The dose rate was 2.5 kGy/hr with a dose rate error of  $\pm 0.02$  kGy. Non-irradiated leeks were used as a control and all samples were stored at  $-18^\circ\text{C}$  until required.

**Reagents** All the reagents used in the experiments were purchased from Sigma Co. (Bellefonte, PA, USA) and Fisher Scientific (Pittsburg, PA, USA). The organic solvents used for extraction and in chromatography were redistilled using a wire spiral packed double distilling apparatus (Normschliff Geratebau, Wertheim, Germany) and purified water was generated with a Milli Q water purification system (Millipore Corporation, Bedford, MA, USA).

**Extraction of volatile compounds from dried leek by simultaneous steam distillation and extraction (SDE)** Leek samples weighing 20 g were homogenized in a blender (MR350CA; Braun, Spain) to which 1 L of purified water was added. The resultant slurry, maintaining a pH of 6.5, was used for the quantitative analysis with 1 mg of n-butyl benzene used as an internal standard. The volatile compounds were extracted for 2 hr with 200 mL of a redistilled n-pentane/diethyl ether (1:1, v/v) mixture, using a SDE (Likens & Nickerson type) apparatus (14), as modified by Schultz *et al.* (15), under atmospheric pressure.

The extract was dehydrated over anhydrous sodium sulfate and concentrated to approximately 2 mL using a Vigreux column. The 2 mL sample was further concentrated to a final volume 0.2 mL under a mild stream of nitrogen. The final sample was then used for gas chromatograph/mass spectrometry (GC/MS) analysis.

**GC/MS analysis** The GC/MS used for quantitative analysis was a Shimadzu GC/MS QP-5000 (Shimadzu, Kyoto, Japan) in the electron impact (EI) mode. The ionization voltage and ion source temperature were 70 eV and  $230^\circ\text{C}$ , respectively. The mass spectrometer scanned from 41 to 450  $m/z$ . A DB-WAX capillary column (60 m  $\times$  0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness; J&W, New Brighton, MN, USA) was used for the separation. The oven temperature program was as follows:  $40^\circ\text{C}$  (isothermal for 3 min) which was ramped to  $150^\circ\text{C}$  at 2/min, and then to  $210^\circ\text{C}$  at  $4^\circ\text{C}/\text{min}$ . The injector and detector temperatures were 250 and  $230^\circ\text{C}$ , respectively. Helium was used as the carrier gas at a flow rate of 1.0 mL/min with an injector volume of 1  $\mu\text{L}$  using a 1:20 split ratio.

**Identification and quantitative analysis of volatile compounds** Identification of volatile compounds was tentatively carried out with the help of our own mass spectral data, those contained within the WILEY 139, NIST 62, and NIST 12 libraries and mass spectral data

books (16, 17) as well as by the comparison of retention indices to reference data (18, 19).

$$\text{Amount of compound (mg/kg)} = \frac{C \times 1000}{A \times B}$$

A : Peak area of internal standard

B : Amount of sample (g)

C : Peak area of each compound in sample.

## Results and Discussion

**Composition of volatile flavor compounds in dried leek** The volatile flavor compounds of irradiated and non-irradiated dried leeks were extracted and concentrated to an appropriate volume. The compounds were detected and identified by GC/MS.

A total of 41 compounds categorized as alcohols (7), sulfur-containing compounds (20), aldehydes (9), esters (2), nitro-containing compounds (2), and miscellaneous compounds (1) were identified. Qualitative and quantitative analysis of volatile flavor compounds of the control showed sulfur-containing compounds to be the main volatile flavor compounds of dried leek. Among these constituents, dimethyl trisulfide (314.9 mg/kg) was the primary compound while (*Z*)-methyl propenyl trisulfide, dimethyl disulfide, and (*E*)-propenyl methyl disulfide present at 155.2, 120.3, and 103.4 mg/kg, respectively, were also detected in large amount. Methyl allyl disulfide (73.5 mg/kg), 3-ethyl-1,2-dithi-5-ene (49.4 mg/kg), and (*Z*)-propenyl methyl disulfide (38.8 mg/kg) were also found in high amount (Table 1). It has been well documented that the sulfur-containing compounds are the primary volatile flavor compounds in the *Allium* genus (20-22). Previous studies documented that, dimethyl disulfide, dimethyl trisulfide, (*Z*)-methyl-1-propenyl trisulfide, and (*E*)-propenyl methyl are characteristically present in fresh leek (23). Bernhard (24) and Lopes *et al.* (25) noted that the main compounds of the essential oil of leek are methyl allyl disulfide, diallyl disulfide, and dimethyl disulfide.

