

Effect of Electron Beam Irradiation on the Volatile Flavor Profile of *Elettaria Cardamomum* (L.) Maton., from Pakistan

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Summary: This study was aimed to investigate the effects of electron beam irradiation on volatile flavor profile of *Elettaria cardamomum* (L.) Maton., from Pakistan. The volatile flavor compounds of non-irradiated and 1, 5, 10 and 20 kGy E-beam irradiated samples were extracted by simultaneous distillation-solvent extraction (SDE) and analyzed by gas chromatography mass spectrometry (GC-MS). Volatile flavor compounds were slightly changed by E-beam irradiation as some of the components showed a minor increase or decrease in concentration after ionization treatment. A total of 136 (10684.15 mg/kg) volatile flavor compounds were detected in non-irradiated control samples which decreased to 133 for 1 kGy (11068.61 mg/kg), remained the same 136 for 5 kGy (10884.10 mg/kg) and 10 kGy (11762.52 mg/kg), and increased to 137 for 20 kGy (10973.78 mg/kg) E-beam irradiated samples. There was no major change observed in the application of E-beam irradiation treatment of the samples compared to the non-irradiated ones. E-beam irradiation was therefore concluded to have no adverse effect on the volatile flavor of *Elettaria cardamomum* (L.) Maton and doses up to 20 kGy were declared not to interfere with the flavor perception of consumers.

Keywords: Volatile flavor, *E. cardamomum* (L.) Maton., E-beam irradiation, simultaneous distillation-solvent extraction (SDE), gas chromatography-mass spectrometry (GC-MS)

Introduction

The “Queen of Spices”, *Elettaria cardamomum* (L.), Maton., is one of the world’s most ancient spices and the third most expensive spice, after saffron and vanilla [1, 2]. It is commonly known as small cardamom, green cardamom or true cardamom [3]. Originally *E. cardamomum* belong to the tropical regions of Asia [4], but presently cultivated around the world especially in India, Sri Lanka, Nepal, Pakistan, Bhutan, Iran, Vietnam and Costa Rica [1, 5, 6]. This spice is a non-perishable perennial herbaceous plant belonging to the ginger family Zingiberaceae, growing from thick rootstalk up to 4 m in height. The commercial part of the plant is the fruit, which is used as spice and a flavoring agent [7]. *E. cardamom* is used for flavor in tea and coffee in Pakistan, the Kakakule in Turkey and Masala tea in India. As a flavoring agent it is also used in the preparation of curries, cakes, breads, pickles, rice, and drinks [3, 6]. In the Middle-East countries, traditionally cardamom is used along with coffee in making of a beverage *Gahwa* [8]. The essential oil of fruit is known for its traditional health care, fine perfumery application, and the use in

cosmetic preparation [6]. As a traditional herbal medicine, it has been prescribed in the treatment of gastrointestinal, stomachic, digestive, antiemetic, and carminative disorders [3, 9, 10].

Spices are often originated in developing countries where harvest and storage conditions are inadequately controlled with respect to food hygiene. Mostly they are dried in the open air and become seriously contaminated by air- and soil-borne bacteria, fungi, and insects. Bacterial plate counts of one to 100 million per gram of spice have been reported in literature [11]. According to WHO, good manufacturing practices during harvest and processing could improve their hygienic quality, but frequently not to an extent sufficient to obtain an acceptable microbiological purity level [12]. Therefore irradiation of spices is recommended around the world to extend their shelf life, improve hygienic quality and promote international trade.

Literature survey revealed that in recent years, there have been several research studies on the

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chemical composition of cardamom fruits from around the world. These included studies from Costa Rica [13], Italy [14], India and Sri-Lanka [10, 15, 16], Pakistan [17], Turkey [2], and Iran [18]. All these studies on the chemical compositions of cardamom are still limited and require further analyses.

Most importantly, to the best of our knowledge, no research work has been reported so far on the effects of irradiation on the volatile flavor compounds of spices such as *Elettaria cardamomum* (L.), Maton. Research studies on the effects of irradiation techniques on volatile flavor constituents of spices are urgently required to ascertain their safety, acceptability and promote international trade. In view of these objectives this study was designed to investigate the volatile flavor profile compounds of *E. cardamomum* and to further evaluate any possible effects of sterilization by irradiations such E-beam on these flavor compounds. From literature 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) [19-21]. This advanced SDE-GC-MS technique was applied for the analysis of volatile flavor compounds of *E. cardamomum* spice fruits collected from Pakistan. The identified volatile flavor compounds of the E-beam irradiated samples at 1, 5, 10 and 20 kGy doses were compared with those from non-irradiated (0 kGy) control ones to find out any possible effects of these irradiation doses on the overall flavor of the subject spice.

Experimental

Samples Collection

E. cardamomum fruit samples were collected in triplicate from a local super market in Peshawar, Khyber Pakhtunkhwa, Pakistan. These were identified by a plant taxonomist at Kohat University of Science & Technology, Kohat, Pakistan. A voucher specimen of the samples was deposited at the university herbarium center for future reference. The collected samples were labeled properly in plastic bags and stored at -22 °C in refrigerator (MICOM CFD-0622, Samsung, Korea) until required for analysis.

Reagents

All the reagents used in this research work were purchased from sigma Co. (St Louis, MO, USA) otherwise stated. The extraction solvents; n-pentane and diethyl ether were purchased from Fisher Scientific (Waltham, MA., USA) and were redistilled using a wire spiral packed double distilling apparatus

(Normschliff Geratebau, Wertheim, Germany) before use. The purified water (Milli-Q) was obtained through a water purification system (Millipore Corp., Bedford, USA). Anhydrous Na₂SO₄ was used for dehydration of organic solvents after burning for overnight at 650 °C in a furnace (F 6000, Barnstead Thermolyne Co., IA, USA) and allowing cooling down in desiccator.

