

Volatile Flavor Compounds of Gamma Irradiated *Cucumis sativus* L. (Cucumber) Seeds by Simultaneous Distillation Extraction and Gas Chromatography-Mass Spectrometry Analysis

(Sebatian Perisa Meruap bagi Biji *Cucumis sativus* L. (Timun) Disinari Gamma dengan Pengekstrakan Penyulingan Serentak dan Analisis Kromatografi Gas-Spektrometri Jisim)

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ABSTRACT

Food irradiation is a technique applied to increase food safety and prolongs shelf life. Irradiation with gamma rays is one of the sterilization and preservation methods used in the food industry. The current study was aimed at analyzing the volatile flavor compounds of *Cucumis sativus* L. seeds and to assess the impact of gamma irradiation on these flavor compounds. The *Cucumis sativus* (cucumber) seeds were gamma irradiated with 1, 5, 10, and 20 kGy doses and analyzed for flavor profile compounds. Simultaneous distillation extraction (SDE) and gas chromatography mass spectrometry (GC-MS) techniques were used to determine the volatile flavor compounds. From the results, a total of 58 volatile compounds in the non-irradiated (control) sample were identified with 1-hexanol (13.2%), methyl valerate (8.97%), and butanoic acid, methyl ester (8.34%) as the most abundant compounds. Among the 58 volatile flavor compounds identified, 23 were those detected in varying quantities in all irradiated samples and non-irradiated control. The number of flavor compounds increased with increasing irradiation doses, from 1 kGy to 10 kGy and then decreased at 20 kGy. The results indicated that high dose of radiation (above 10 kGy) has direct impact on concentration and profile of volatile flavor compounds. Conclusively, gamma-irradiation up to 10 kGy doses can be employed for food sterilization to increase shelf life of analyzed seeds.

Keywords: *Cucumis sativus* L.; gamma-irradiation; gas chromatography-mass spectrometry; simultaneous distillation-extraction; volatile compounds

ABSTRAK

Penyinaran makanan adalah teknik yang digunakan untuk meningkatkan keselamatan makanan dan memanjangkan jangka hayat. Penyinaran dengan sinar gamma adalah salah satu kaedah pensterilan dan pengawetan yang digunakan dalam industri makanan. Kajian semasa ini bertujuan untuk menganalisis sebatian rasa meruap biji *Cucumis sativus* L. dan untuk menilai kesan penyinaran gamma ke atas sebatian perasa ini. Biji *Cucumis sativus* (timun) telah disinari gamma dengan dos 1, 5, 10 dan 20 kGy dan dianalisis untuk sebatian profil perisa. Teknik pengekstrakan penyulingan serentak (SDE) dan kromatografi gas-spektrometri jisim (GC-MS) telah digunakan untuk menentukan sebatian rasa meruap. Daripada keputusan tersebut, sejumlah 58 sebatian meruap dalam sampel tidak disinari (kawalan) telah dikenal pasti dengan 1-heksanol (13.2%), metil valerat (8.97%) dan asid butanoik, metil ester (8.34%) sebagai sebatian yang paling banyak. sebatian. Antara 58 sebatian rasa meruap yang dikenal pasti, 23 adalah yang dikesan dalam kuantiti yang berbeza-beza dalam semua sampel yang disinari dan kawalan tidak disinari. Bilangan sebatian perisa meningkat dengan peningkatan dos penyinaran, daripada 1 kGy kepada 10 kGy dan kemudian menurun pada 20 kGy. Keputusan menunjukkan bahawa dos sinaran yang tinggi (melebihi 10 kGy) mempunyai kesan langsung ke atas kepekatan dan

profil sebatian rasa meruap. Kesimpulannya, penyinaran gamma sehingga 10 kGy dos boleh digunakan untuk pensterilan makanan untuk meningkatkan jangka hayat benih yang dianalisis.

Kata kunci: *Cucumis sativus* L.; kromatografi gas-spektrometri jisim; penyinaran gamma; penyulingan-pengekstrakan serentak; sebatian meruap

INTRODUCTION

Cucumis sativus L. (cucumber) seeds, belonging to Cucurbitaceae family, have high oil contents, with a nutty flavor. Cucumber seed oil are used as cooking oil, which could increase high-density cholesterol and decrease low-density cholesterol and thus serum cholesterol levels, thereby preventing cardiovascular diseases (Sharma, Sharma & Sandhu 2020). Being one of Charmagaz seeds (watermelon, melon, cucumber and pumpkin seeds), cucumber seeds have the potential to improve cognitive brain processes and aid in the recovery of both physical and mental strength following a chronic illness (Lawal 2011; Showkat et al. 2024).

Cucumber seeds has served as a good source of proteins, fats, minerals, calcium and secondary compounds and can also be used as food and water additives (Abiodun & Adeleke 2010; Mandey et al. 2019). Gill and Bali (2012) have reported in their study about the cooling, tonic, diuretic, and anthelmintic behavior of cucumber seeds. Different parts of the *Cucumis sativus* plant including peel, pulp, and seeds have been found to have good nutritional potential for human beings (Niyi, Jonathan & Ibukun 2019). Due to the presence of phytochemicals and other inorganic components, cucumber seed oil has got the potential applications as a raw material for medicinal and cosmetic formulations (Oragwu et al. 2021).

Cucumber seeds are packed in multiwalled packets and kept in cold storage, but when these are exposed, the seeds get moldy, caked, and rancid due to microbial contamination, which results in an adverse impact on the chemical composition of the seeds (Packirisamy et al. 2016). These seeds emit an 'oily' odor when rancidity starts and this odor cannot be removed simply by boiling or frying (Diprossimo & Malek 1996; Showkat et al. 2024). It is possible to treat these kinds of food goods with high energy radiation such as gamma rays to extend their shelf life and increase safety by effectively damaging the microbial DNA. So far, sixty countries worldwide have authorized the use of irradiations for food safety (Di Stefano et al. 2014).