**Comparison of the volatile flavor compounds produced in non-irradiated and irradiated dried leeks** The GC-MS chromatographic profiles of volatile flavor compounds of control and irradiated samples showed similarity in their constituents (Fig. 1). The volatile flavor compounds of 1 kGy irradiated dried leek consisted of alcohols (6), sulfur-containing compounds (22), aldehydes (11), esters (3), nitro-containing compounds (2), and miscellaneous compounds (7) (Table 2). Among the sulfur-containing compounds, dimethyl trisulfide, (*Z*)-methyl propenyl trisulfide, (*E*)-propenyl methyl disulfide, and dimethyl disulfide were detected as the dominant compounds. Methyl allyl disulfide, 3-ethyl-1,2-dithi-5-ene, and (*E*)-methyl propenyl trisulfide were also detected in large amount. In 3 kGy irradiated dried leek, the flavor compounds consisted of alcohols (8), sulfur-containing compounds (20), aldehydes (9), esters (2), nitro-containing compounds (2), and miscellaneous compounds (4). Among the sulfur-containing compounds, dimethyl trisulfide was the primary

**Table 1. Volatile compounds identified in non-irradiated and 1, 3, and 10 kGy irradiated dried leeks**

Peak no.	RI <sup>1)</sup>	Compound name	MF <sup>2)</sup>	MW <sup>3)</sup>	Relative amount (mg/kg)			
					control	1 kGy	3 kGy	10 kGy
1	813	Ethyl formate	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	74	0.9	2.4	2.7	3.6
2	832	2-Propenal	C <sub>3</sub> H <sub>4</sub> O	56	0.1	0.9	0.1	0.9
3	874	Ethyl acetate	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	88	3.0	8.2	6.4	7.7
4	930	Ethanol	C <sub>2</sub> H <sub>6</sub> O	46	2.0	2.1	2.7	1.3
5	946	2-Ethyl furan	C <sub>6</sub> H <sub>8</sub> O	96	-	0.1	0.2	0.2
6	949	Allyl methyl sulfide	C <sub>4</sub> H <sub>8</sub> S	88	1.6	1.4	1.5	1.6
7	962	2,5-Dimethylfuran	C <sub>6</sub> H <sub>8</sub> O	96	-	0.2	0.2	-
8	973	2,3-Butanedione	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>	86	-	0.3	0.3	-
9	974	n-Pentanal	C <sub>5</sub> H <sub>10</sub> O	86	0.4	0.4	0.3	0.1
10	1038	2-Butenal	C <sub>4</sub> H <sub>6</sub> O	70	-	0.3	-	-
11	1073	Dimethyl disulfide	C <sub>2</sub> H <sub>6</sub> S <sub>2</sub>	94	120.3	80.0	115.0	92.7
12	1081	n-Hexanal	C <sub>6</sub> H <sub>12</sub> O	100	0.6	0.5	0.7	0.4
13	1103	2,3,4-Trimethyl-1,4-pentadiene	C <sub>8</sub> H <sub>14</sub>	110	-	0.1	-	-
14	1108	Allyl formate	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>	86	-	0.5	-	-
15	1113	2-Propenol	C <sub>3</sub> H <sub>6</sub> O	58	1.4	3.0	1.7	3.1
16	1137	2-Methyl-4-pentenal	C <sub>6</sub> H <sub>10</sub> O	98	0.3	0.2	0.2	-
17	1142	2,4-dimethyl hexene	C <sub>8</sub> H <sub>16</sub>	112	-	0.1	-	-
18	1144	Methyl ethyl disulfide	C <sub>3</sub> H <sub>8</sub> S <sub>2</sub>	103	-	0.3	0.3	0.1
19	1158	2-Methyl-2-pentenal	C <sub>6</sub> H <sub>10</sub> O	98	0.6	0.3	0.4	0.6
20	1184	Pyridine	C <sub>5</sub> H <sub>5</sub> N	79	0.9	0.2	0.3	0.7
21	1198	n-Heptanal	C <sub>7</sub> H <sub>14</sub> O	114	0.8	0.7	0.3	0.3
22	1216	(E)-2-Hexenal	C <sub>6</sub> H <sub>10</sub> O	98	11.1	10.6	11.7	4.1