Samples Irradiation

Samples were irradiated at 1, 5, 10 and 20 kGy doses by E-beam accelerator (model ELV 4, 2.5 MeV, EB-Tech., Ltd., Daejeon, Korea). The beam currents used were 0.47 mA (0.25 kGy), 1.3 mA (0.5 kGy), 2.96 mA (1 kGy) and 3.7 mA (3 kGy), and velocities of 20 m/min (0.25, 0.5, 1 kGy) and 10 m/min (3 kGy). The absorbed doses were investigated with cellulose triacetate (CTA) dosimeter. The irradiated samples were stored at -22 °C in refrigerator until required for analysis. The non-irradiated samples were used as control [22, 23].

Extraction of Volatile Flavor Compounds

Each 30.0 g of *E. cardamomum* sample was homogenized using a blender (MR 350CA, Braun, Spain) and mixed well with 500 mL distilled water. The pH was adjusted at 7.0, using 0.1 N NaOH and 0.1 N HCl through pH meter (HM-30P, DKK-TOA Corp., Tokyo, Japan). Then as internal standard, 10 mL of n-butyl benzene (110 ppm in n-pentane) was added and the resultant slurry so obtained was used for the extraction of flavor compounds. The volatile compounds were extracted for 3 hours with 100 mL redistilled n-pentane/diethyl ether mixture (1:1, v/v), using a simultaneous steam distillation and extraction (SDE, Likens & Nickerson type) apparatus [24], as modified under atmospheric pressure by Schultz *et al.* [25]. The extract obtained was dehydrated with 10 g anhydrous Na₂SO₄ for overnight and concentrated to final volume of approximately 2.5 mL using a vigreux column. Finally it was concentrated to 0.5 mL under mild stream of N₂ gas. This sample obtained was used for the gas chromatography-mass spectrometry (GC-MS) analysis [26, 27].

Analysis of Volatile Organic Compounds by GC/MS

The quantitative analysis was done by using gas chromatography-mass spectrometry instrument (GC-MS-QP2010, Shimadzu, Japan) in the electron impact ionization (EI) mode. The voltage of ionization and temperature of injector and ion source were 70 eV, 230 °C and 250 °C, respectively. The mass spectrometer was scanned from 45 to 450 m/z. The capillary column used for separation was DB-WAX, 60 m length × 0.25 mm diameter, 0.25 μm

film thickness (Agilent, J&W, USA). The oven temperature was programmed as: 40 °C (isothermal for 3 min) which was ramped to 180 °C (isothermal for 5 min) at 2 °C/min, to 200 °C (isothermal for 10 min) at 4 °C/min, to 220 °C (isothermal for 5 min) at 5 °C/min and then to 250 °C (isothermal for 10 min) at 5 °C/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) [26, 27].

Table-1: GC-MS conditions and operating parameters as applied for volatile flavor compounds of *Elettaria cardamomum* (L.) Maton., fruits from Pakistan

GC-MS	GCMS-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 °C (3 min), to 2 °C/min-180°C(5 min), to 4 °C/min-200 °C (10 min), to 5°C/min-220 °C (5 min) and to 5 °C/min-250 °C (10 min)
Injector temperature	240 °C
Ion source temperature	250 °C
Ionization	Electron Impact (EI)
Ionization voltage	70 eV
Mass range	50 ~ 400 (m/z)
Injection volume	1µL
Split ratio	1:100

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 (C₇-C₄₀), which appeared above and below the solute [28]. Mass spectra of volatile organic compounds were identified with the help of our own mass spectral data and those contained within the FFNSC 2.0, NIST 05 and Willey 7 spectral libraries provided with the GCMS instrument and mass spectral data books [29, 30], as well as by the comparison of retention indices to reference data [31] and online available data from The Pherobase [32]. The similarity ratio more than 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 [26, 27]:

$$\text{Component content (mg/kg)} = \frac{C \times 1000 \text{ g}}{A \times B \text{ g}}$$

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

Statistical Analysis

Data were reported as average of three replicates (n=3) as mg/kg of the sample. 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 different superscript letters.

Results and Discussion

Volatile flavor profile compounds were extracted from non-irradiated and electron beam irradiated samples of *E. cardamomum* fruits from Pakistan and identified by comparing their spectral data and retention indices. The average results of three replicates so obtained are reported in Table-2, according to their elution order on DB-WAX column with ranges of their amounts corresponding to irradiation doses. Table-3 gives content of functional groups of volatile flavor compounds identified in non-irradiated and E-beam irradiated samples and Table-4 shows the effect of E-beam irradiation doses on major volatile flavor compounds from *E. cardamomum* fruits.

The GC-MS chromatograms of non-irradiated and electron beam irradiated samples at 1, 5, 10 and 20 kGy doses showed very close similarity in their volatile flavor constituents (Fig. 1). A total of 136 volatile flavor compounds were detected in non-irradiated and 5, 10 kGy irradiated samples which decreased to 133 for 1 kGy and increased to 137 for 20 kGy irradiated samples. The relative contents of the detected compounds were: 10684.15 mg/kg (0 kGy), 11068.61 mg/kg (1 kGy), 10884.10 mg/kg (5 kGy), 11762.52 mg/kg (10 kGy), and 10973.78 mg/kg (20 kGy) (Table-1). Thus a slight variation in the number of volatile compounds was observed for E-beam irradiation of *E. cardamomum* fruit samples. These results were well supported by literature on irradiated foods by Giyawali et al., 2008 [26]. Two compounds *p*-anisaldehyde and geranic acid were detected only in irradiated samples, *α*-cubebene was detected at 1 kGy irradiated samples, cryptone, cuminaldehyde and isomenthone disappeared only at 1 kGy doses, (*S*)-carvone acetate appeared at 0 and 20 kGy, 2,5-dimethy-1,5,7-octatrien-3-ol was detected at 0, 5 and 20 kGy, nonanoic acid was absent at 1 and 5 kGy, and geranyl acetone disappeared only at 20 kGy dose of e-beam irradiated samples. One inter conversion of peaks was detected due to e-beam irradiation. The peak (RI = 1453) was detected as *α*-cubebene only at 1 kGy dose while at 0, 5, 10 and 20 kGy (RI = 1456) its peak area decreased and identified as isomenthone (Table-2).

