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Impact of γ-irradiation on the Aroma Volatiles, Antioxidant and Antimicrobial Activities of Black and White Pepper (*Piper nigrum* L.)

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ABSTRACT

The aim of this study was to investigate the effect of γ-irradiation on (I) aroma volatiles, (ii) antioxidant activity, (iii) total phenolic content of black and white pepper seeds at 6, 8 and 10 kGy. The evaluation of antimicrobial activity of black and white pepper essential oils (EOs).GC and GC/ MS of black pepper EO revealed that, α-pinene, limonene, δ-carene and β-caryophyllene were the predominant aroma compounds in both control and irradiated samples. However, the area % of the α-pinene, limonene, δ-carene was decreased and β-caryophyllene increased with respect to γ-irradiation dose increased. White pepper EO has caryophyllene oxide as a dominant volatile compound at 10 kGy. Both DPPH and β-carotene were used to determine the antiradical activity of black and white EOs and their extracts. Ethanol extract of irradiated black and white pepper samples at 10 kGy showed a higher antioxidant activity with 72.4% and 76.2% in DPPH and β-carotene assays. Also, ethanol extract of black and white pepper has a higher TPC with 182.5 and 179.47 mgGAE/ 100g DW. The antibacterial activity was measured by agar disc diffusion and MIC method. Black and white pepper showed antimicrobial activity against all tested bacteria with zone of inhibition ranged from 8-20mm. maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (20mm) and minimum against Gram negative bacteria *Escherichia coli* (8mm). Also, the MIC ranged from (0.25– 1.0µl/ml) and the treatment of γ-irradiation (10 kGy) showed no significant effect on the antimicrobial activity of the EOs.

Key words: Black pepper, White pepper, *Piper nigrum*, Antibacterial, Antioxidant, Food irradiation, GC, GC/MS, Phytochemical.

Introduction

In the last years, the irradiation of food and agricultural products has been authorised in about 40 countries, in order to extend the shelf-life of foodstuffs and reduce food loss. It was shown that the food irradiation treatment improves the quality of the products, since it inhibits – besides pathogens – the replication of insects, parasites, bacteria, saprophytic moulds, and yeasts (Bendini *et al.* 1998).

In fact, spices, herbs, and dried vegetable seasonings are currently treated with ionising radiation to eliminate microbial contamination, this, however, may alter the chemical composition and subsequently the flavour of spices in the dependence on the radiation dose used. According to the authors (Wilkinson & Gould 1998), these commodities are commonly dry products and they are relatively resistant to ionizing radiation; in general, they can tolerate doses up to 10 kGy, without any significant changes in flavour. Farkas (1998; 2004; 2006) has detected the threshold doses, causing organoleptic changes of black pepper, which ranged from > 9.0 kGy to 10.0 kGy.

Comparing the gamma irradiation with the heat treatment, it has been unambiguously confirmed that the treatment with ionizing energy is more effective against bacteria than the thermal treatment and does not leave chemical residues in the food product (Tjaberg *et al*. 1972; Loaharanu 1994; Byun *et al.* 1996; Thayer *et al*. 1996; Olson 1998). Thus, ethylene oxide and methyl bromide treatments can be effectively replaced by food irradiation, which is less harmful to the spices than heat sterilisation, which implicates the loss of thermolabile aromatic volatiles and/or causes additional thermally induced changes (e.g. thermal decomposition or production of thermally induced radicals). The practice of food processing industry points out that the heat treatment of spices significantly reduces the content of essential oil by one third on average. Since essential oils are responsible for the organoleptic quality of spices (taste and odour), this fact is a weighty argument for the use of irradiation technology for the purposes of spices sterilization chemical composition of spices essential oils irradiated at various doses have been studied in several publications (Emam *et al*. 1995; Farag *et al.,* 1996; Antonelli *et al*., 1998; Sádecká, 2010).

Due to the extension of global demand on naturally occurring functional foods, researches have focused on herbs and spices, not only for their sensory properties, but also for their antioxidant activity. Pepper (*piper nigrum* L.) is one of the world's most important spices, used for both its aroma and pungency. The main constituent responsible for its aroma is the steam volatile oil, which should normally yield between 1 and 3 % black, green and white peppers which are the main three different forms available in the market, although most of the pepper oil in commerce is distilled from black pepper (Pino *et al.,* 1990).