23	1231	Methyl propyl disulfide	C <sub>4</sub> H <sub>10</sub> S <sub>2</sub>	122	1.0	0.6	1.1	0.8
24	1252	2,4-Dimethylthiophene	C <sub>6</sub> H <sub>8</sub> S	112	1.0	0.7	0.9	1.0
25	1254	n-Pentanol	C <sub>5</sub> H <sub>12</sub> O	88	0.2	0.2	0.1	0.5
26	1258	2-Butanethiol	C <sub>4</sub> H <sub>10</sub> S	90	-	0.4	-	-
27	1263	(Z)-Propenyl methyl disulfide	C <sub>4</sub> H <sub>8</sub> S <sub>2</sub>	120	38.8	29.5	34.0	30.1
28	1280	Methyl allyl disulfide	C <sub>4</sub> H <sub>8</sub> S <sub>2</sub>	120	73.5	52.7	60.7	43.4
29	1288	(E)-Propenyl methyl disulfide	C <sub>4</sub> H <sub>8</sub> S <sub>2</sub>	120	103.4	82.1	95.0	81.8
I.S. <sup>4)</sup>	1311	Butyl benzene	C <sub>10</sub> H <sub>14</sub>	134	-	-	-	-
30	1381	Dimethyl trisulfide	C <sub>2</sub> H <sub>6</sub> S <sub>3</sub>	126	314.8	225.8	265.4	217.6
31	1386	(Z)-3-Hexenol	C <sub>6</sub> H <sub>12</sub> O	100	0.6	0.2	1.2	0.7
32	1392	(Z)-2-Hexenol	C <sub>6</sub> H <sub>12</sub> O	100	-	-	1.0	-
33	1392	n-Nonanal	C <sub>9</sub> H <sub>18</sub> O	142	1.1	1.0	-	0.8
34	1429	(E)-Propenyl propyl disulfide	C <sub>6</sub> H <sub>12</sub> S <sub>2</sub>	148	0.2	0.1	0.2	0.6
35	1455	Methional	C <sub>4</sub> H <sub>8</sub> OS	104	8.3	6.3	4.0	3.7
36	1463	Furfural	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	96	0.4	0.4	0.9	1.4
37	1466	3-Ethyl-1,2-dithi-4-ene	C <sub>6</sub> H <sub>10</sub> S <sub>2</sub>	146	15.2	13.0	12.8	9.5
38	1481	Diallyl disulfide	C <sub>6</sub> H <sub>10</sub> S <sub>2</sub>	146	17.3	13.8	12.5	8.9
39	1487	3-Ethyl-1,2-dithi-5-ene	C <sub>6</sub> H <sub>10</sub> S <sub>2</sub>	146	49.4	42.3	41.0	32.2
40	1493	2-Ethyl hexanol	C <sub>8</sub> H <sub>18</sub> O	130	0.4	-	0.4	-
41	1523	Benzaldehyde	C <sub>7</sub> H <sub>6</sub> O	106	-	0.4	0.6	0.6
42	1526	N,N-dimethylthioacetamide	C <sub>4</sub> H <sub>9</sub> NS	103	1.1	1.6	1.1	1.4
43	1531	Methyl propyl trisulfide	C <sub>4</sub> H <sub>10</sub> S <sub>3</sub>	154	1.1	3.0	7.0	1.1
44	1551	Linalool	C <sub>10</sub> H <sub>18</sub> O	154	0.7	1.2	0.8	0.9
45	1561	Cyclohexyl ethyl ether	C <sub>8</sub> H <sub>16</sub> O	128	-	0.6	-	-
46	1583	Dimethyl sulfoxide	C <sub>2</sub> H <sub>6</sub> OS	78	-	1.1	0.7	0.4
47	1592	(Z)-Methyl propenyl trisulfide	C <sub>4</sub> H <sub>8</sub> S <sub>3</sub>	152	155.1	116.9	108.1	91.6
48	1598	(E)-Methyl propenyl trisulfide	C <sub>4</sub> H <sub>8</sub> S <sub>3</sub>	152	34.1	31.6	26.1	28.0
49	1675	S-Methyl methylthiosulphonate	C <sub>2</sub> H <sub>6</sub> O <sub>2</sub> S <sub>2</sub>	126	1.2	0.6	-	0.5
50	1681	2-Butyl-4-methylthiazole	C <sub>8</sub> H <sub>13</sub> NS	155	0.3	-	-	-
51	1689	Cyclohexyl methyl sulfide	C <sub>7</sub> H <sub>14</sub> S	130	3.8	3.6	3.5	2.8
52	1792	Diallyl trisulfide	C <sub>6</sub> H <sub>10</sub> S <sub>3</sub>	178	9.6	8.7	6.0	4.2
53	1832	Hexanoic acid	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	116	1.2	0.2	0.2	1.0
54	1975	1-Phenyl-1-butanol	C <sub>10</sub> H <sub>14</sub> O	150	1.6	1.3	1.0	0.9
Total					979.4	752.7	831.3	683.8