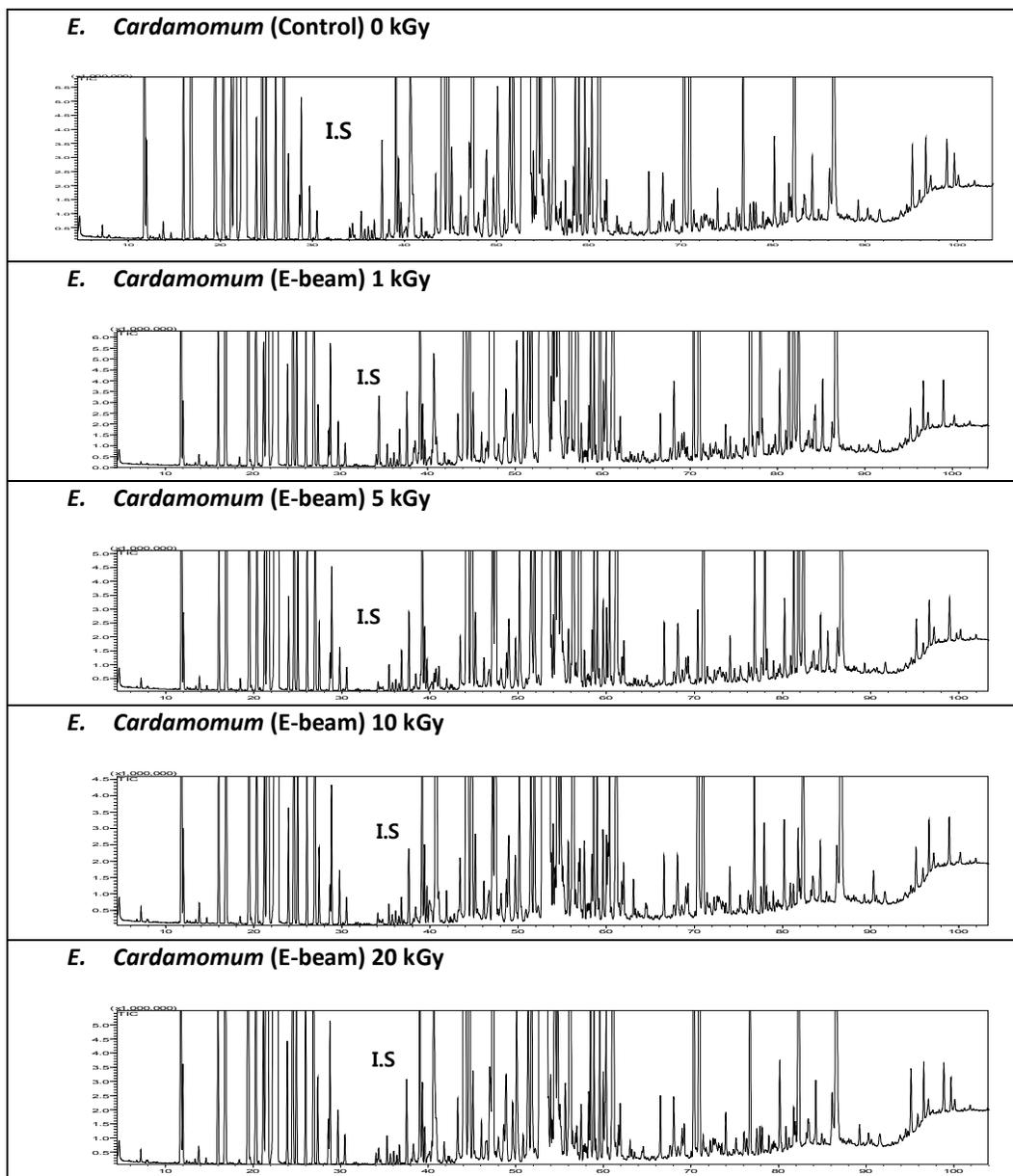


Fig. 1: GC-MS Chromatograms of volatile flavor compounds of non-irradiated and E-beam irradiated *Elettaria cardamomum* (L.) Maton., from Pakistan.

Table-2: Volatile flavor compounds identified in non-irradiated and E-beam irradiated fruits of *Elettaria cardamomum* (L.) Maton. From Pakistan.