Besides the wide applications of cucumber seeds in human nutrition, very limited information regarding cucumber seeds composition is available. Moreover, to the best of our knowledge, there is no authentic research to report the volatile flavor compounds in cucumber seeds. Also, as these seeds are expensive, their decay due

to microbial contamination results in substantial financial losses. Therefore, irradiation such as gamma rays can be employed to extend their shelf life and avoid their financial loss but any impacts of irradiation on the seed's composition such as flavor profile compounds needs to be determined. Consequently, the current study was aimed at determining the volatile organic compounds of cucumber seeds and to assess any effects of gamma radiations on these compounds. Simultaneous distillation extraction (SDE) and gas chromatography-mass spectrometry (GC-MS) are the recommended techniques applied for analysis volatile compounds in different food samples around the world (Gyawali et al. 2008; Islam et al. 2020; Khan et al. 2019, 2017, 2015; Reineccius 1993; Sheibani et al. 2016; Young et al. 2022). These advanced techniques of SDE and GC-MS were used to analyze the volatile flavor compounds in the fresh cucumber seeds. The seed samples were irradiated in triplicate to dosages of 1, 5, 10, and 20 kGy gamma rays. The non-irradiated seed samples were used as control for comparison to evaluate any impact of the applied gamma irradiation on the analyzed subject seeds.

MATERIALS AND METHODS

SAMPLES COLLECTION AND IRRADIATION

Cucumber seeds were collected from natural sources in Kohat, Khyber Pakhtunkhwa, Pakistan, and identified by a plant taxonomist. Seed samples were exposed to gamma irradiation for one hour, in properly arranged stainless containers, at doses of 1, 5, 10, and 20 kGy at 12 ± 1 °C, using a cobalt-60 gamma-irradiator encapsulated in a stainless-steel capsule (ISOGAMMA LLCO type). The irradiation dose rate was kept at 2.5 kGy/h and monitored with a dosimeter. All the samples were properly labelled, stored in plastic bags at -20 °C in a deep freezer (MICOM CFD0622, Samsung, Korea) until they were required for analysis. The non-irradiated (0 kGy) samples served as control (Jeong et al. 2014).

SIMULTANEOUS DISTILLATION EXTRACTION OF VOLATILE FLAVOR COMPOUNDS

500 mL distilled water was added to each 30.0 g homogenized seed sample. The pH was adjusted to 7.0 by using 0.1 N HCl and NaOH solutions. Then, 10 mL of

internal standard, n-butyl benzene solution (110 ppm in n-pentane) was added, and the resultant mixture obtained was applied for the extraction. The extraction of volatile flavor compounds was done for three hours, using 100 mL solvent mixture of diethyl ether/n-pentane (1:1, v/v) (Schultz et al. 1977), and using a Likens & Nickerson type equipment intended for simultaneous steam distillation and extraction (SDE) (Ribeiro et al. 2021; Teixeira et al. 2007). The extract was dehydrated by adding 10 g of anhydrous Na₂SO₄ for overnight before being concentrated to 1.5 mL by a Vigreux column. At the end, the N₂ gas stream was used to concentrate it to 0.5 mL for volatile flavor compounds analysis by GC-MS system (Majcher & Jeleń 2009).

VOLATILE FLAVOR COMPOUNDS BY GC-MS ANALYSIS

The analysis of volatile flavor compounds in the cucumber seeds extracts was carried out in the electron impact ionization mode (EI mode) of the gas chromatography-mass spectrometry system (GC-MS-QP2010, Shimadzu, Japan). The ionization voltage was 70 eV, while temperatures of 230 °C and 250 °C were applied to the injector and ion source, respectively. The mass spectrometer was scanned for 50 and 400 m/z range. For separation, a DB-WAX capillary column measuring 60 meters in length, 0.25 millimeters in diameter and 0.25 micrometers in thickness was employed (Stashenko et al. 2015). The oven operation was configured as follows: a 40 °C for 3 min of isothermal operation, ramping up to 180 °C for 5 min of isothermal operation at 2 °C/min, then to 200 °C for 10 min at 4 °C/min to 220 °C for 5 min at 5 °C/min, and finally to 250 °C for 10 min at 5 °C/min (Wu et al. 2004).

IDENTIFICATION AND QUANTIFICATION OF VOLATILE FLAVOR COMPOUNDS

Retention index (RI) values were used as identifying parameters for volatile flavor compounds from GC-chromatograms by comparing the retention time (RT) of standard n-alkanes (C₇-C₄₀) mixture, that appeared above and below the compounds (Young et al. 2022). Mass spectra of all the samples were identified with the help of mass spectral data and those contained within the FFNSC 2.0, Willey 7, and NIST 05 spectral libraries available with the GC-MS instrument and mass spectral data books (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 more than 85% was considered enough for the identification of volatile flavor compounds in the seed samples. The relative quantitative analysis was done with the help of peak area percent of n-butylbenzene (internal standard) as reported in literature (Gyawali et al. 2008).

RESULTS AND DISCUSSION

GC-MS chromatograms of gamma irradiated, and non-irradiated control samples are given in Figure 1, while major flavor compounds identified in cucumber seed samples are shown in Figure 2. Volatile flavor compounds identified are mentioned in Table 1 and the relative concentrations of the functional groups of the volatile flavor compounds are given in Table 2. The list of flavor compounds that are commonly found in both irradiated and non-irradiated seeds is shown in Table 3 and major flavor compounds in all cucumber seed samples are given in Table 4.

From the results of the current study, it was reported that the number of volatile flavor compounds increased from 58 for non-irradiated seed sample (0 kGy) to 65, 59, and 60 in samples that were exposed to gamma radiation at 1, 5, and 10 kGy, respectively, and decreased to 33 at 20 kGy (Tables 1 & 2). These results findings were in line with the research findings on South Korean gamma-irradiated licorice root (*Glycyrrhiza uralensis* Fischer) (Gyawali et al. 2008). Similarly in another study by Kim, Choi and Oh (2006), it has also been reported that sensory characteristics of food decline when exposed to radiation dose higher than 10 kGy. In literature, the degradation in structural properties of fibrous carbohydrates due to higher radiation dose (more than 10 kGy) has been reported which support the present research findings (Crawford & Ruff 1996; Dionísio, Gomes & Oetterer 2009; Miller 2005). From the present results it was found that although slight change in concentrations and formation of some new volatile flavor compounds was observed after irradiation, the volatile flavor profile of control sample mostly remained unchanged after irradiation up to recommended irradiation dose of 10 kGy. A research study in literature has reported that when dried ginger was exposed to radiation, the amount of the flavor compounds changed slightly with irradiation, but the volatiles' profiles remained the same (No et al. 2005).

