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EFFECT OF GAMMA-IRRADIATION ON THE VOLATILE FLAVOR PROFILE OF FENNEL (*FOENICULUM VULGARE* MILL.) FROM PAKISTAN

**NAEEM KHAN[1,2*], NARGIS JAMILA[1], JI YEON CHOI[2], EUN YEONG NHO[2],
IQBAL HUSSAIN[3] AND KYONG SU KIM[2**]**

[1] Department of Chemistry, Kohat University of Science & Technology, Kohat 26000, Khyber Pakhtunkhwa, Pakistan
[2] Department of Food and Nutrition, Chosun University, Gwangju 501-759, Republic of Korea
[3] Department of Chemistry, Islamia College University, Peshawar 25000, Khyber Pakhtunkhwa, Pakistan
*Corresponding author's e-mail:*nkhan812@gmail.com; **kskim@chosun.ac.kr*

Abstract

The volatile flavor compounds of non-irradiated and 1, 5, 10 and 20 kGy γ-irradiated seeds of fennel (*Foeniculum vulgare* Mill.) from Pakistan were isolated by simultaneous distillation-extraction (SDE) and were analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 82 compounds were identified in the non irradiated fennel, with *E*-Anethole (36.74%), Estragole (26.31%), and *β*-Limonene (15.99%) as the major compounds. The irradiation doses caused slight variations in the number and contents of the volatile components. Though several volatile compounds showed increase after γ-irradiation, the contents of major compounds such as *β*-Limonene and estrgole were decreased. The overall number of the volatile compounds showed increase up to the recommended irradiation doses of 10 kGy but their contents decreased. In general no major change was noted in the overall major flavor compounds of the subject spice. Therefore the application of γ-irradiation is feasible without any significant qualitative or quantitative loss of volatile flavor compounds when exposed to 10 kGy γ-irradiation.

Key words: *Foeniculum vulgare* Mill., Volatile flavor compounds, SDE, GC-MS, Pakistan.

Introduction

Fennel *(Foeniculum vulgare* Mill) is a well-known medicinal and aromatic plant belonging to the family Apiaceae (Umbelliferaceae). It is a perennial herb cultivated throughout the temperate and tropical regions for its light green anise-flavored aromatic seeds (Diao *et al*., 2014; Ikram *et al.,* 2015). Many cultures in Pakistan, India, Afghanistan, Iran and the Middle East use mature fennel seeds as flavoring agents in food products such as sauces, pickles, bread, sweet desserts, salads and cheese (Agarwal *et al*., 2008; Diao *et al*., 2014). Especially after meals fennel seeds are chewed because of their aroma which works as breath fresheners and also added to some natural tooth pastes. They can relax intestines and reduce bloating caused by indigestion. The fennel water when mixed with sodium bicarbonate form the domestic Gripe Water, commonly used to ease flatulence in infants. Also its syrup is used to treat babies with colic or painful teething (Türkyilmaz *et al*., 2008; Telcia *et al*., 2009).

The microbial food poisoning and spoilage are still the most serious problems faced by the food industry and consumers, even in developed countries (Lücking *et al.,* 2013). To avoid food contamination by microbes, food irradiation has been recognized in the recent past as an effective technique of food preservation by consumers, food industries and governments. Around the world 60 countries have approved the use of irradiations for more than 200 food items (Chauhan *et al*., 2009). In this method of food processing, the high energy ionizing radiations (e.g., gamma rays, high energy electrons or X-rays) damage the DNA very effectively, inactivating the living cells and therefore insect gametes and microorganisms are being prevented from reproduction, resulting in various food preservative effects (Lado and Yousef 2002). However, any possible chemical changes which may be caused by high energy ionizing irradiations in food need careful monitoring to avoid adverse effects on the food quality (Hwang *et al*., 2014).

In literature several research studies have been reported in the recent past on the fennel chemical composition across the world. These include studies on the samples extraction methods (Díaz-Maroto, *et al*., 2005), relative concentration of compounds based on environmental conditions (Carrubba *et al*., 2005), geographical locations and various parts (Díaz-Maroto *et al*., 2006), variations along growth stages (Telci *et al*., 2009), comparison of wild and cultivated varieties (Shahat *et al*., 2012), metals contents (Khan *et al*., 2014) and mechanism of action of oil seeds (Diao *et al*., 2014). All these studies on the chemical compositions of fennel are still limited and require further analyses. Most importantly, to the best of our knowledge, still no work has been reported so far on the effects of irradiation on the volatile flavor compounds of spices such as *F. vulgare*. Therefore, this study was designed to investigate the volatile flavor profile compounds of *F. vulgare* and to evaluate further any possible effects of irradiation such as gamma ray on these flavor compounds. The well-known method of choice for volatile flavor studies involves extraction by simultaneous distillation-extraction (SDE) and analyses by gas chromatography-mass spectrometry (GC-MS) (Reineccius, 1993; Bosch-Fusté *et al*., 2007; Gholivand *et al*., 2013). This advanced technique of SDE-GC-MS was applied for the volatile flavor compounds of *F. vulgare* spice seeds collected from Pakistan. Keeping in view the recommended irradiation dose of 10 kGy for spices, the samples were irradiated at 1, 5, 10 and 20 kGy doses and the identified compounds were compared with those from non-irradiated ones to find out any effects of these irradiations on the overall flavor of the subject spice.

Materials and Methods

Samples collection: Samples were collected in triplicate from a local super market in Peshawar, Khyber Pakhtunkhwa, Pakistan. These were identified by plant taxonomist at Department of Botany, University of Peshawar. Voucher specimens were deposited at the university herbarium center for future reference. All the samples were properly labeled and sent by express mail service (EMS) to food analysis laboratory, Dept. of Food and Nutrition, Chosun University, Gwangju, Korea. These were stored in plastic bags at -20ºC in refrigerator (MICOM CFD-0622, Samsung, Korea) until analysis.