No.	RI ¹⁾	Compound name	MF ²⁾	Relative contents (mg/kg)				
				0 kGy	1 kGy	5 kGy	10 kGy	20 kGy
1	808	Acetone ^{d,3)}	C ₃ H ₆ O	0.10 ^{a,4)}	0.05 ^a	0.08 ^a	0.07 ^a	0.07 ^a
2	878	Ethyl acetate ^a	C ₄ H ₈ O ₂	0.04 ^{a,4)}	0.33 ^b	1.30	1.71	1.39
3	907	2-Methylbutanal ^b	C ₅ H ₁₀ O	0.03 ^a	0.12 ^b	0.26	0.31	0.27
4	911	3-Methylbutanal ^b	C ₅ H ₁₀ O	0.03 ^a	0.12 ^b	0.26	0.33	0.28
5	1006	Tricyclene ^f	C ₁₀ H ₁₆	0.12	0.07	0.10	0.12	0.15
6	1016	α -Pinene ^f	C ₁₀ H ₁₆	133.58 ^b	74.80 ^a	90.38	114.98	116.85
7	1021	α -Thujene ^f	C ₁₀ H ₁₆	20.55 ^a	9.12	10.62	11.57	12.05
8	1030	Toluene ^f	C ₇ H ₈	0.11	0.21	0.32	0.27	0.26
9	1037	2-Methyl-3-buten-2-ol ^e	C ₅ H ₁₀ O	0.09	0.11	0.18	0.19	0.17
10	1045	3-Hexanone ^d	C ₆ H ₁₂ O	0.05	0.09	0.12	0.14	0.06
11	1051	α -Fenchene ^f	C ₁₀ H ₁₆	0.87	0.42	0.50	0.50	0.57

Table-2 Continue. . .