In non-irradiated samples, a total of 58 volatile flavor compounds were found belonging to different functional groups with relative concentration (to internal standard) of 53.40 mg/kg. Among the 58 volatile compounds identified, there were 16 alcohols (31.13%), followed by 11 hydrocarbons (18.98%), 10 carboxylic acids (20.68%), 8 aromatic compounds (5.94%), 6 terpenes (8.42%), 4 esters (10.61%), 1 ether (2.80%), 1 furan compound (1.33%) and 1 aldehyde (0.07%). Compared to non-irradiation seeds, the concentrations of alcohols and carboxylic acids were higher in the irradiated samples while the other functional group contents were almost the same. These findings were in line with the Seo and Shim's results on volatile extracts from irradiated samples (Seo et al. 2007; Shim et al. 2009).

According to Rignakos et al. (1999), radiation causes an increase in the synthesis of oxidation products such as alcohols, aldehydes, and carboxylic acids which can also

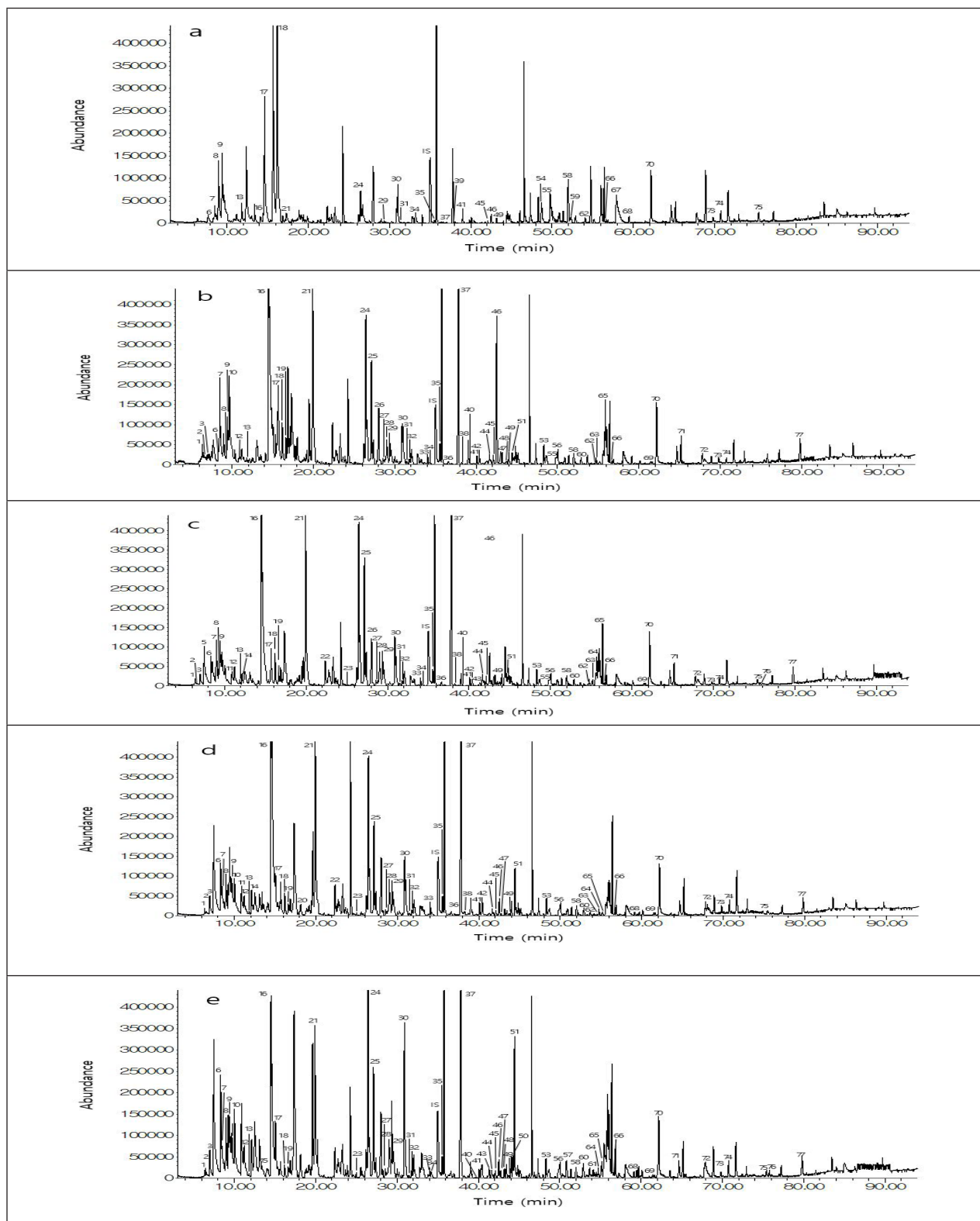


FIGURE 1. GC-MS chromatograms of volatile flavor compounds of non-irradiated and gamma irradiated seeds of cucumber. (a) non-irradiated seed sample (0 kGy), (b) seeds exposed to 1 kGy, (c) seeds exposed to 5 kGy, (d) seeds exposed to 10 kGy, and (e) seeds exposed to 20 kGy

TABLE 1. List of volatile flavor compounds in non-irradiated and irradiated seeds of cucumber (*Cucumis sativus* L.)