Reagents: The reagents used were purchased from sigma Co. (St Louis, MO, USA). The organic solvents; diethyl ether, and n-pentane used for extraction were purchased from Fisher Scientific (Waltham, Massachusetts, USA) and were redistilled using a wire spiral packed double distilling apparatus (Normschliff Geratebau, Wertheim, Germany) and Milli-Q water was generated through a water purification system (Millepore Corporation, Bedford, USA). Anhydrous Na_2SO_4 was used for dehydration of organic solvents after being burnt for overnight at 650ºC in a furnace (F 6000, Barnstead Thermolyne Co., IA, USA) and allowed to cool down in desiccator.

Samples irradiation: Samples were irradiated at 1, 5, 10, and 20 kGy doses of gamma ray, at 12±1°C using a Cobalt-60 γ-irradiator (Sailor 100,000 Ci, IR-79, Nordion International Ltd., Ontario, Canada, 100 kCi) at Korea Atomic Energy Research Institute, Daejeon. The dose rate was 2.5 kGy/h, with an error rate of ± 0.02 kGy. Absorbed doses were monitored with either a radical or a ceric-cerous dosimeter. The irradiated samples and non-irradiated control were stored at -20°C in refrigerator until required for analysis. The non-irradiated samples were used as control (Jeong *et al*., 2014).

Extraction of volatile flavor compounds: Each 30.0 g of sample was homogenized in a blender (MR 350CA, Braun, Spain) and mixed with 500 mL distilled water. For maintaining the pH at 7.0, by pH meter (HM-30P, DKK-TOA Corp., Tokyo, Japan), 1 N NaOH and 1 N HCl were used. Then 10 mL of n-butyl benzene (110 ppm in n-pentane) was added as internal standard and the resultant slurry was used for the quantitative analysis. The volatile compounds were extracted for 3 hours with 100 mL redistilled n-pentane/diethylether (1:1, v/v) mixture, using a simultaneous steam distillation and extraction (SDE, Likens & Nickerson type) apparatus (Nikerson & Likens, 1966) as modified later on under atmospheric pressure (Schultz *et al*., 1977). The extract was dehydrated for overnight with 10 g anhydrous Na_2SO_4 and concentrated to final volume of approximately 1.5 mL using a vigreux column. Finally it was concentrated to 0.5 mL under mild stream of N_2 gas. This sample so obtained was used for spectrometric analysis.

Analysis of volatile organic compounds by GC-MS: GC-MS-QP2010 (Shimadzu, Japan) in the EI (electron impact ionization) mode was used for the quantitative analysis. The ionization voltage and temperature of injector and ion source were 70 eV, 230°C and 250°C respectively. The mass spectrometer scanned from 50 to 400 m/z. DB-WAX capillary column (60 m length × 0.25 mm diameter, 0.25 µm film thickness, Agilent J&W, USA) was used for the separation. The oven temperature was programmed as: 40ºC (isothermal for 3 min) which was ramped to 180ºC (isothermal for 5 min) at 2ºC /min, to 200ºC (isothermal for 10 min) at 4ºC /min, to 220ºC (isothermal for 5 min) at 5ºC/min and then to 250ºC (isothermal for 10 min) at 5ºC/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min and the sample injector volume was 1.0 µL using a 1:30 split ratio (Table 1).

Identification and quantification of volatile flavor compounds: Retention index was used as parameter for checking of a solute from chromatogram by comparing the retention time of standard n-alkanes (C_7-C_{40}), that appeared above and below the solute (Kovats, 1958). Mass spectra of volatile organic compounds were identified with the aid of our own mass spectral data and those contained within the Willey 7, NIST 05 and FFNSC 2.0 spectral libraries provided with the GC-MS instrument and mass spectral data books (Anon., 1996; Davies, 1990) as well as by the comparison of retention indices to reference data (Robert, 1995) and online available data from The Pherobase (El-Sayed, 2014). The similarity ratio > 90% was considered effective for the identification of volatile compounds in the samples. The quantitative analysis was carried out with the help of peak area percent of internal standard (n-butylbenzene) by using following formula (Giyawali *et al*., 2008):

$$\text{Compound relative contents (mg/kg)} = \frac{C \times 1000\ g}{A \times B\ g}$$

where:
A = Peak area of each sample of internal standard
B g = Amount of sample
C = Peak area of each component in sample

Statistical analysis: Data were reported as average of 3 replicates (mean ± standard deviation). Significant differences ($p<0.05$) within means of same compounds among different irradiated dose samples were analyzed by one way analysis of variance (ANOVA), following Tukey's honestly significant difference (HSD) test and Duncan test, in the Statistical Package for Social Sciences (SPSS), Software Version 20 (IBM, New York, USA). The concentrations of chemical compounds significantly different ($p<0.05$) were represented by superscript letters.

Table 1. GC-MS conditions and operating parameters as applied for volatile flavor compounds of *Foeniculum vulgagre* Mill. seeds from Pakistan.

GC-MS	GC-MS-QP2010, Shimadzu, Japan.
Column	DB-Wax (60 m Length, 0.25 mm Diameter, 0.25 µm thickness)
Carrier gas	He (1.0 mL/min)
Temperature program	40 ºC (3 min), to 2 ºC/min-180ºC(5 min), to 4 ºC/min-200 ºC (10 min), to 5ºC/min-220 ºC (5 min) and to 5 ºC/min-250 ºC (10 min)
Injector temperature	240 ºC
Ion source temperature	250 ºC
Ionization	Electron Impact (EI)
Ionization voltage	70 eV
Mass range	50 ~ 400 (m/z)
Injection volume	1µL
Split ratio	1:100

Results and Discussion

Volatile flavor profile compounds were extracted from non-irradiated and γ-irradiated seeds of *F. vulgare* from Pakistan and identified by comparing their spectral data and retention indices. The GC-MS chromatograms of the non-irradiated control and γ-irradiated samples (1, 5, 10 and 20 kGy doses) are shown in Fig. 1; the identified compounds are reported in Table 2, according to their elution order on DB-WAX column with ranges of their amounts corresponding to irradiation doses, and Table 3 shows the number and relative contents of the functional groups of identified compounds as varied with the irradiation doses.