12	1059	Camphene ^f	C ₁₀ H ₁₆	2.63	1.78	1.89	2.64	2.36
13	1075	n-Hexanal ^b	C ₆ H ₁₂ O	0.59	0.55	0.64	0.68	0.71
14	1099	β -Pinene ^f	C ₁₀ H ₁₆	40.73 ^a	21.78	25.76	28.09	29.89
15	1115	Sabinene ^f	C ₁₀ H ₁₆	309.69 ^a	137.71	169.14	145.67	179.95
16	1143	δ -3-Carene ^f	C ₁₀ H ₁₆	0.54 ^a	1.54	2.01	1.03	0.69 ^b
17	1152	4-Methylhexanal ^b	C ₇ H ₁₄ O	0.05	0.04	0.05	0.05	0.06
18	1160	Myrcene ^f	C ₁₀ H ₁₆	181.39 ^a	95.02	106.67	106.52	114.52
19	1164	ρ -Mentha-1(7),8-diene ^f	C ₁₀ H ₁₆	0.26 ^a	1.07	0.77	0.94	0.28 ^a
20	1173	α -Terpinene ^f	C ₁₀ H ₁₆	36.31	47.62	43.07	50.47	53.80
21	1178	Heptanal ^b	C ₇ H ₁₄ O	0.29	0.23	0.26	0.26	0.29
22	1186	Dehydro-1,8-cineole ^g	C ₁₀ H ₁₆ O	16.56 ^a	24.17	25.68	29.74	28.69
23	1194	Limonene ^f	C ₁₀ H ₁₆	467.73	443.32	353.08	396.02	414.44
24	1211	1,8-Cineole ^g	C ₁₀ H ₁₈ O	2637.88	1878.54	2206.57	2226.50	2243.51
25	1225	2-Amyl furan ⁱ	C ₉ H ₁₄ O	0.11	0.15	0.14	0.16	0.16
26	1229	(Z)- β -Ocimene ^f	C ₁₀ H ₁₆	13.20	15.22	13.68	15.16	15.71
27	1239	γ -Terpinene ^f	C ₁₀ H ₁₆	77.03	86.27	83.55	105.91	101.65
28	1246	(E)- β -Ocimene ^f	C ₁₀ H ₁₆	26.13	28.40	26.54	29.62	30.22
29	1262	ρ -Cymene ^f	C ₁₀ H ₁₄	24.09	24.93	30.21	29.01	25.49
30	1268	Hexyl acetate ^a	C ₈ H ₁₆ O ₂	0.13	0.17	0.15	0.11	0.14
31	1275	α -Terpinolene ^f	C ₁₀ H ₁₆	70.16 ^a	135.49 ^a	108.66	103.99	98.98
32	1282	Octanal ^b	C ₈ H ₁₆ O	13.08	9.17	10.09	10.07	11.07
33	1299	(E)-4,8-Dimethyl-nona-1,3,7-triene ^f	C ₁₁ H ₁₈	8.20 ^a	5.22	5.51	5.20	5.74
I.S	1302	Butyl benzene	C ₁₀ H ₁₄	-	-	-	-	-
34	1329	Sulcatone ^d	C ₈ H ₁₄ O	3.00	3.78	3.76	3.92	4.08
35	1335	Hexylpropionate ^a	C ₉ H ₁₈ O ₂	0.06 ^a	0.01	0.01	0.03	0.07 ^b
36	1346	Melonal ^b	C ₉ H ₁₆ O	0.14	0.21	0.19	0.21	0.21
37	1349	Amylcarbinol ^c	C ₆ H ₁₄ O	0.23	0.25	0.26	0.28	0.28
38	1353	Nonanal ^b	C ₉ H ₁₈ O	0.10	0.09	0.10	0.14	0.11
39	1364	(E,Z)-Alloocimene ^f	C ₁₀ H ₁₆	0.09	0.17	0.13	0.15	0.16
40	1383	ρ -1,3,8-Menthatriene ^f	C ₁₀ H ₁₄	0.90 ^a	1.97	1.45	1.60	1.57
41	1387	Fenchone ^g	C ₁₀ H ₁₆ O	0.64 ^b	1.73	0.41 ^a	0.39 ^a	3.02
42	1401	6,7-Epoxy myrcene ^g	C ₁₀ H ₁₆ O	5.57 ^a	3.28	3.89	2.73	3.70
43	1407	Perillene ^g	C ₁₀ H ₁₄ O	1.03	1.30	1.33	1.43	1.50
44	1420	(E)-2-Octenal ^b	C ₈ H ₁₄ O	1.35	0.97	1.05	1.06	1.22
45	1425	ρ -Cymene ^f	C ₁₂ H ₁₂	1.18 ^a	5.61	6.02	3.64 ^b	2.54 ^b
46	1432	α -Thujone ^g	C ₁₀ H ₁₆ O	0.10	0.07	0.09	0.11	0.09
47	1438	(Z)-Linalool oxide ^g	C ₁₀ H ₁₈ O ₂	9.39	11.57	12.02	13.09	13.39
48	1447	Furfural ^b	C ₅ H ₄ O ₂	0.40 ^a	0.58	0.56	0.85	0.82
49	1450	(E)-Limonene oxide ^g	C ₁₀ H ₁₆ O	3.00	3.12	3.27	4.17	3.94
50	1453	α -Cubebene ^h	C ₁₅ H ₂₄	ND ⁵⁾	6.26	ND	ND	ND
51	1456	Isomenthone ^g	C ₁₀ H ₁₈ O	0.13	ND	0.10	0.18	0.23
52	1462	(E)-Sabinene hydrate ^g	C ₁₀ H ₁₈ O	91.18 ^a	46.68	62.63	42.94	46.93
53	1466	(E)-Linalool oxide ^g	C ₁₀ H ₁₈ O ₂	8.45	9.91	10.03	11.43	11.07
54	1470	Octyl acetate ^a	C ₁₀ H ₂₀ O ₂	4.44	4.02	4.58	5.01	4.80
55	1479	α -Campholenal ^b	C ₁₀ H ₁₆ O	2.29	1.48	2.61	1.59	3.49
56	1483	Methyl-2-hydroxy-3-methyl pentanoate ^a	C ₇ H ₁₄ O ₃	2.34	2.01	2.40	2.25	2.35
57	1485	α -Copaene ^h	C ₁₅ H ₂₄	0.40 ^a	32.85 ^c	5.16 ^b	167.01 ^d	57.37 ^c
58	1490	Decanal ^b	C ₁₀ H ₂₀ O	4.27	5.03	4.34	4.63	4.56
59	1504	Benzaldehyde ^j	C ₇ H ₆ O	1.30	2.71	1.89	5.29 ^a	3.29
60	1514	Methyl-2-hydroxy-4-methylpentanoate ^a	C ₇ H ₁₄ O ₃	0.78	0.66	0.73	0.34	0.78
61	1530	(Z)-4-Decenal ^b	C ₁₀ H ₁₈ O	10.12	9.82	10.01	11.71	11.77
62	1544	Linalool ^g	C ₁₀ H ₁₈ O	794.13	827.75	875.03	1041.68	942.12
63	1553	Linalyl acetate ^g	C ₁₂ H ₂₀ O ₂	292.20	187.19	293.76	257.61	265.73
64	1559	ρ (Z)-Mentha-2-en-1-ol ^g	C ₁₀ H ₁₈ O	13.87	13.68	13.14	14.52	14.79
65	1562	Isogeranial ^b	C ₁₀ H ₁₆ O	0.77	0.91	0.73	1.04	0.85
66	1568	Nopinone ^d	C ₉ H ₁₄ O	0.10	0.16	0.06	0.02 ^a	0.06
67	1575	α -Fenchyl acetate ^g	C ₁₂ H ₂₀ O ₂	4.74	4.83	4.92	5.29	5.51
68	1583	Elemene ^h	C ₁₅ H ₂₄	4.58	5.31	5.42	8.68	6.11
69	1590	(E)-Caryophyllene ^h	C ₁₅ H ₂₄	7.01 ^a	260.41 ^b	57.12	33.64	22.59
70	1595	Terpinen-4-ol ^g	C ₁₀ H ₁₈ O	282.12	300.56	302.98	347.35	339.04
71	1606	4-Terpinenyl acetate ^g	C ₁₂ H ₂₀ O ₂	4.35	4.54	4.80	5.50	5.62
72	1617	Aromadendrene ^h	C ₁₅ H ₂₄	6.67	6.02	7.49	8.16	7.50
73	1621	(E)- ρ -Mentha-2,8-dien-1-ol ^g	C ₁₀ H ₁₈ O	16.13	21.34	18.07	20.07	20.75
74	1634	L(-)-Menthof ^g	C ₁₀ H ₂₀ O	8.26	9.96	10.45	12.88	11.73
75	1643	δ -Terpinyl acetate ^g	C ₁₂ H ₂₀ O ₂	27.75	28.92	30.38	33.64	33.25
76	1655	ρ -Allylanisole ^j	C ₁₀ H ₁₂ O	1.58 ^a	3.00	2.21	2.71	4.46 ^b
77	1660	Cryptone ^d	C ₉ H ₁₄ O	0.77	ND	1.93 ^a	2.71 ^a	0.85
78	1665	δ -Terpineol ^g	C ₁₀ H ₁₈ O	30.33	76.23 ^a	52.42	43.31	39.02
79	1671	Neral ^b	C ₁₀ H ₁₆ O	56.11	63.55	57.27	60.22	59.95
81	1699	α -Terpinyl acetate ^g	C ₁₂ H ₂₀ O ₂	3716.98	3592.20	3930.33	4205.27	3851.49
82	1709	Germaacrene D ^h	C ₁₅ H ₂₄	9.06	12.48	10.12	12.84	10.73
83	1712	δ -Cadinene ^h	C ₁₅ H ₂₄	2.73 ^a	7.47	4.46 ^b	8.61	5.77 ^b
84	1717	Neryl Acetate ^a	C ₁₂ H ₂₀ O ₂	83.72	126.70	110.85	135.81	113.03
85	1723	(E)-Citral ^g	C ₁₀ H ₁₆ O	81.37	121.89	89.83	92.57	79.96
86	1728	5-Dodecenal ^b	C ₁₂ H ₂₂ O	9.48	10.55	11.76	12.24	11.30
87	1735	2,6-Dimethyl-1,5,7-octatrien-3-ol ^g	C ₁₀ H ₁₆ O	0.15	ND	0.22	ND	0.30
88	1739	(E)-Piperitol ^g	C ₁₀ H ₁₈ O	7.24	12.93	10.40	12.48	12.12

Table-2 Continue. . . .