NO	RT (Apx)	RI (Apx)	Compound names	MF	MW	0 kGy	1 kGy	5kGy	10kGy	20 kGy
1	6.355	600	<i>n</i> -Hexane ^a	C ₆ H ₁₄	86.18	0.13±0.03	0.39±0.02	0.11±0.02	0.31±0.04	ND
2	6.99	700	<i>n</i> -Heptane ^a	C ₇ H ₁₆	100.2	0.51±0.08	0.18±0.04	0.32±0.05	0.66±0.07	ND
3	7.475	727	1-Heptene ^a	C ₇ H ₁₄	98.19	3.23±0.36	0.29±0.01	1.48±0.12	4.05±0.44	ND
4	7.659	741	2-Methyl-heptane ^a	C ₇ H ₁₄	114.22	ND	0.28±0.01	ND	ND	ND
5	7.734	746	4-Methyl-heptane ^a	C ₇ H ₁₄	114.22	ND	0.73±0.06	ND	ND	ND
6	8.332	788	<i>n</i> -Octane ^a	C ₈ H ₁₈	114.23	1.15±0.06	0.27±0.02	0.68±0.02	2.00±0.15	0.10±0.01
7	8.516	800	2,4-dimethyl- heptane ^a	C ₈ H ₁₈	128.5	0.75±0.09	2.45±0.06	0.68±0.03	0.94±0.06	0.50±0.06
8	9.274	832	1-Octene ^a	C ₉ H ₂₀	112.21	0.63±0.01	0.17±0.02	0.36±0.01	1.16±0.07	ND
9	9.684	848	4-methyl- octane ^a	C ₇ H ₁₄ O	128.25	1.34±0.07	3.25±0.30	1.66±0.12	1.51±0.10	1.79±0.11
10	10.03	861	2-Octene, (E)- ^a	C ₈ H ₁₆	112.21	1.44±0.21	ND	0.97±0.28	2.35±0.18	ND
11	10.37	874	2,4-Dimethyl-1-heptene ^a	C ₉ H ₁₈	126.23	ND	0.23±0.03	0.26±0.12	ND	ND
12	10.88	891	<i>n</i> -Nonane ^a	C ₉ H ₂₀	128.25	0.82±0.02	0.18±0.03	0.48±0.01	1.69±0.06	ND
13	11.83	919	Methyl isobutyrate ^d	C ₅ H ₁₀ O ₂	102.13	0.10±0.01	0.19±0.01	0.14±0.01	0.08±0.01	0.59±0.02
14	12.14	927	E-1-Methoxy-3-hexene ^a	C ₇ H ₁₄ O	114.18	ND	0.17±0.02	0.37±0.02	ND	ND
15	13.96	971	1,6-Heptadiene ^a	C ₇ H ₁₂	96.17	ND	ND	ND	0.24±0.09	ND
16	14.51	983	Butanoic acid, methyl ester ^e	C ₅ H ₁₀ O ₂	102.13	4.45±0.29	5.23±0.32	4.91±0.09	5.13±0.37	0.24±0.07
17	15.70	1007	2-Methyl-butanoic acid ^c	C ₅ H ₁₀ O ₂	102.13	0.96±0.07	2.07±0.33	1.19±0.05	0.65±0.31	5.70±0.21
18	16.18	1017	Methyl isovalerate ^c	C ₆ H ₁₂ O ₂	116.15	0.59±0.01	1.11±0.17	0.86±0.05	0.48±0.02	8.71±0.47
19	16.64	1025	2-Butanol ^f	C ₄ H ₁₀ O	74.12	0.43±0.05	0.65±0.07	0.48±0.01	0.40±0.01	ND
20	18.15	1053	1,3-Octadiene ^a	C ₇ H ₁₀ O	110.15	ND	ND	0.34±0.12	ND	ND
21	19.93	1082	Methyl valerate ^d	C ₆ H ₁₂ O ₂	116.15	4.79±0.38	5.21±0.11	5.51±0.20	4.08±0.36	0.28±0.07
22	22.36	1121	3-Methyl-2-Butanol ^b	C ₄ H ₁₀ O	74.12	ND	1.01±0.04	0.75±0.04	ND	ND
23	24.69	1158	Beta-Myrcene	C ₁₀ H ₁₆	136.23	ND	ND	0.06±0.01	0.05±0.01	ND
24	26.46	1184	Hexanoic acid,methyl ester ^e	C ₇ H ₁₄ O ₂	130.18	4.24±0.30	4.38±0.04	5.16±0.28	5.25±0.36	0.70±0.01
25	27.14	1193	D-Limonene ⁱ	C ₁₀ H ₁₆	136.23	2.67±0.08	3.01±0.15	3.79±0.10	2.86±0.08	ND