The GC-MS chromatograms of volatile flavor profiles of non-irradiated and γ-irradiated samples at 1, 5, 10 and 20 kGy doses showed great similarity in their constituents (Fig. 1). A total of 82 volatile flavor compounds were detected in non-irradiated samples which increased to 97, 96, 103 and 102 for 1, 5, 10 and 20 kGy γ-irradiated samples respectively (Table 3). Thus an increasing trend in the number of volatile compounds was observed in accordance to what was reported for γ-irradiated root of licorice (*Glycyrrhiza uralensis* Fischer) from South Korea by Giyawali *et al*. (2008). The important variations were observed for flavor compounds which showed increase with irradiation doses up to 10 kGy but decreased at 20 kGy. Three compounds including *n*-decanal, *Z*-4-decenal and *L*–(-)-menthol were detected only for high irradiation doses of 10 and 20 kGy doses respectively. The *ar*-Tumerone with retention index value of 2244 (RI = 2244) was the only compound not detected for 20 kGy irradiation doses. This means *ar*-Tumerone was sensitive to γ-irradiation dose of 20 kGy. There were fifteen (15) compounds detected in very small concentrations only in irradiated samples indicating their synthesis as a result of γ-irradiation of the subject seed spice samples (Table 2). This production of small concentration of the flavor compounds by gamma irradiation was in accordance to published literatures (Jo and Ahn 2000; Giyawali *et al*., 2008). The total relative contents of the detected compounds were: 7946.22 mg/kg (0 kGy), 6414.11 mg/kg (1 kGy), 5895.55 mg/kg (5 kGy), 6260.75 mg/kg (10 kGy), and 6042.44 mg/kg (20 kGy). Hence irradiation decreased the overall relative contents of the identified compounds.

The identified compounds were grouped into various functional group classes as mentioned in Table 3. From these flavor compounds belonging to esters (3), oxygenated sesquiterpens (4) and miscellaneous (4) were detected in same numbers in non-irradiated and all irradiated samples. It means compounds belonging to these thee classes were unchanged at least by numbers for all the irradiation doses. However, identified compounds from other classes were found to show either increase or decrease both in number and relative contents. In general, oxygenated monoterpenes were detected in highest numbers, followed by monoterpene hydrocarbons, aromatic compounds and aldehydes. However based on relative percentage, aromatic compounds were the highest (58.123-64.202%), followed by monoterpene hydrocarbons (18.172-22.001%) and oxygenated monoterpenes (11.150-20.379%) (Table 3). There were nine (9) major flavor compounds which contributed more than 90% of the total relative volatile flavor contents of the samples. On the basis of elution order, these were including *α*-pinene, eugenol, *E*-anethole, *α*-terpenyl acetate, estragole, fenchone, 1,8-cineole, *β*-limonene and *ρ*-anisaldehyde. Among these the most abundant were *E*-anethole, estragole and *β*-limonene contributing around 70% or more of the identified flavor compounds for 0-20 kGy irradiated samples analyzed (Table 4). All these major compounds were approximately in accordance to the reported literatures for *F. vulgare* seeds from around the world including Pakistan (Gulfraz *et al*., 2008), Turkey (Çetin *et al*., 2010), Egypt (Shahat *et al*., 2012), and China (Diao *et al*., 2014). Estragole and *β*-limonene were the two compounds which showed major decrease as a result γ-irradiation compared to other flavor compounds (Fig. 2).

Table 2. Volatile flavor compounds identified in non-irradiated and γ-irradiated seeds of *F. vulgare* from Pakistan.