89	1749	Geranyl acetate ^g	C ₁₂ H ₂₀ O ₂	168.90	214.52	202.72	259.29	227.67
90	1754	Decanol ^f	C ₁₀ H ₂₂ O	1.54 ^a	1.21 ^a	0.06	0.09	0.12
91	1760	Sesquiphellandrene ^h	C ₁₅ H ₂₄	0.4 ^a	117.93	69.13	6.88 ^c	2.32 ^b
92	1763	α -Curcumene ⁱ	C ₁₅ H ₂₂	0.52 ^a	81.50	52.85	9.41 ^c	3.37 ^b
93	1767	Cuminaldehyde ^j	C ₁₀ H ₁₂ O	0.46	ND	0.12 ^a	0.72	0.40
94	1772	Phellandren-8-ol ^g	C ₁₀ H ₁₆ O	3.66 ^a	7.31	6.08	11.63	7.96
95	1777	(Z)-Sabinol ^g	C ₁₀ H ₁₆ O	1.37	1.77	1.93	1.88	1.97
96	1783	Myrtenol ^g	C ₁₀ H ₁₆ O	1.24	1.88	1.76	2.12	1.91
97	1787	(Z)-4-Decenyl-1-ol ^f	C ₁₀ H ₂₀ O	8.27	9.37	9.23	9.12	9.79
98	1791	Nerol ^g	C ₁₀ H ₁₈ O	23.48	29.79	27.30	30.65	30.81
99	1801	2-Tridecanone ^d	C ₁₃ H ₂₆ O	2.68	2.79	2.97	3.03	3.02
100	1810	Anethole ⁱ	C ₁₀ H ₁₂ O	15.26	16.98	14.76	14.44	23.86 ^a
101	1818	Germairene B ^h	C ₁₅ H ₂₄	9.91	13.53	12.69	12.72	12.61
102	1824	(E)-Carveol ^g	C ₁₀ H ₁₆ O	19.83	30.05	26.93	23.86	30.92
103	1828	Terpinyl butyrate ^k	C ₁₄ H ₂₄ O ₂	1.31	1.39	1.53	1.87	1.81
104	1839	Geraniol ^g	C ₁₀ H ₁₈ O	246.11	288.24	281.79	296.04	287.02
105	1845	(E)-Geranylacetone ^g	C ₁₃ H ₂₂ O	1.86	0.64	0.67	0.78	ND
106	1851	(E)-2-Dodecenal ^h	C ₁₄ H ₂₂ O	3.98	3.22	4.40	5.23	4.99
107	1855	(Z)-Carveol ^g	C ₁₀ H ₁₆ O	6.01	7.86	7.48	7.81	7.69
108	1876	(Z)- ρ -Mentha-1(7), 8-dien-2-ol ^g	C ₁₀ H ₁₆ O	1.50	1.59	1.12	6.35 ^b	3.00 ^a
109	1879	Dodecadienal ^b	C ₁₂ H ₂₀ O	1.64	1.18	1.17	1.35	1.29
110	1903	Z-7-Dec-ylacetate ^a	C ₁₂ H ₂₂ O ₂	1.83	1.90	1.78	1.41	1.60
111	1959	Dodecanol ^f	C ₁₂ H ₂₆ O	0.20	0.27	0.29	0.38	0.35
112	1964	Cubebol ⁱ	C ₁₅ H ₂₆ O	2.51	3.34	3.38	1.82 ^a	1.77 ^a
113	1972	Caryophyllene oxide ^l	C ₁₅ H ₂₄ O	8.62 ^a	19.31	15.57	13.33	12.34
114	1991	Perilla alcohol ^g	C ₁₀ H ₁₆ O	3.33 ^a	6.25	5.57	5.06	4.89
115	1995	E-7-Tetradecenol ^g	C ₁₄ H ₂₈ O	4.65	5.60	5.48	4.95	5.57
116	1999	ρ -Anisaldehyde ^j	C ₈ H ₈ O ₂	ND	1.96	1.03	0.10 ^a	0.29 ^a
117	2012	2-Pentadecanone ^d	C ₁₅ H ₃₀ O	0.72	0.62	0.64	0.98	1.04
118	2018	Cinnamaldehyde ^l	C ₉ H ₈ O	2.33 ^a	45.06 ^c	12.39 ^a	332.92	138.53
119	2031	Z-Nerolidol ⁱ	C ₁₅ H ₂₆ O	236.54	311.06	299.52	314.17	307.33
120	2040	ρ -Mentha-1,4-dien-7-ol ^g	C ₁₀ H ₁₆ O	2.66	3.64	3.64	3.81	4.73
121	2043	1,10-Diepicubenol ⁱ	C ₁₅ H ₂₆ O	0.99	1.50	1.40	2.22 ^a	1.73
122	2057	Z-Methylcinnamate ^j	C ₁₀ H ₁₀ O ₂	1.79 ^a	3.08	2.52	3.41	2.57
123	2083	ρ -Cymen-7-ol ^g	C ₁₀ H ₁₄ O	1.02	1.26	1.40	1.45	1.35
124	2144	Eugenol ⁱ	C ₁₀ H ₁₂ O ₂	24.92	388.70 ^a	28.02	34.69	46.80
125	2164	Thymol ⁱ	C ₁₀ H ₁₄ O	3.98	95.40 ^a	6.86	14.23 ^b	4.16
126	2168	Germairene-4-ol ⁱ	C ₁₅ H ₂₆ O	4.10	6.91	52.07 ^a	3.99	3.87
127	2174	Nonanoic acid ^f	C ₉ H ₁₈ O ₂	0.56	ND	ND	1.99	0.70
128	2188	α -Cadinol ⁱ	C ₁₅ H ₂₆ O	0.61	1.16	1.03	1.68	1.38
129	2191	Carvacrol ⁱ	C ₁₀ H ₁₄ O	1.31	1.70	1.62	1.68	1.84
130	2204	Z,E-Farnesal ⁱ	C ₁₅ H ₂₄ O	12.26	15.76	14.74	15.21	15.52
131	2220	Intermedol ⁱ	C ₁₅ H ₂₆ O	3.15	5.04	4.38	4.14	4.29
132	2227	Curlone ⁱ	C ₁₅ H ₂₂ O	1.74	76.67 ^a	45.79 ^a	4.12	2.28
133	2239	ar-Tumerone ⁱ	C ₁₅ H ₂₀ O	5.25 ^a	177.56 ^c	118.65 ^b	12.68 ^a	7.20 ^a
134	2244	Carvone acetate ^k	C ₁₂ H ₁₈ O ₃	2.84 ^a	ND	ND	ND	3.95 ^b
135	2253	E,E-Farnesal ⁱ	C ₁₅ H ₂₄ O	43.12 ^a	57.88 ^a	60.80 ^a	60.96 ^a	58.18 ^a
136	2277	Caprylic acid ^f	C ₁₀ H ₂₀ O ₂	3.11 ^a	6.48 ^b	6.30 ^b	6.83 ^b	6.99 ^b
137	2329	Geranic acid ^f	C ₁₀ H ₁₆ O ₂	ND	8.62 ^a	11.93 ^{ab}	15.39 ^b	13.19 ^{ab}
138	2340	E,E-Farnesol ⁱ	C ₁₅ H ₂₆ O	78.39 ^a	74.62 ^a	71.09 ^a	92.62 ^a	101.14 ^a
139	2524	Geranyl Linalool ^k	C ₂₀ H ₃₄ O	12.71 ^a	13.12 ^a	13.41 ^a	14.73 ^a	14.65 ^a
Total				10684.15	11068.61	10884.10	11762.52	10973.78