<sup>1)</sup>Retention index. <sup>2)</sup>Molecular formula. <sup>3)</sup>Molecular weight. <sup>4)</sup>Internal standard.

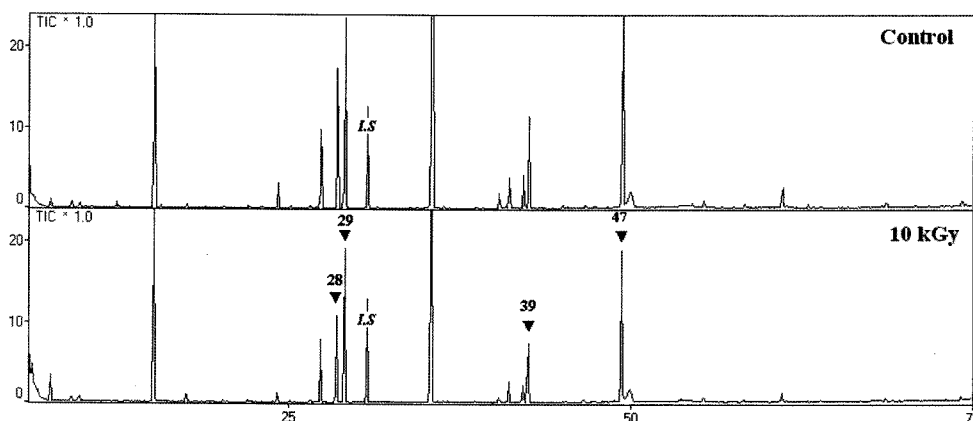


Fig. 1. GC/MS chromatograms of the volatile flavor compounds from non-irradiated (control) and 10 kGy irradiated dried leeks.

compound while dimethyl disulfide, (*Z*)-methyl propenyl trisulfide, (*E*)-propenyl methyl disulfide, methyl allyl disulfide, and 3-ethyl-1,2-dithi-5-ene were detected as well. In 10 kGy irradiated dried leek, the flavor compounds consisted of alcohols (6), sulfur-containing compounds (21), aldehydes (9), esters (2), nitro-containing compounds (2), and miscellaneous compounds (2). Similar types of compounds were detected in 10 kGy irradiated samples as in 1 and 3 kGy irradiated samples. These results show that the sulfur-containing compounds detected in non-irradiated and irradiated samples are the main compounds responsible for the flavor of dried leek.

The volatile compounds detected in irradiated dried leeks were similar to those of non-irradiated samples, but the peak intensities were clearly different in non-irradiated and irradiated samples. The total concentration of volatile flavor compounds of non-irradiated dried leek was approximately 979.4 mg/kg, whereas samples irradiated with 1, 3, and 10 kGy contained 752.4, 831.6, and 683.7 mg/kg, respectively (Table 1).