No.	RI$^{1)}$	Compound name	MF$^{2)}$	Relative contents (mg/kg)				
				0 kGy	1 kGy	5 kGy	10 kGy	20 kGy
1.	881	Ethyl acetate$^{a, 3)}$	$C_4H_8O_2$	$0.38^{a\ 4)}$	0.46^{b}	0.42^{ab}	0.45^{b}	0.45^{b}
2.	909	2-Methylbutanal^{b}	$C_5H_{10}O$	0.06^{ab}	0.04^{a}	0.06^{ab}	0.09^{b}	0.09^{b}
3.	913	3-Methylbutanal^{b}	$C_5H_{10}O$	0.04^{ab}	0.02^{a}	0.04^{ab}	0.06^{b}	0.06^{b}
4.	1003	Tricyclene^{f}	$C_{10}H_{16}$	0.52^{a}	0.59^{a}	0.54^{a}	0.58^{a}	0.58^{a}
5.	1019	α-Pinene^{f}	$C_{10}H_{16}$	149.25^{b}	121.24^{a}	112.59^{a}	116.78^{a}	116.78^{a}
6.	1024	α-Thujene^{f}	$C_{10}H_{16}$	1.50^{c}	1.30^{b}	1.10^{a}	1.38^{b}	1.38^{b}
7.	1040	2-Methyl-3-buten-2-ol^{d}	$C_5H_{10}O$	0.11^{a}	0.11^{a}	0.09^{a}	0.11^{a}	0.11^{a}
8.	1053	α-Fenchene^{f}	$C_{10}H_{16}$	1.58^{a}	1.78^{b}	1.64^{a}	1.72^{ab}	1.72^{ab}
9.	1061	Camphene^{f}	$C_{10}H_{16}$	15.73^{a}	17.30^{a}	16.17^{a}	16.86^{a}	16.86^{a}
10.	1077	n-Hexanal^{b}	$C_6H_{12}O$	0.23^{a}	0.26^{a}	0.25^{a}	0.28^{a}	0.28^{a}
11.	1101	β-Pinene^{f}	$C_{10}H_{16}$	10.60^{a}	10.43^{a}	9.56^{a}	10.09^{a}	10.09^{a}
12.	1116	Sabinene^{f}	$C_{10}H_{16}$	35.97^{a}	32.56^{a}	29.29^{a}	33.33^{a}	33.33^{a}
13.	1145	δ-3-Carene^{f}	$C_{10}H_{16}$	0.16^{a}	0.21^{a}	0.23^{a}	0.28^{b}	0.28^{b}
14.	1161	β-Myrcene^{f}	$C_{10}H_{16}$	74.34^{a}	69.28^{a}	64.83^{a}	67.29^{a}	67.29^{a}
15.	1175	α-Terpinene^{f}	$C_{10}H_{16}$	0.78^{a}	1.60^{c}	1.09^{b}	1.82^{c}	1.82^{c}
16.	1178	Pyridine^{j}	C_5H_5N	0.12^{a}	0.12^{a}	0.12^{a}	0.13^{a}	0.13^{a}
17.	1181	n-Heptanal^{b}	$C_7H_{14}O$	0.05^{a}	0.05^{a}	0.05^{a}	0.06^{a}	0.06^{a}
18.	1188	2,3-Dehydro-1,8-cineole^{g}	$C_{10}H_{16}O$	0.24^{a}	0.26^{a}	0.25^{a}	0.37^{a}	0.37^{a}
19.	1197	β-Limonene^{f}	$C_{10}H_{16}$	1270.77^{c}	804.11^{b}	743.49^{a}	761.31^{a}	761.31^{a}
20.	1203	β-Phallendrene^{f}	$C_{10}H_{16}$	41.17^{b}	27.53^{a}	26.49^{a}	26.49^{a}	26.49^{a}
21.	1206	1,8-Cineole^{g}	$C_{10}H_{18}O$	96.73^{a}	176.95^{b}	114.87^{a}	204.14^{b}	204.14^{b}
22.	1227	2-Amyl furan^{k}	$C_9H_{14}O$	0.15^{a}	0.20^{a}	0.20^{a}	0.20^{a}	0.20^{a}
23.	1232	*Z*-β-Ocimene^{f}	$C_{10}H_{16}$	74.62^{b}	52.39^{a}	49.70^{a}	50.46^{a}	50.46^{a}
24.	1242	γ-Terpinene^{f}	$C_{10}H_{16}$	31.66^{b}	15.36^{a}	13.61^{a}	15.14^{a}	15.14^{a}
25.	1248	*E*-β-Ocimene^{f}	$C_{10}H_{16}$	3.26^{a}	4.19^{b}	3.93^{b}	4.13^{b}	4.13^{b}
26.	1265	ρ-Cymene^{f}	$C_{10}H_{14}$	26.78^{b}	15.09^{a}	14.56^{a}	15.67^{a}	15.67^{a}
27.	1277	α-Terpinoline^{f}	$C_{10}H_{14}$	7.74^{a}	11.79^{b}	10.62^{b}	12.82^{b}	12.82^{b}