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For more than a century, many studies have been devoted to the chemical composition of pepper oil and these were reviewed by several authors (Plessi *et al.*, 2002; Singh *et al.*, 2004; Nisha *et al.*, 2009). The monoterpene hydrocarbons account for as much as 70-80% with smaller amounts of sesquiterpene hydrocarbons (20-30%), which appear to possess the main desirable attributes of pepper flavour. Although the oxygenated terpenes are relatively, minor constituents, comprising less than 4% they contribute to the characteristic odour of pepper oil (Pino *et al.*, 1990). Pepper essential oil plays an important role in the manufacture of perfumery and confectionery products. It has been already reported that spice volatile oils and aromatic plant extracts possess strong antioxidant activity (Singh *et al.*, 2004; Suhaj *et al.*, 2006). Also, the effect of γ- and microwave irradiation on the essential oil of black pepper was investigated by(Emam *et al.*, 1995), besides the effect of microwave irradiation on essential oils of black and white pepper and their antioxidant activities were investigated by (Abd Elmageed *et al.*, 2011).

Urbain (1986) showed that the products with low moisture content (10%) are prone to the formation of free radicals by irradiation which leads to increase the production of oxides and alcohols. In addition, the configuration of the side groups on the terpene skeleton, especially the position of double bonds and functional groups, can result in a variety of the compounds produced. The research has demonstrated that gamma irradiation at the dose of 10 kGy (toxicologically and nutritionally confirmed maximum safe dose) can eliminate microbial load of spices without causing any significant organoleptic or chemical alterations (Farkas, 1985, 1987; Ito *et al*., 1985; Narvaiz *et al*., 1989; Sádecká *et al*., 2004, 2005a, b).

Erdogdu and Ekiz (2013), determinated the potential of far infrared and ultraviolet radiation as an alternative technique for the reduction of microbial load as surface pasteurization of black pepper seeds.

The effect of gamma-irradiation at different doses on the antioxidant activities of many spices and herbs were investigated by many authors (Topuz and Ozdemir, 2004; Calucci *et al*., 2003; ;Franco *et al*., 2004;Suhaj *et al*., 2006; Polovka *et al*., 2006, 2009, 2010).

Irradiation is an effective method to decontaminate medicinal plants; however, it may affect active components of the plants. Additionally, the heat generated during steam sterilization may also have affects on the active compounds in plants (Piyanuch and Jarunee 2012). So, the objective of the present study was to determine the effects of γ- irradiation on the aroma compounds, antioxidant activity, total phenolic content, total flavonide content and antimicrobial activity of black and white pepper essential (EOs) oils at different doses 6, 8 and 10 kGy compared with control samples.

Materials and Methods

Materials:

Black and white pepper samples were purchased from local market. HPLC-grade ethanol, anhydrous sodium sulphate, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteu reagent, β-carotene, sodium nitrite, sodium carbonate, sodium hydroxide, aluminium chloride, tween-40 (polyoxyethylene sorbitan monopalmitate), n-alkanes (C7-C21), tert.-butyl hydroquinone (TBHQ). Also, linoleic acid and chloroform (HPLC) were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA).

Methods:

γ- Irradiation process:

Black and white pepper samples were packaged in a sanitized brown glass capped bottles (1L) and irradiated in Nuclear Research Centre, Cairo, Egypt by γ- cell, cobalt-60 γ- irradiator at dose rate 1.29744 KGY/ hours. The applied doses in this study were 0, 6, 8 and 10 kGy. The actual doses were within 75.4% of the target dose (Choi et al., 2010). The irradiation room temperature was 18 ^{o}C. The non-irradiated control was placed outside the irradiation chamber to have the same environmental temperature effect with the irradiating sample. The irradiated black and white samples were transferred and kept in dry place.

Extraction of black and white pepper essential oils:

Black and white pepper seeds control and irradiated at 6, 8 and 10 kGy were separately grounded and powdered in domestic Mixi and hydrodistilled in a Clevenger's apparatus to obtain essential oil (EOs). EOs were stored at 4±1 ºC till used.