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26	28.04	1206	Eucalyptol ^f	C ₁₀ H ₁₈ O	154.24	1.50±0.10	1.59±0.11	ND	ND	ND	ND
27	28.58	1215	Butanoic acid, butyl ester ^e	C ₈ H ₁₆ O ₂	144.21	0.17±0.01	0.20±0.04	0.13±0.01	0.24±0.01	0.24±0.01	ND
28	29.03	1222	2-Ethoxy-ethanol ^f	C ₄ H ₁₀ O ₂	90.12	1.16±0.13	0.81±0.03	1.07±0.10	0.89±0.04	0.89±0.04	ND
29	29.38	1228	2-Pentyl-furan ⁱ	C ₉ H ₁₄ O	138.20	0.71±0.01	0.66±0.05	0.74±0.02	1.40±0.03	1.40±0.03	0.04±0.01
30	30.90	1251	1-Pentanol ^f	C ₅ H ₁₂ O	88.14	1.11±0.10	0.88±0.01	1.10±0.09	3.33±0.62	3.33±0.62	0.28±0.02
31	31.47	1259	1-Ethyl-4-methyl-benzene ^h	C ₉ H ₁₂	120.19	0.05±0.04	0.10±0.01	0.09±0.01	0.05±0.01	0.05±0.01	0.10±0.04
32	31.8	1264	Butanoic acid ^e	C ₉ H ₁₈ O ₂	158.23	0.16±0.06	0.15±0.05	0.15±0.01	0.16±0.05	0.16±0.05	ND
33	32.06	1268	p-Cymene ⁱ	C ₁₀ H ₁₄	134.21	0.52±0.02	0.52±0.03	0.52±0.01	0.65±0.02	0.65±0.02	ND
34	33.26	1284	Heptanoic acid ^e	C ₈ H ₁₆ O ₂	144.21	0.18±0.03	0.15±0.01	ND	0.40±0.01	0.40±0.01	0.25±0.01
I.S	35.00	1309	n-Butyl- benzene, ^h	C ₁₀ H ₁₄	134.21	1.98±0.01	1.98±0.01	1.99±0.01	1.99±0.04	1.99±0.04	1.98±0.01
35	35.55	1318	2-Heptanol ^f	C ₇ H ₁₆ O	116.20	2.22±0.22	0.54±0.03	0.46±0.01	0.50±0.01	0.50±0.01	0.07±0.03
36	36.63	1335	Mesitylene ^h	C ₉ H ₁₂	120.19	0.11±0.01	0.11±0.01	0.12±0.01	ND	ND	ND
37	37.81	1353	1-Hexanol ^f	C ₆ H ₁₄ O	102.17	7.04±1.49	4.94±0.04	6.43±0.39	13.8±0.90	13.8±0.90	1.40±0.08
38	38.25	1360	Pentanoic acid ^e	C ₁₀ H ₂₀ O ₂	172.26	0.09±0.03	0.09±0.01	0.05±0.01	ND	ND	ND
39	39.86	1383	3-Hexen-1-ol, (Z)- ^f	C ₆ H ₁₂ O	100.15	ND	ND	ND	ND	ND	0.08±0.01
40	39.22	1374	2,4,6-trimethyl-pyridine ^h	C ₈ H ₁₁ N	121.17	0.06±0.02	0.03±0.03	ND	0.04±0.03	0.04±0.03	ND
41	40.10	1387	Octanoic acid, methyl ester ^e	C ₉ H ₁₈ O ₂	158.2	0.20±0.08	0.18±0.02	0.21±0.03	0.19±0.01	0.19±0.01	0.20±0.07
42	40.41	1405	3-Octanol ^f	C ₈ H ₁₈ O	130.22	0.33±0.04	0.16±0.03	0.17±0.04	ND	ND	ND
43	41.35	1405	3-Octen-2-one, (E)- ^s	C ₈ H ₁₄ O	126.19	ND	0.05±0.01	ND	0.19±0.01	0.19±0.01	ND
44	41.66	1410	Hexanoic acid, butyl ester ^e	C ₁₀ H ₂₀ O ₂	172.26	0.15±0.01	0.20±0.02	0.19±0.05	0.15±0.01	0.15±0.01	ND
45	42.13	1418	2-Octanol ^f	C ₈ H ₁₈ O	130.22	0.11±0.01	0.15±0.01	0.18±0.05	0.16±0.01	0.16±0.01	0.05±0.01
46	42.53	1424	1,3-Di-tert-butylbenzene	C ₁₄ H ₂₂	190.32	0.32±0.07	2.15±0.29	0.63±0.03	0.32±0.06	0.32±0.06	0.24±0.07
47	42.74	1428	2-Octenal, (E)- ^b	C ₈ H ₁₄ O	126.19	0.03±0.01	ND	0.12±0.01	0.13±0.04	0.13±0.04	ND
48	43.46	1439	1-Tridecene ^a	C ₁₃ H ₂₆	182.34	0.11±0.02	ND	ND	0.28±0.01	0.28±0.01	ND
49	44.03	1448	1-Octen-3-ol ^f	C ₈ H ₁₆ O	128.21	0.22±0.04	0.19±0.03	0.29±0.01	0.34±0.02	0.34±0.02	0.04±0.01
50	44.23	1451	Acetic acid ^e	C ₂ H ₄ O ₂	60.05	ND	ND	ND	0.33±0.04	0.33±0.04	ND
51	44.45	1455	1-Heptanol ^f	C ₇ H ₁₆ O	116.20	1.01±0.06	0.19±0.03	1.03±0.18	2.62±0.08	2.62±0.08	ND
52	46.05	1479	Diallyl disulphide	C ₆ H ₁₀ S ₂	146.27	ND	ND	ND	ND	ND	ND
53	48.57	1519	Camphor ⁱ	C ₁₀ H ₁₆ O	152.23	0.10±0.02	0.19±0.01	0.07±0.01	0.13±0.05	0.13±0.05	ND

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54	48.72	1522	Benzaldehyde ^b	C ₇ H ₆ O	106.12	ND	ND	ND	ND	0.59±0.09
55	49.79	1540	2,3-Butanediol ^f	C ₄ H ₁₀ O ₂	90.12	0.24±0.06	0.24±0.04	ND	ND	0.56±0.02
56	50.06	1544	Linalool ⁱ	C ₁₀ H ₁₈ O	154.24	0.42±0.09	0.45±0.04	0.60±0.11	0.54±0.03	ND
57	50.30	1548	Propanoic acid ^c	C ₃ H ₆ O ₂	74.07	ND	ND	ND	0.19±0.04	ND
58	52.01	1576	2,3-Butanediol ^f	C ₄ H ₁₀ O ₂	90.12	0.37±0.09	0.52±0.02	0.31±0.02	0.26±0.03	0.72±0.01
59	52.35	1582	Dimethyl Sulfoxide	C ₂ H ₆ OS	78.13	ND	ND	ND	ND	0.11±0.03
60	53.68	1603	Terpinen-4-ol ^f	C ₁₀ H ₁₈ O	154.24	0.10±0.01	0.30±0.08	0.09±0.02	0.16±0.05	ND
61	54.29	1614	1,9-Decadiene ^a	C ₁₀ H ₁₈	138.24	ND	ND	ND	0.06±0.02	ND
62	54.76	1622	Benzoic acid, methyl ester ^h	C ₈ H ₈ O ₂	136.14	0.16±0.05	0.24±0.09	0.14±0.02	ND	1.28±0.06
63	55.17	1630	Butyrolactone ^g	C ₄ H ₆ O ₂	86.08	0.15±0.04	0.11±0.03	0.17±0.04	ND	ND
64	55.69	1639	Butanoic acid ^c	C ₄ H ₈ O ₂	88.10	ND	0.60±0.04	0.35±0.08	0.35±0.08	ND
65	55.87	1642	Levomenthol ⁱ	C ₁₀ H ₂₀ O	156.26	0.66±0.02	1.88±0.05	0.76±0.01	1.26±0.05	ND
66	56.88	1659	1-Nonanol ^f	C ₉ H ₁₈ O	142.23	0.21±0.02	0.11±0.05	0.22±0.01	0.62±0.04	0.05±0.01
67	58.12	1680	2-Methyl-butanoic acid ^c	C ₅ H ₁₀ O ₂	102.13	ND	0.76±0.03	ND	ND	0.22±0.04
68	59.50	1703	Gamma-caprolactone ^g	C ₆ H ₁₀ O ₂	114.14	ND	0.04±0.04	0.06±0.03	0.20±0.06	0.15±0.01
69	61.64	1743	Naphthalene ^h	C ₁₀ H ₈	128.17	0.06±0.01	0.82±0.07	0.05±0.01	0.05±0.05	ND
70	62.18	1753	Methoxy-phenyl-oxime ^h	C ₈ H ₉ NO ₂	151.16	1.50±0.04	0.78±0.07	1.48±0.11	1.64±0.07	1.28±0.04
71	65.48	1813	Benzenemethanol ^h ,	C ₈ H ₁₀ O	122.16	0.03±0.01	0.17±0.02	ND	0.06±0.01	ND
72	68.01	1862	Hexanoic acid ^c	C ₆ H ₁₂ O ₂	116.15	0.37±0.07	0.32±0.02	0.33±0.01	0.74±0.09	ND
73	68.90	1878	Benzyl alcohol ^h	C ₇ H ₈ O	108.13	0.36±0.02	0.10±0.06	0.28±0.01	0.675±0.04	1.20±0.07
74	70.76	1914	Phenylethyl Alcohol ^h	C ₈ H ₁₀ O	122.16	0.25±0.08	0.06±0.03	0.16±0.01	0.34±0.01	0.30±0.01
75	75.43	1987	Phenol ^h	C ₆ H ₆ O	94.11	ND	0.06±0.01	0.19±0.05	0.18±0.01	0.27±0.02
76	75.80	2013	Methyl Eugenol ^h	C ₁₁ H ₁₄ O ₂	178.22	ND	0.10±0.01	ND	0.14±0.04	ND
77	79.81	2079	p-Cresol ^h	C ₇ H ₈ O	108.13	0.38±0.01	0.29±0.01	0.44±0.03	0.47±0.01	ND
Total						53.40	55.92	52.22	70.46	30.23