28.	1280	2-Methylbutyl-2-methylbutanoate^{a}	$C_6H_{12}O_2$	0.24^{a}	0.91^{b}	0.81^{b}	0.84^{b}	0.84^{b}
29.	1284	n-Octanal^{b}	$C_8H_{16}O$	0.09^{a}	0.46^{c}	0.30^{b}	0.51^{c}	0.51^{c}
30.	1294	3-Methylbutyl-3-methylbutanoate^{a}	$C_6H_{12}O_2$	0.87^{a}	2.31^{c}	2.11^{b}	2.29^{c}	2.29^{c}
IS	**1305**	**n-Butylbenzene**	$\mathbf{C_{10}H_{14}}$	-	-	-	-	-
31.	1351	n-Hexanol^{d}	$C_6H_{14}O$	ND$^{5)}$	0.08^{a}	0.06^{a}	0.06^{a}	0.08^{a}
32.	1369	*E,E*-Alloocimene^{f}	$C_{10}H_{16}$	1.83^{b}	0.96^{a}	0.86^{a}	0.93^{a}	0.82^{a}
33.	1394	Fenchone^{g}	$C_{10}H_{16}O$	546.00^{a}	574.73^{a}	556.95^{a}	546.73^{a}	541.12^{a}
34.	1409	6,7-Epoxymyrecene^{g}	$C_{10}H_{16}O$	ND	0.27^{b}	0.21^{a}	0.34^{c}	0.31^{c}
35.	1411	Perillene^{g}	$C_{10}H_{14}O$	0.19^{a}	0.25^{b}	0.24^{b}	0.27^{b}	0.25^{b}
36.	1426	ρ-Cymenene^{f}	$C_{12}H_{12}$	ND	0.34^{a}	0.41^{b}	0.63^{c}	0.29^{a}
37.	1441	*Z*-Limonene oxide^{g}	$C_{10}H_{16}O$	4.23^{b}	2.08^{a}	2.42^{a}	2.56^{a}	2.15^{a}
38.	1443	3-Butenyl-isothiocyanate^{j}	C_5H_7NS	ND	ND	0.80^{b}	0.42^{a}	0.26^{a}
39.	1451	Furfural^{b}	$C_5H_4O_2$	0.14^{a}	0.23^{b}	0.26^{b}	0.30^{b}	0.24^{b}
40.	1454	*E*-Limonene oxide^{g}	$C_{10}H_{16}O$	3.45^{b}	2.40^{a}	2.21^{a}	2.26^{a}	2.04^{a}
41.	1463	*Z*-Myroxide^{g}	$C_{10}H_{16}O$	1.15^{c}	0.41^{a}	0.49^{a}	0.85^{b}	0.36^{a}
42.	1465	*E*-Sabinene hydrate^{g}	$C_{10}H_{18}O$	2.50^{a}	5.41^{b}	4.95^{b}	7.65^{c}	7.00^{c}
43.	1468	Z-Linalool oxide^{g}	$C_{10}H_{18}O_2$	ND	0.30^{a}	0.22^{a}	0.36^{b}	0.49^{c}
44.	1482	α-Campholenal^{b}	$C_{10}H_{16}O$	1.02^{b}	0.52^{a}	0.53^{a}	0.55^{a}	0.52^{a}
45.	1488	α-Fenchyl acetate^{g}	$C_{12}H_{20}O_2$	8.76^{a}	18.24^{b}	18.11^{b}	16.80^{b}	19.00^{b}
46.	1491	n-Decanal^{b}	$C_{10}H_{20}O$	ND	ND	ND	0.17^{a}	0.13^{a}
47.	1508	Camphor^{g}	$C_{10}H_{16}O$	21.75^{a}	21.23^{a}	21.00^{a}	20.20^{a}	20.29^{a}
48.	1531	Non-(2*E*)-enal^{b}	$C_9H_{16}O_2$	0.19^{b}	0.12^{a}	0.11^{a}	0.09^{a}	0.14^{a}
49.	1535	Dec-(4*Z*)-enal^{b}	$C_{10}H_{18}O$	ND	ND	ND	0.27^{b}	0.20^{a}
50.	1545	*L*-Linalool^{g}	$C_{10}H_{18}O$	34.00^{a}	68.11^{c}	53.91^{b}	39.04^{a}	53.30^{b}
51.	1547	*E*-Sabinene hydrate^{g}	$C_{10}H_{18}O$	3.60^{a}	ND	ND	4.35^{a}	4.02^{a}
52.	1553	Linalyl acetate^{g}	$C_{12}H_{20}O_2$	2.19^{a}	3.93^{b}	4.05^{b}	13.19^{c}	9.19^{c}
53.	1555	1-Octanol^{d}	$C_8H_{18}O$	ND	0.26^{b}	ND	0.13^{a}	0.59^{c}
54.	1569	β-Fenchol^{g}	$C_{10}H_{18}O$	0.42^{b}	0.65^{c}	0.33^{a}	0.29^{a}	0.33^{a}
55.	1577	Bornyl acetate^{g}	$C_{12}H_{20}O_2$	0.67^{a}	1.26^{c}	0.98^{b}	1.09^{b}	1.22^{c}