1) RI= Retention index

2) MF= Molecular formula

3) a-k Superscripts represent the functional group of compound (a= ester; b= aldehyde; c= acid; d= ketone; e= alcohols;

f= monoterpene hydrocarbon; g= oxygenated monoterpene; h= sesquiterpene hydrocarbon; i= oxygenated sesquiterpene;

j= aromatic hydrocarbons; k= miscellaneous)

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

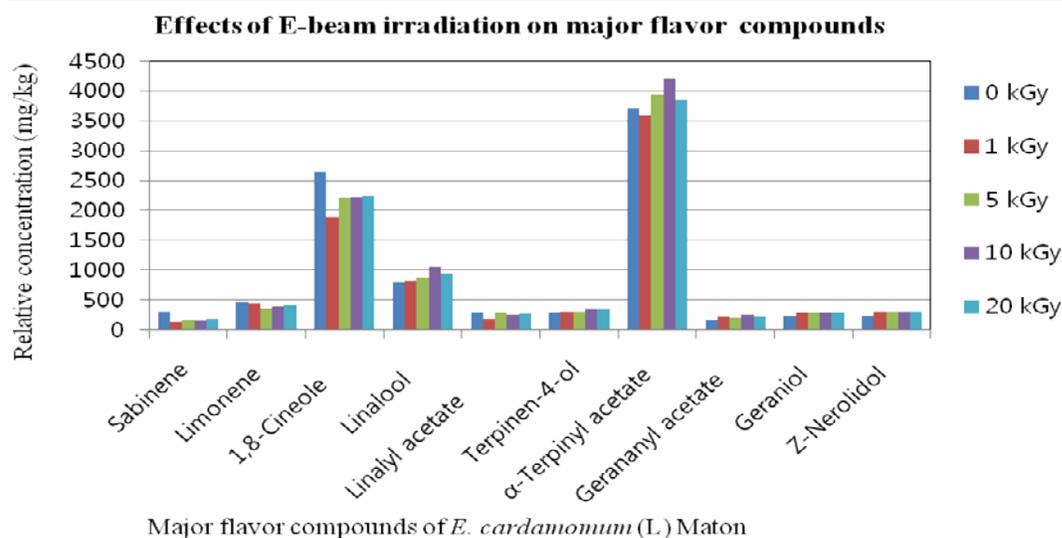
5) ND= not detected

Table-3: Relative content of functional groups of volatile flavor compounds identified in non-irradiated and E-beam irradiated *E. cardamomum* (L.) Maton. fruits from Pakistan.