Gas chromatographic (GC) analysis:

GC analysis was performed using Hewlett-Packard model 5890 equipped with a flame ionization detector (FID). A fused silica capillary column DB-5 (60mx0.32 mm id,) was used. The oven temperature was maintained initially at 50°C for 5 min, then programmed from 50 to 250°C at a rate of 4°C/min. Helium was used as the carrier gas, at flow rate of 1.1 ml/min. The injector and detector temperatures were 220 and 250°C,

respectively. The linear retention indices (LRI) of the separated volatile components were calculated using hydrocarbons (C_7-C_{21}, Aldrich Co.) as references.

Gas chromatographic - mass spectrometric (GC-MS) analysis:

The analysis was carried out using a coupled gas chromatography [Hewlett-Packard model (5890)]/ mass spectrometry [Hewlett-Packard MS (5970)]. The ionization voltage was 70 eV, mass range m/z 39-400 a.m.u. The GC condition was carried out as mentioned above. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology) and compared with those of authentic compounds and published data (Adams 2001). The quantitative determination was carried out based on peak area integration.

The antioxidant activity assays:

Black and white pepper EOs and their extracts used for the determination of the antioxidant activity assays, and total phenolic content (TPC) were prepared as follows: 1 g of respective solid Black and White pepper was mixed with 100 ml 80% (v/v) water/ methanol or ethanol solution and the suspension was shaken for 1 hour using a laboratory shaker at 1000 rpm. The solid phase was separated using filtration and this step was carried out in triplicate and the final extracts were stored in closed vials in darkness at 4°C.

2, 2-Diphenyl-2- picrylhydrazyl Scavenging assay(DPPH):

Each extract (100–200 µg/ml) in methanol was mixed with 4 ml of methanolic solution containing DPPH radicals. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517nm using spectrophotometer (Shimadzu, UV-160-IPC, Japan) against a blank (Najjaa *et al*, 2011). The inhibition % (I%) was calculated as follow:
I% = [(ΔA517 C–ΔA517 S)/ ΔA517 S] x 100
Where, ΔA: average absorbance; C: control and S: sample.

β-Carotene scavenging activity assay:

The antioxidant activity of ethanolic and methanolic extracts of black and white pepper at 0, 6, 8 and 10 kGy was performed using β-carotene bleaching assay (BCBA) according to Iqbal et al. (2007). β-carotene (0.1 mg) in 0.2 ml of chloroform, 10 mg of linoleic acid and 100 mg of Tween-40 were mixed. The solvent was removed at 40 0C under vacuum and the resulting mixture was diluted with 10 ml of water and was mixed well. To this mixture, 20 ml of oxygenated water was added. Four milliliter aliquots mixtures were pipetted into different test tubes containing 100µL of each extract (100 and 200 µg/ml) and the same concentrations of TBHQ (100 and 200 µg/ml) in ethanol. All determinations were carried out in triplicate. The antioxidant activity (AA) at 0, 6, 8 and 10 kGy was evaluated in terms of bleaching of the β-carotene using the following formula.
% Inhibition = [(AB-AA)/AB] X100
Where: AB: absorption of blank sample (t=0 min) and AA: absorption of sample solution (t=60 min). The results were expressed in % basis in preventing bleaching of β-carotene.

Total phenolic content assay (TPC):

The total polyphenol content (TPC), determined using the Folin Ciocalteu assay according to Singleton (1998). Gallic acid in methanol (50–2500 mg/L) served as an external standard. Samples, standards, and blanks were made in triplicate. The sample absorbance (indicative for polyphenols) was determined photometrically at 760 nm. Results are expressed as milligrams of gallic acid equivalents per 100 g DW (mg GAE/100 g DW).

Antimicrobial assay:

Bacterial cultures:

The tested microorganisms were provided by the culture collections of the Microbiological Dept. National Research Center (NRC) Dokki, Giza, Egypt. These include Gram positive bacteria: *Staphylococcus aureus* (ATCC 43300), *Streptococcus faecalis* (ATCC 12755) and *Bacillus cereus* (ATCC 11778) and Grame negative bacteria: *Salmonella typhimurium* (ATCC 13311), *Listeria monocytogens* (ATCC 35152) and *Escherichia coli* (ATCC 27325).