Table 2. (Cont'd.).

No.	RI[1]	Compound name	MF[2]	Relative contents (mg/kg)				
				0 kGy	**1 kGy**	**5 kGy**	**10 kGy**	**20 kGy**
56.	1579	*α*-Fenchol^{g}	$C_{10}H_{18}O$	3.44^{c}	0.65^{b}	0.39^{a}	0.37^{a}	0.56^{b}
57.	1585	6-Methyl-3,5-heptadien-2-one^{k}	$C_{8}H_{12}O$	0.33^{b}	0.24^{ab}	0.17^{a}	0.12^{a}	0.32^{b}
58.	1593	*E*-Caryophyllene^{h}	$C_{15}H_{24}$	9.77^{a}	6.71^{a}	14.29^{b}	40.87^{c}	8.61^{a}
59.	1597	4-Terpineol^{g}	$C_{10}H_{18}O$	2.49^{a}	9.36^{b}	4.99^{a}	11.97^{b}	15.09^{c}
60.	1599	*Z*-Dihydrocarvone^{g}	$C_{10}H_{16}O$	ND	0.49^{a}	1.12^{b}	1.40^{c}	0.59^{a}
61.	1621	*E*-Dihydrocarvone^{g}	$C_{10}H_{16}O$	1.39^{a}	1.39^{a}	5.80^{c}	7.00^{d}	2.01^{b}
62.	1624	*E*-*ρ*-Mentha-2,8-dien-1-ol^{g}	$C_{10}H_{18}O$	ND	3.10^{b}	2.77^{a}	2.72^{a}	2.79^{a}
63.	1628	*Z*-*β*-Terpineol^{g}	$C_{10}H_{18}O$	0.30^{a}	0.40^{a}	0.32^{a}	0.32^{a}	0.32^{a}
64.	1636	*L*-(-)-Menthol^{g}	$C_{10}H_{20}O$	ND	ND	ND	0.28^{a}	0.35^{a}
65.	1648	*δ*-Terpinyl acetate^{g}	$C_{12}H_{20}O_{2}$	ND	1.57^{a}	ND	1.75^{a}	1.57^{a}
66.	1668	Estragole^{e}	$C_{10}H_{12}O$	2090.64^{b}	962.70^{a}	914.80^{a}	925.71^{a}	919.48^{a}
67.	1665	*Z*-*ρ*-Mentha- 2,8-dien-1-ol^{g}	$C_{10}H_{18}O$	ND	4.80^{b}	3.56^{a}	3.67^{a}	5.51^{c}
68.	1673	*α*-Humulene^{h}	$C_{15}H_{24}$	2.93^{b}	ND	1.70^{a}	5.90^{c}	ND
69.	1676	*E*-Verbenol^{g}	$C_{10}H_{16}O$	1.75^{a}	2.15^{b}	1.52^{a}	2.37^{b}	2.36^{b}
70.	1681	1,8-Menthadien-4-ol^{g}	$C_{10}H_{16}O$	1.27^{b}	1.36^{b}	0.87^{a}	0.93^{a}	0.81^{a}
71.	1691	*α*-Terpinenyl acetate^{g}	$C_{12}H_{20}O_{2}$	108.74^{a}	187.50^{b}	108.83^{a}	288.08^{c}	295.63^{c}
72.	1694	1-Borneol^{g}	$C_{10}H_{18}O$	ND	0.12^{a}	0.16^{b}	0.13^{a}	0.18^{b}
73.	1704	Germacrene-D^{h}	$C_{15}H_{24}$	7.00^{c}	2.57^{b}	2.54^{b}	2.71^{b}	1.85^{a}
74.	1718	Zingiberene^{h}	$C_{15}H_{24}$	10.67^{d}	0.59^{ab}	0.26^{a}	2.59^{c}	1.04^{b}
75.	1727	Carvone^{g}	$C_{10}H_{14}O$	35.40^{b}	19.16^{a}	17.18^{a}	18.26^{a}	19.89^{a}
76.	1746	*Z*-Anethole^{e}	$C_{10}H_{12}O$	23.78^{a}	24.03^{a}	26.69^{ab}	31.42^{b}	38.41^{c}
77.	1754	Geranyl acetate^{g}	$C_{12}H_{20}O_{2}$	ND	6.36^{b}	5.45^{a}	5.23^{a}	5.76^{ab}
78.	1760	Methyl salicylate^{e}	$C_{8}H_{8}O_{3}$	ND	0.46^{b}	0.49^{b}	0.45^{b}	0.35^{a}
79.	1770	*β*-Sesquisabinene^{h}	$C_{15}H_{24}$	14.94^{d}	4.50^{b}	4.57^{b}	7.84^{c}	1.61^{a}
80.	1790	*Z*-Isocarveol^{g}	$C_{10}H_{16}O$	0.83^{b}	1.64^{c}	0.45^{a}	0.46^{a}	0.53^{a}
81.	1799	*Z*-Sabinol^{g}	$C_{10}H_{16}O$	1.20^{b}	1.67^{c}	ND	0.48^{a}	0.65^{a}
82.	1826	*E*-Anethole^{e}	$C_{10}H_{12}O$	2919.20^{a}	2831.10^{a}	2554.24^{a}	2502.35^{a}	2437.69^{a}
83.	1840	Geraniol^{g}	$C_{10}H_{18}O$	ND	6.26^{b}	4.04^{a}	7.46^{b}	7.80^{b}
84.	1859	*Z*-Carveol^{g}	$C_{10}H_{16}O$	2.90^{a}	2.74^{a}	2.56^{a}	2.60^{a}	2.57^{a}
85.	1864	Benzyl alcohol^{e}	$C_{7}H_{8}O$	0.47^{a}	0.56^{a}	1.08^{b}	0.91^{b}	0.87^{b}
86.	1978	Caryophyllene oxide^{i}	$C_{15}H_{24}O$	3.59^{c}	0.61^{a}	0.86^{a}	1.35^{b}	0.68^{a}
87.	1996	Perilla alcohol^{g}	$C_{10}H_{16}O$	0.44^{b}	0.27^{a}	0.25^{a}	0.27^{a}	0.30^{a}
88.	2000	Methyl eugenol^{e}	$C_{11}H_{14}O_{2}$	1.27^{b}	0.52^{a}	0.51^{a}	0.51^{a}	0.56^{a}
89.	2009	*ρ*-Anisaldehyde^{b}	$C_{8}H_{8}O_{2}$	110.14^{b}	99.46^{a}	121.68^{b}	116.98^{b}	108.83^{b}
90.	2023	*E*-Cinnamaldehyde^{b}	$C_{9}H_{8}O$	7.26^{a}	12.13^{a}	12.47^{a}	2.24^{a}	7.03^{a}
91.	2034	*E*-Nerolidol^{i}	$C_{15}H_{26}O$	1.32^{a}	1.99^{b}	1.91^{b}	2.30^{c}	2.16^{c}
92.	2085	Cumin alcohol^{g}	$C_{10}H_{14}O$	ND	0.46^{a}	0.55^{a}	0.53^{a}	0.53^{a}
93.	2137	*ρ*-Acetylanisole^{e}	$C_{9}H_{10}O_{2}$	ND	4.14^{a}	7.912^{c}	7.48^{bc}	6.30^{b}
94.	2149	Eugenol^{e}	$C_{10}H_{12}O_{2}$	20.48^{a}	81.14^{b}	128.37^{c}	177.42^{d}	77.55^{b}
95.	2169	Thymol^{e}	$C_{10}H_{14}O$	42.44^{c}	29.88^{b}	16.71^{a}	34.12^{b}	23.46^{ab}
96.	2191	1-(4-methoxyphenyl)- 1-propanone^{e}	$C_{10}H_{12}O_{2}$	ND	4.88^{a}	5.18^{a}	4.95^{a}	4.56^{a}
97.	2194	Carvacrol^{e}	$C_{10}H_{14}O$	ND	0.51^{a}	0.38^{a}	0.53^{a}	0.52^{a}
98.	2214	Elemicin^{e}	$C_{12}H_{16}O_{3}$	1.51^{a}	1.12^{a}	1.36^{a}	1.30^{a}	1.20^{a}
99.	2233	Curlone^{i}	$C_{15}H_{22}O$	7.54^{c}	0.61^{ab}	0.54^{a}	1.03^{b}	0.38^{a}
100.	2244	*ar*-Tumerone^{i}	$C_{15}H_{20}O$	11.50^{c}	1.78^{a}	1.81^{a}	3.38^{b}	ND
101.	2248	Myristcin^{k}	$C_{11}H_{12}O_{3}$	3.20^{c}	2.09^{ab}	2.23^{b}	1.82^{a}	2.55^{b}
102.	2316	Chavicol^{e}	$C_{9}H_{10}O$	1.82^{c}	0.89^{a}	1.46^{b}	1.44^{b}	1.12^{a}
103.	2338	Dillapiole^{k}	$C_{12}H_{14}O_{4}$	19.64^{b}	16.77^{a}	22.42^{c}	21.54^{c}	19.49^{b}
		Total		**7946.22**	**6414.11**	**5895.55**	**6260.75**	**6042.44**