Peak area reported by mean ± standard deviation (n=3); RT = Retention time in minutes; RI = Retention index; MF = Molecular formula; MW = Molecular weight; ND = Not detected; The superscripts a-j on name compounds represents the functional group of compounds. (a)=hydrocarbons, b=aldehydes, c=carboxylic acids, d=esters, e=ether, f=alcohols, g=ketones, h=aromatics, i=terpenes, j=furane compounds

be confirmed from the present research findings. The most significant functional groups of volatile flavor compounds including terpenes, esters and ethers were nearly the same in both irradiated (up to 10 kGy dose) and non-irradiated seed samples (Table 2). These results were the same as reported for *Houttuynia cordata* Thumb., that irradiation has no effect on the contents of volatile compounds belonging to terpenes, ethers, and esters (Ryu et al. 2008).

From the results of the current study, the volatile flavor compounds of the irradiated samples were mostly found to be identical to those of the non-irradiated control. However, their concentration showed slight changes with the irradiated doses. Twenty three volatile flavor compounds were found common in all irradiated and non-irradiated seeds (Table 3). Amongst these 23 volatile compounds 1-hexanol (13.19%), methyl valerate (8.97%) and butanoic acid, methyl ester (8.34%) were found to be the most abundant compounds (Table 4). These results of slight changes in concentration and formation new compounds were consistent with the earlier studies reported in literature for volatile compounds in irradiated food samples (Gyawali et al. 2008; Shim et al. 2009).

1-Hexanol, the most abundant volatile flavor compound reported in cucumber seeds, is known to give alcoholic and fruity flavor; also called as green leaf-flavor. This volatile alcohol has also been reported in literature as the main component in essential oils (EOs) of perilla, guava, cherry, and olive (Kyoui et al. 2023). In another research work on buckwheat flower, 1-hexanol has been found as the major volatile component which also showed good antimicrobial activities against various pathogenic microbes (Zhao et al. 2018). Methyl valerate, the second most abundant flavor

compound, has been reported in literature for grapes, pineapples and apples, contributing to their fruity aroma and flavors. Volatile flavor compounds present in wine also include methyl valerate, contributing to the aroma profile of beverages (Welke et al. 2012).

The methyl ester of butyric acid, also known as methyl butyrate, has a strawberry, apple, and pineapple scent (Garlapati & Banerjee 2013). Liu et al. (2011) have carried out GC-MS analysis to evaluate the scent components of pineapple fruits that had ripened in the spring, summer, autumn, and winter seasons. They have reported the presence of eleven different types of fragrance components in fall apples. The most common ones were hexanoic acid methyl ester, butyric acid, methyl ester, and butanoic acid 2-methyl ester with relative contents of 57.67, 8.76, and 18.52%, respectively.

The relative concentration of the aforementioned three major compounds in mg/kg, found in control seeds of cucumber were mostly not affected in samples when these were exposed to irradiation of 1 kGy (1-hexanol 6.43, methyl valerate 5.21 butanoic acid, methyl ester 5.23); 5 kGy (1-hexanol 4.94, methyl valerate 5.51, butanoic acid, methyl ester 4.91), and 10 kGy (1-hexanol 13.82, methyl valerate 4.08, butanoic acid, methyl ester 5.13). However, for 20 kGy their concentration decreased to 1.40 mg/kg (1-hexanol), 0.28 mg/kg (methyl valerate), and 0.24 mg/kg (butanoic acid, methyl ester) (Figure 2).