No	Functional group	0 kGy		1 kGy		5 kGy		10 kGy		20 kGy	
		Area%	No	Area%	No	Area%	No	Area%	No	Area%	No
1	Esters	0.87	8	1.23	8	1.12	8	1.25	8	1.13	8
2	Aldehydes	0.98	18	0.97	19	0.97	19	0.95	19	1.03	19
3	Acids	0.03	2	0.14	2	0.17	2	0.21	3	0.19	3
4	Ketones	0.07	7	0.07	6	0.09	7	0.09	7	0.08	7
5	Alcohols	0.14	6	0.15	6	0.14	6	0.13	6	0.15	6
6	Monoterpene hydrocarbons	13.25	21	10.28	21	9.92	21	9.80	21	11.00	21
7	Oxygenated monoterpenes	79.89	39	70.29	37	78.41	39	77.18	38	78.28	38
8	Sesquiterpene hydrocarbons	0.39	9	4.91	10	2.06	09	2.28	09	1.17	09
9	Oxygenated sesquiterpenes	3.72	12	6.78	11	6.33	12	4.48	12	4.71	12
10	Aromatic hydrocarbons	0.50	11	5.05	11	0.66	11	3.49	11	2.07	11
11	Miscellaneous	0.16	3	0.13	2	0.14	2	0.14	2	0.19	3
Total		100	136	100	133	100	136	100	136	100	137

Table-4: Effects of E-beam irradiation on major volatile flavor compounds of *E. cardamomum* (L.) Maton from Pakistan

Major flavor compounds	0 kGy		1 kGy		5 kGy		10 kGy		20 kGy	
	Contents (mg/kg)	Percent (%)								
Sabinene	309.69	2.90	137.71	1.24	169.14	1.55	145.67	1.24	179.95	1.64
Limonene	467.73	4.38	443.32	4.00	353.08	3.24	396.02	3.37	414.44	3.78
1,8-Cineole	2637.88	24.70	1878.54	16.97	2206.57	20.27	2226.50	18.93	2243.51	20.44
Linalool	794.13	7.43	827.75	7.48	875.03	8.04	1041.68	8.86	942.12	8.59
Linalyl acetate	292.20	2.74	187.19	1.69	293.76	2.70	257.61	2.19	265.73	2.42
Terpinen-4-ol	282.12	2.64	300.56	2.72	302.98	2.78	347.35	2.95	339.04	3.09
α -Terpinyl acetate	3716.98	34.79	3592.20	32.45	3930.33	36.11	4205.27	35.75	3851.49	35.10
Geranyl acetate	168.90	1.58	214.52	1.94	202.72	1.86	259.29	2.20	227.67	2.08
Geraniol	246.11	2.30	288.24	2.60	281.79	2.59	296.04	2.52	287.02	2.62
Z-Nerolidol	236.54	2.21	311.06	2.81	299.52	2.75	314.17	2.67	307.33	2.80
Total	9152.27	85.66	8181.08	73.91	8914.91	81.91	9489.59	80.68	9058.31	82.54

Fig. 2: Effects of E-beam irradiation on major volatile flavor profile compounds of *E. cardamomum* (L.) Maton., fruits from Pakistan.

According to the detected functional group classes of volatile flavor compounds, the detected compounds which remained unchanged in number by irradiation however showed slight variations in their relative contents. These included monoterpene hydrocarbons (21), esters (8), alcohols (6) and aromatic hydrocarbons (11). One aldehyde was produced by irradiations, as its number changed from 18 for control, to 19 for all irradiated samples. The noticeable variation was observed for the contents of aromatic hydrocarbons. These were 0.50% for 0 kGy, while 5.05, 0.66, 3.49, and 2.07% for 1, 5, 10 and 20 kGy irradiated samples, respectively. All other volatile flavor compounds both individually and together in their respective classes was detected to show only minor variations (Table-3). There were ten (10) major flavor compounds which contributed more than 70% of the total relative volatile flavor contents of the samples. On the basis of elution order, these were including sabinene, limonene, 1,8-cineole, linalool, linalyl acetate, terpinen-4-ol, α -terpinyl

acetate, geranyl acetate, geraniol and Z-nerolidol (Table-4). All these major compounds were approximately in accordance to the reported literatures for *E. cardamomum* fruits from around the world including Italy [14], India [15], Pakistan [17], Turkey [2], and Iran [18]. In all these studies α -terpinyl acetate was the principal compound, followed by 1,8-cineole and limonene. The same were reported in the present study for *E. cardamomum* from Pakistan.

The E-beam irradiation caused slight variations in the contents of aforementioned major flavor profile compounds. Among these sabinene, limonene, 1,8-cineole and linalyl acetate showed slight increase while linalool, terpinen-4-ol, gernalyl acetate, geraniol and N-nerolidol showed slight decrease due to E-beam irradiation doses (1, 5, 10 and 20 kGy). α -Terpinyl acetate first decreased from 34.79% (0 kGy) to 32.45 (1 kGy) and then increased to 36.11, 35.75, and 35.10% for 5, 10 and 20 kGy E-beam irradiation doses respectively (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 [26]. Similarly Jo and Ahn 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 [33]. In another study on irradiation of foods with higher doses (>10 kGy), the decrement of some of volatile flavor compounds was also reported [34]. These research findings, verify the slight variation in volatile flavor contents of *E. cardamomum* fruits by E-beam irradiation in present study from Pakistan.

Conclusion

The E-beam irradiation of *E. cardamomum* (L.) Maton., fruit samples at 1, 5, 10, and 20 kGy doses caused only slight variations in the number and relative contents of volatile flavor profile compounds. To a greater extent similarity in occurrence of volatile flavor constituents in non-irradiated and irradiated samples was reported. The slight variation reported in the number of volatile compounds was belonging to aldehydes, ketones, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. There was no major change and the volatile flavor of the subject spice remained unaffected by E-beam irradiation technique. All the major flavor compounds responsible for characteristic flavor remained unchanged to a greater extent. Therefore irradiation was declared quite safe up to the recommended dose of 10 kGy. The application E-beam for sterilization was therefore recommended for spices which will extend their shelf life and additionally would also slightly increase their volatile flavor profile compounds.

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