1. RI= Retention index

2. MF= Molecular formula

3. $^{a-k}$ Superscripts represent the functional group of compound (a= esters; b= aldehydes; d= alcohols; e= aromatic compounds; f= monoterpene hydrocarbons; g= oxygenated monoterpenes; h= sesquiterpene hydrocarbons; i= oxygenated sesquiterpene; j= nitrogenous compounds; k= miscellaneous)

4. Numbers with different superscripts (a-d) in the same row are not significant ($p<0.05$) with one another in the same row.

5. ND= not detected

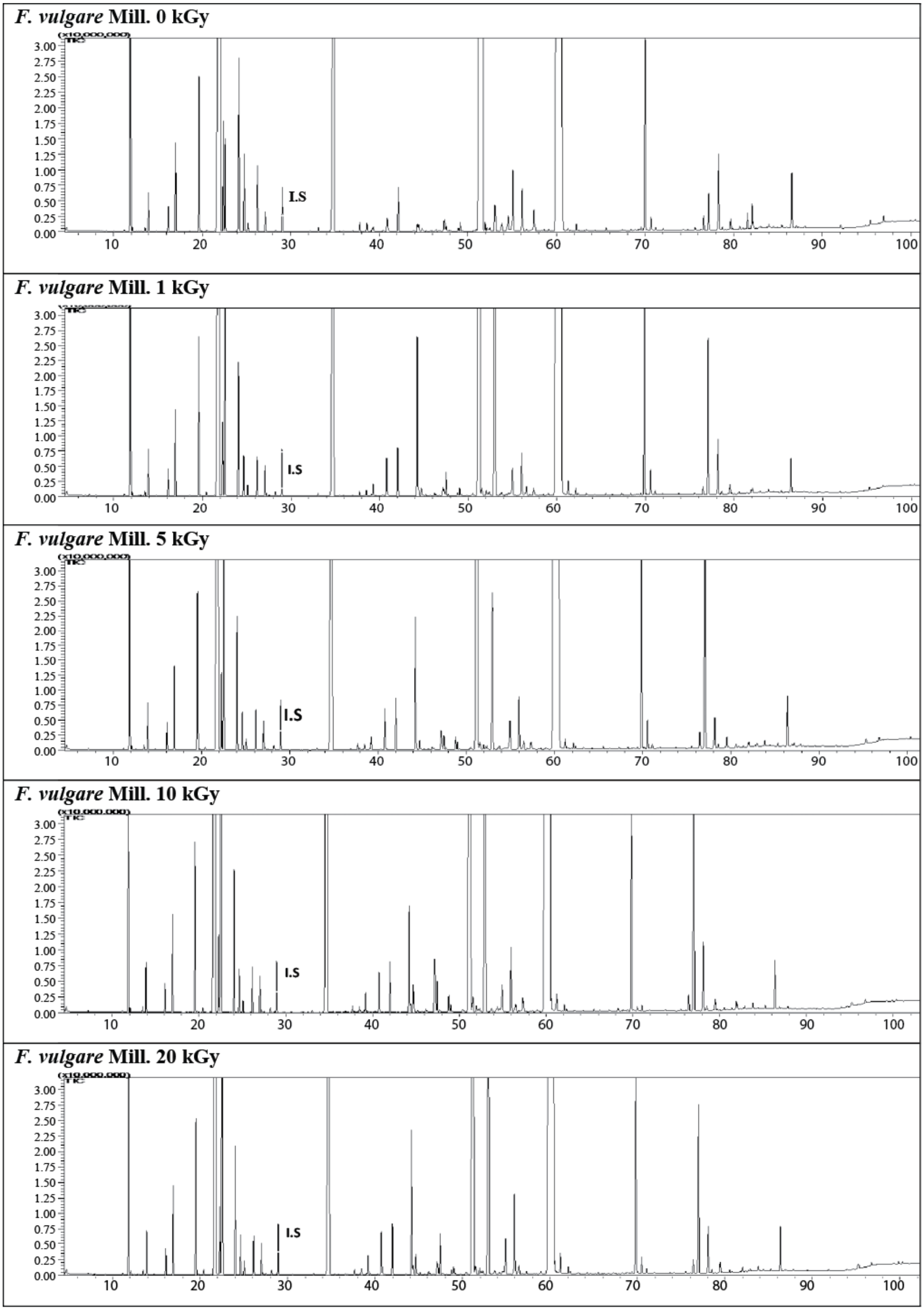

Fig. 1. GC-MS chromatograms of volatile flavor compounds of non-irradiated and γ-ray irradiated *F. vulgare* Mill. seeds from Pakistan.

Table 3. Relative content of functional groups of volatile flavor compounds identified in non-irradiated and γ-irradiated *F. vulgare* seeds from Pakistan.