In general, the amounts of volatile flavor constituents did not show any drastic changes at radiation dosages of 1 kGy, 5 kGy, and 10 kGy, which have been determined by toxicological and nutritional studies to be a non-dangerous maximum dose. However, major change in the decrease of

TABLE 2. List of relative contents of functional groups of volatile flavor compounds identified in *Cucumis sativus* L. (cucumber) seeds

S.NO	Major compounds	0 kGY		1 kGY		5 kGY		10 kGY		20 kGY	
		Area%	total	Area%	total	Area%	total	Area%	total	Area%	total
1	Hydrocarbons	18.9	11	15.5	12	14.8	12	21.6	12	10.1	5
2	Aldehydes	0.07	1	ND*	ND	0.24	1	0.19	1	ND	ND
3	Carboxylic acids	20.8	10	25.7	12	24.3	10	20.6	13	24.8	6
4	Esters	10.6	4	11.6	4	12.8	4	5.90	2	31.7	3
5	Ether	2.80	1	1.43	1	2.84	1	2.33	1	4.28	1
6	Alcohols	31.1	16	21.6	17	24.9	14	33.9	12	15.8	11
7	Ketones	ND	ND	0.08	1	ND	ND	0.27	1	ND	ND
8	Aromatic Compounds	5.94	8	11.7	10	7.07	8	4.79	9	12.8	5
9	Terpenes	8.42	6	11.1	6	11.5	7	8.14	7	ND	ND
10	Furan compounds	1.37	1	1.29	2	1.55	2	2.28	2	0.52	1
	Total	100	58	100	65	100	59	100	60	100	33

*ND = Not detected

TABLE 3. List of flavor compounds found common in all non-irradiated and gamma irradiated (1, 5, 10, 20 kGy) cucumber seeds

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	<i>n</i> -Octane	1.15	2.15	0.27	0.49	0.68	1.31	2.00	2.84	0.10	0.34
2	2,4-Dimethylheptane	0.75	1.42	2.45	4.39	0.68	1.31	0.94	1.33	0.50	1.67
3	4-Methyl-octane	1.34	2.50	3.25	5.81	1.66	3.19	1.51	2.15	1.79	5.95
4	Methyl isobutyrate	0.10	0.19	0.19	0.34	0.14	0.27	0.08	0.12	0.59	1.96
5	Methyl butyrate	4.45	8.34	5.23	9.36	4.91	9.41	5.13	7.28	0.24	0.81
6	2-Methylbutanoic acid,	0.96	1.80	2.07	3.70	1.19	2.28	0.65	0.93	5.70	18.9
7	Methyl isovalerate	0.59	1.10	1.11	2.00	0.86	1.64	0.48	0.69	8.71	28.8
8	Methyl valerate	4.79	8.97	5.21	9.32	5.51	10.56	4.08	5.79	0.28	0.94
9	Methyl hexanoate,	4.24	7.95	4.38	7.83	5.16	9.89	5.25	7.46	0.70	2.32
10	2-Pentyl-furan	0.71	1.33	0.66	1.18	0.74	1.42	1.40	1.99	0.04	0.14
11	1-Pentanol	1.11	2.08	0.88	1.57	1.10	2.11	3.33	4.73	0.28	0.93
12	<i>p</i> -Ethyltoluene	0.05	0.10	0.10	0.18	0.09	0.17	0.05	0.07	0.10	0.33
13	2-Heptanol	2.22	4.17	0.54	0.98	0.46	0.89	0.50	0.71	0.07	0.23
14	1-Hexanol	7.04	13.2	4.94	8.83	6.43	12.3	13.8	19.6	1.40	4.63
15	Methyloctanoate	0.20	0.38	0.18	0.32	0.21	0.41	0.19	0.27	0.20	0.66
16	2-Octanol	0.11	0.21	0.15	0.28	0.18	0.34	0.16	0.24	0.05	0.17
17	1,3-Di-tert- butylbenzene	0.32	0.61	2.15	3.84	0.63	1.21	0.32	0.46	0.24	0.80
18	1-Octen-3-ol	0.22	0.41	0.19	0.35	0.29	0.56	0.34	0.48	0.04	0.16
19	2,3-Butanediol	0.37	0.70	0.52	0.94	0.31	0.60	0.26	0.38	0.72	2.39
20	1-Nonanol	0.21	0.39	0.11	0.20	0.22	0.42	0.62	0.88	0.05	0.18
21	Methoxyphenyl oxime,	1.50	2.82	0.78	1.41	1.48	2.84	1.64	2.33	1.28	4.25
22	Benzyl alcohol	0.36	0.67	0.10	0.18	0.28	0.54	0.67	0.95	1.20	3.98
23	Phenylethyl Alcohol	0.25	0.46	0.06	0.12	0.16	0.31	0.34	0.49	0.30	0.99

*Relative concentration based on internal standard peak

TABLE 4. List of major flavor compounds identified seed samples of cucumber (*Cucumis sativus* L.)

S.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	Octane	1.15	2.15	0.27	0.49	0.68	1.31	2.00	2.84	0.10	0.34
2	4-Methyl- octane,	1.34	2.50	3.25	5.81	1.66	3.19	1.51	2.15	1.79	5.95
3	Butanoic acid, methyl ester	4.45	8.34	5.23	9.36	4.91	9.41	5.13	7.28	0.24	0.81
4	Methyl valerate	4.79	8.97	5.21	9.31	5.51	10.6	4.08	5.79	0.28	0.92
5	Hexanoic acid, methyl ester	4.24	7.95	4.38	7.83	5.16	9.89	5.25	7.46	0.70	2.32
6	1-Pentanol	1.11	2.08	0.88	1.57	1.10	2.11	3.33	4.73	0.28	0.93
7	2-Heptanol	2.22	4.17	0.54	0.98	0.46	0.89	0.50	0.71	0.07	0.23
8	1-Hexanol	7.04	13.2	4.94	8.83	6.43	12.3	13.8	19.6	1.40	4.63
9	Methoxy- phenyl- Oxime-	1.50	2.82	0.78	1.41	1.48	2.84	1.64	2.33	1.28	4.25