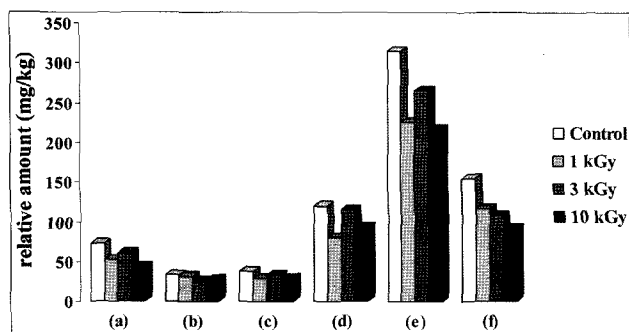
The major effect of irradiation was clearly found in the sulfur-containing compounds. Before irradiation, the concentration of sulfur-containing compounds was 950.1 mg/kg in non-irradiated dried leek. After irradiation of samples with 1, 3, and 10 kGy, the concentration of sulfur-containing compounds decreased to 714.3, 795.7, and

652.5 mg/kg, respectively (Table 2). Yang *et al.* (26) reported similar results regarding sulfur-containing compounds in dried shiitake that were reduced by irradiation. Lai *et al.* (27) performed studies on dry shiitake irradiated with 1-10 kGy and concluded that the amount of sulfur-containing compounds in non-irradiated dry shiitake was considerably higher than those irradiated with 1-10 kGy. Gyawalli *et al.* (28) reported that in comparison with non-irradiated dried Welsh onion, sulfur-containing compounds were reduced by irradiation with 10 and 20 kGy.

These results suggest that an optimal dose of irradiation increases the number of volatile compounds but decreases their concentrations. Such variation may be due to the fact that when molecules absorb ionizing energy they become reactive and form ions or free radicals that react to form stable radiolysis products (29). Kim *et al.* (30) reported that volatile compounds in salted and fermented anchovy sauce were highly increased by irradiation at 5 and 7.5 kGy, but slightly decreased at high dose irradiation with 10 kGy. The volatile oil of black pepper was also slightly changed due to radiation (31). Similarly, Wu *et al.* (32) noted that diallyl disulfide decreased after radiation treatment. Diallyl disulfide contributes to the harsh and pungent flavor of garlic (33, 34). On the contrary, Variyar *et al.* (35) noted that no qualitative and major quantitative

Table 2. Relative amount of functional groups in identified volatile compounds from non-irradiated and 1, 3, and 10 kGy irradiated dried leeks

Functional group	Relative amount (mg/kg)							
	0		1		3		10	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Alcohols	7	7.0	6	8.0	8	9.0	6	7.4
Aldehydes	9	15.3	11	15.7	9	15.3	9	9.3
Esters	2	3.8	3	11.1	2	9.1	2	11.3
Sulfur-containing compounds	20	950.1	22	714.3	20	795.7	21	652.5
Nitro-containing compounds	2	2.0	2	1.9	2	1.5	2	2.1
Miscellaneous	1	1.2	7	1.7	4	0.7	2	1.2
Total	41	979.4	51	752.7	45	831.3	42	683.8



**Fig. 2.** Comparison of sulfur-containing compounds from dried leeks following various radiation doses. (a) methyl allyl disulfide, (b) (*E*)-methyl propenyl trisulfide, (c) (*Z*)-propenyl methyl disulfide, (d) dimethyl disulfide, (e) dimethyl trisulfide, (f) (*Z*)-methyl propenyl trisulfide.

changes were observed in the essential oil constituents of clove and cardamom irradiated with a dose of 10 kGy. Chatterjee *et al.* (36) reported that no detectable differences were observed between the aroma impact compounds of non-irradiated and irradiated turmeric.

Aldehydes were detected as the second largest chemical class of volatiles from dried leek compounds, including *n*-pentanal, *n*-hexanal, *n*-heptanal, and (*E*)-2-hexenal, etc. Among them, (*E*)-2-hexenal was affected more than other aldehydes, being decreased to 4.1 mg/kg at 10 kGy from 11.1 mg/kg without irradiation, following the same pattern seen with sulfur-containing compounds.

In conclusion, the concentration of sulfur-containing compounds in dried leeks was considerably higher in non-irradiated than irradiated leeks up to 10 kGy. Although the number of volatile compounds of dried leeks increased after irradiation, the total concentrations of volatile compounds from leeks decreased by 23.19, 15.09, and 30.23% at 1, 3, and 10 kGy doses, respectively. These changes due to irradiation are of significance and require further investigation.

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