No.	Functional group	Control		1kGy		5kGy		10kGy		20kGy	
		Area%	No	Area%	No	Area%	No	Area%	No	Area%	No
1.	Esters	0.019	3	0.058	3	0.057	3	0.057	3	0.059	3
2.	Aldehydes	1.500	10	1.766	10	2.303	10	1.943	12	1.954	12
3.	Alcohols	0.001	1	0.007	3	0.003	2	0.005	3	0.013	3
4.	Aromatic compounds	64.202	9	61.457	13	62.067	13	58.916	13	58.123	13
5.	Monoterpene hydrocarbons	22.001	18	18.522	19	18.671	19	18.172	19	18.821	19
6.	Oxygenated monoterpenes	11.150	27	17.585	36	15.978	34	19.435	38	20.379	38
7.	Sesquiterpene hydrocarbons	0.570	5	0.224	4	0.396	5	0.957	5	0.217	4
8.	Oxygenated sesquiterpenes	0.302	4	0.078	4	0.087	4	0.129	4	0.053	4
9.	Nitrogenous compounds	0.002	1	0.002	1	0.016	2	0.009	2	0.007	2
10.	Miscellaneous	0.253	4	0.301	4	0.422	4	0.377	4	0.373	4
	Total	**100**	**82**	**100**	**97**	**100**	**96**	**100**	**103**	**100**	**102**

Table 4. Effects of γ-irradiation doses (1, 5, 10 & 20 kGy) on major volatile flavor compounds of *F. vulgare* seeds from Pakistan.

No.	Major compounds	0 kGy		1 kGy		5 kGy		10 kGy		20 kGy	
		Contents (mg/kg)	Percent (%)	Contents (mg/kg)	Percent (%)	Contents (mg/kg)	Percent (%)	Contents (mg/kg)	Percent (%)	Contents (mg/kg)	Percent (%)
1.	*α*-Pinene	149.25	1.88	121.24	1.89	112.59	1.91	116.78	1.87	116.78	1.93
2.	Eugenol	20.48	0.26	81.14	1.27	128.37	2.18	177.42	2.83	77.55	1.28
3.	*E*-Anethole	2919.20	36.74	2831.1	44.14	2554.24	43.32	2502.35	39.97	2437.69	40.34
4.	*α*-Terpinenyl acetate	108.74	1.37	187.5	2.92	108.83	1.85	288.08	4.60	295.63	4.89
5.	Estragole	2090.64	26.31	962.7	15.01	914.8	15.52	925.71	14.79	919.48	15.22
6.	Fenchone	546	6.87	574.73	8.96	556.95	9.45	546.73	8.73	541.12	8.96
7.	1,8-Cineole	96.73	1.22	176.95	2.76	114.87	1.95	204.14	3.26	204.14	3.38
8.	*β*-Limonene	1270.77	15.99	804.11	12.54	743.49	12.61	761.31	12.16	761.31	12.60
9.	*ρ*-Anisaldehyde	110.14	1.39	99.46	1.55	121.68	2.06	116.98	1.87	108.83	1.80
	Total	**7311.95**	**92.02**	**5838.93**	**91.03**	**5355.82**	**90.84**	**5639.5**	**90.08**	**5462.53**	**90.40**

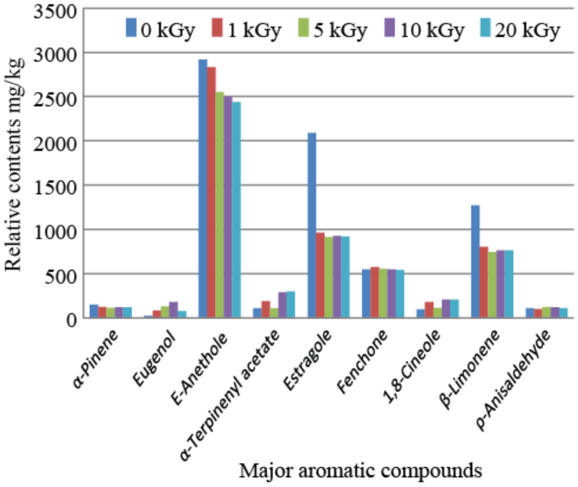

Fig. 2. Variation of major volatile flavor compounds *F. vulgare* Mill. seeds with γ-irradiation doses.

The γ-irradiation caused slight variations in the contents of aforementioned major flavor profile compounds. Among these only *β*-limonene and estragole showed slight decrease while all other seven major flavor compounds either more or less remain unchanged or increased due to irradiation doses (Table 4; Fig.2).In published literatures it has been reported that irradiation treatments may cause splitting the chemical bonds in the target volatile flavor compounds causing their free radicals formation and then recombination of the free radicals may produce possible variations in both the kinds and amounts of flavor. For dry Welsh onion one aldehyde compound was produced after 3 kGy dose of *γ*-irradiation compounds (Giyawali *et al*., 2008). Similarly Jo and Ahn (2000) have also reported that in amino acids containing irradiated oil emulsion, several new aldehydes were produced at a dose-dependent rate up to 10 kGy. In another study on irradiation of foods with higher doses (>10 kGy), the decrement of some of volatile flavor compounds was also reported (Kim *et al*., 2004). These research findings, verify the slight variation in volatile flavor contents of *F. vulgare* in present study.

Conclusions

The γ-irradiation of *F*. vulgare seeds at 1, 5, 10, and 20 kGy doses resulted in an increase in the number of volatile flavor compounds. The relative contents of volatile flavor profile compounds however showed decrease by irradiation and slight variation among the applied doses. All major flavor compounds which are giving characteristic flavor to the samples remained unchanged to a greater extent. Estragole and *β*-limonene however showed major decrease as a result γ-irradiation compared to other compounds. In general greater similarity in occurrence of volatile flavor constituents in non-irradiated and irradiated samples was noted. Irradiations caused variations usually in the number and contents of compounds belonging to aldehydes, monoterpene hydrocarbons, oxygenated monoterpenes and aromatic compounds. However there were no major changes and the volatile flavor of the subject spice was judged unaffected by gamma irradiation technique. Therefore irradiation of *F. vulgare* was declared quite safe up to the recommended dose of 10 kGy which would also slightly increase their volatile flavor profile compounds.

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