*Relative concentration based on internal standard peak

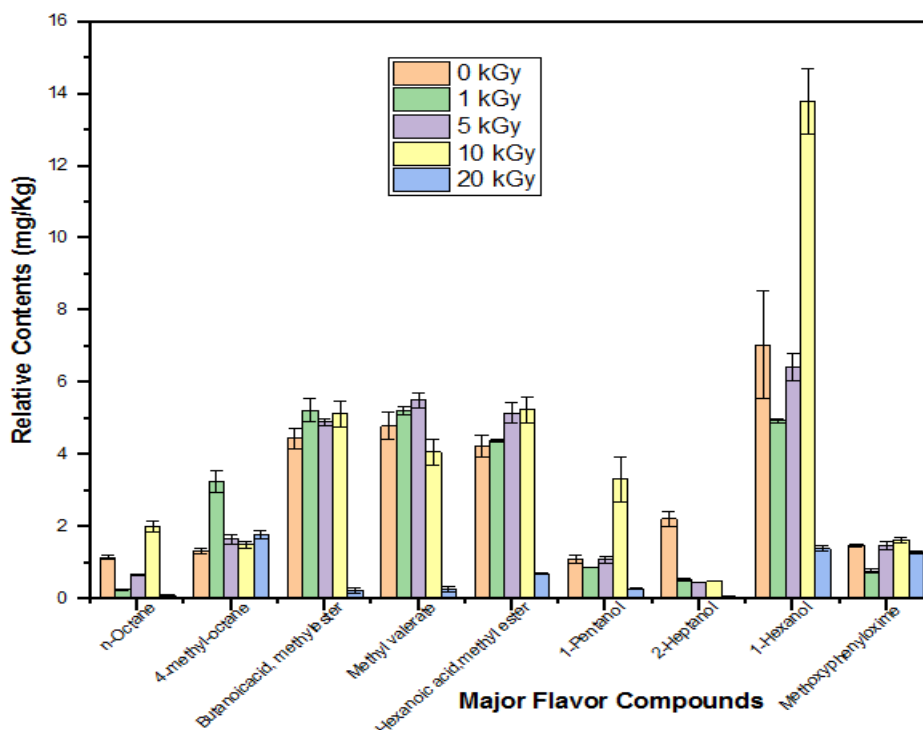


FIGURE 2. Variation of major volatile flavor compounds found in both irradiated and non-irradiated cucumber seed samples

TABLE 5. Compounds produced by gamma irradiation (1, 5, 10, & 20 kGy) in cucumber seeds with properties reported in literature

No	Name of compounds	Gamma ray doses	Properties	References
1	3-Octene-2-one, (E)	1 kGy	Spicy, green, or herbal note to flavors	(Hu et al. 2023)
	1,3-Octadiene	5 kGy	Anti-inflammatory and antioxidant properties; & sweet, floral aroma	(Bozok et al. 2015)
	E-1-Methoxy-3-hexene	1 & 5 kGy	A fresh, green, or fruity note	(Kim et al. 2006)
	3-Methyl-2-butanol	1 & 5 kGy	A sweet, fruity aroma often found in flavors and fragrances	(Choi et al. 1997)
2	β -Myrcene	5 & 10 kGy	Anti-inflammatory, and analgesic; with fruity, herbal, balsamic aroma	(Kovach 2019)
3	Acetic acid	10 kGy	Antimicrobial, and antifungal; sharp, vinegar-like flavor	(Deshmukh & Manyar 2021)
4	Propanoic acid	10 kGy	Anti-microbial and anti-inflammatory; artificial flavor	(Eş et al. 2017)
5	Methyl eugenol	1 & 10 kGy	Yellowish liquid with a clove-like aroma; possess antifungal activity	(Nejad 2014)
6	Dimethyl sulfoxide	20 kGy	Anti-inflammatory, antioxidant; flavor compound in canned corn	(Ralls et al. 1965)
7	Benzaldehyde	20 kGy	Natural flavor compound	(Dionísio et al. 2012)
8	3-Hexene-1-ol	20 kGy	A green, or floral aroma	(Meng et al. 2020)
	2-Methylbutanoic acid	1 & 20 kGy	A strong, fruity, or cheese-like aroma	(Moreira & De Maria 2005)
9	Butanoic acid	1, 5 & 10 kGy	Anti-inflammatory; a strong, rancid, or cheesy odor	(Jiang et al. 2018)
10	Gamma caprolactone	1, 5, 10, & 20 kGy	Antimicrobial properties; creamy, coconut-like aroma	(Silva et al. 2021)
11	Phenol	1, 5, 10, & 20 kGy	Antiseptic, anesthetic, and antioxidant properties	(Putri et al. 2023)

flavor compounds from 58 to 33 was found for irradiated seed samples at 20 kGy (Tables 1 & 2). A similar research finding has also been reported by Wilkinson and Gould (1996), that flavor and aroma changes at 15 kGy dose, depending on a number of factors, including the particular product, the age of seasoning, storage temperature, humidity, and packaging. Most of the identified new compounds produced by the gamma irradiation doses (1, 5, 10, and 20 kGy) in the cucumber seeds were those already reported for several biological activities and flavor contribution as mentioned in Table 5.

Results from the current study suggested that gamma irradiation at doses up to 10 kGy can be employed for food sterilization to increase their shelf life with a slight change in the volatile flavor compounds. The same can be traced from literature that radiation up to 10 kGy dose does not alter or bring changes in both micro and macronutrients

(Arapcheska, Spasevska & Ginovska 2020). Khan et al. (2015) has also reported that gamma irradiation does not bring a major change in the volatile flavor profile of spice samples and declared 10 kGy as a recommended dose of irradiation to increase shelf life of food. Moreover, formation of some new volatile flavor compounds after gamma irradiation was also found in the current study. These formation of new compounds due to irradiation was the same as reported for red peppers by Lee et al. (2004).

Conclusively, the results from current study were largely in line with the published literature from around the world. The volatile flavor compounds were found to have slight changes in concentration due to irradiation. The number of volatile flavor compounds increased due to irradiation up to 10 kGy and then decreased for 20 kGy. Gamma irradiation up to 10 kGy can be the recommended dose of gamma irradiation for food safety.

CONCLUSIONS

A slight variation in concentrations of flavor compounds and formation of some new volatile flavor compounds were found due to gamma irradiation doses. There was an increase in the number of volatile flavor compounds up to 10 kGy and then decrease for 20 kGy. The main flavoring compounds that give the samples their unique flavor was found as unchanged. Thus, 10 kGy is the recommended dose of gamma radiation and can be used for seeds preservation and to improve the shelf life and minimize economic loss. Based on the current study findings of food irradiation, like other preservation techniques, can be called as appropriate method for extending the shelf life, shielding cucumber seeds from microorganisms, along with maintenance of their chemical composition and sensory quality of food.

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