Disc diffusion method:

Standard experimental set-up as described by Lopez *et al*. (2005) was applied. Briefly, a 100 µl portion of each suspension containing approximately 10^6cfu/ml was spread over the surface of TSA/PDA plate and allowed to dry. A paper disc (diameter 6 mm, Sigma Aldrich) was laid on the inside surface of the upper lid and 10 µl essential oil was placed on each disc. The plate inoculated with microorganisms were immediately inverted on top of the lid and sealed with parafilm to prevent leakage of essential oil vapour. Plates were incubated at 37 °C for 24 h and the diameter of the resulting inhibition zone in the bacterial lawn was measured.

Determination of minimum inhibitory concentration (MIC):

The determination of MIC of the essential oil of black pepper on the test bacterial strain was done using broth dilution method as explained by Hammer *et al*.(1999) with different concentrations of oil. The cultures of the test strains were prepared by inoculating the test strain in sterilized test tube containing 5 ml nutrient broth. The tubes were incubated overnight at (37±1) °C. The MIC was defined as the lowest concentration of the test compound to inhibit the growth of microorganisms and the MBC was defined as the lowest concentration of the test compound to kill the microorganisms. The test tubes containing 10 ml of sterilized Tryptic Soy Broth (TSB) with 0.5% (v/v) tween-80 were inoculated with different concentration of black pepper oil ranging from 0.5% – 0.015% (v/v). TSB with 0.5% (v/v) tween 80 without oil was used as positive growth control. An aliquot of bacterial suspension (25 µl) was added uniformly to each tube. The tubes were incubated at (37±1) °C for 24 hours then 48 hours. The tubes were observed for turbidity after the period of incubation. The lowest concentration at which no visible growth occurs in either culture tubes was taken as MIC. Then the tubes showing no increase in the turbidity at each time interval 24-48 hours were streaked on nutrient agar plates to check the bacterial growth. Each trial was repeated thrice.

Results and Discussion

Aroma constituents of control and irradiated black and white pepper at 6, 8 and 10 KGy:

Thirty two volatile compounds were identified in both black pepper and white pepper EOs. All these compounds are listed with their area percentages in (Table 1, 2). Identification of the volatile components was identified by LRI values and MS spectra (Adams, 2001).

The typical gas chromatograms of the volatiles in HD oil of raw and irradiated samples at different doses 6, 8, 10 KGy from black and white pepper seeds are shown in (Fig, 1, 2).The total area percentages of the main chemical classes of the HD volatile oil of raw and irradiated samples of black and white pepper are shown in (Fig. 3). The volatile profile of raw HD oil of black pepper consisted mainly of δ - careen (27.85%) followed by limonene (24.07). β -caryophyllene (15.96%), sabinene (14.04%), $\propto$ -pinene (9.12%) and β -pinene (2.11%) (Table 1). These results are in accordance with those reported by Abd El mageed *et al*., 2011, Nisha *et al*., 2009; Saad *et al*., 2007; Singh *et al*., 2004).

γ -Irradiation caused a remarkable decrease in the total yield of monoterpenes in different doses of irradiated samples 6, 8,10 KGy recorded 39.39%; 38.52% and 43.01%, respectively, which represent the half concentration of their total yield in raw or control sample 78.83%, at the same time irradiation caused a more than double increase in the total yield of sesquiterpene in three irradiated samples recording 52.55%, 51.6% and 48-83%, respectively, compared to their content 20.07% in control sample. Also γ -irradiation caused drastic increase in oxygenated terpenes recording 5.73%, 6.88% and 7.09%, respectively compared to their content 0.76% in unirradited sample. It is clear that these results indicated no significant differences between the three irradiated samples 6, 8, 10 KGy in the total yield of mmoterpenes, sesquiterpenes and oxygenated terpenes (Fig. 3).

These results are in accordance with those found by Emam *et al*., 1995; Farkas, 2006 and Sadecka 2010. The considerable increase in sesquiterpenes contents of the three irradiated samples were due to the increase in β -elemene , β-farnesene, β -selinene, $\propto$ -muurolene, β -bisabolene, δ -cadinene, β -sesquiphellandrene and β -caryophyllene, the major sesquiterpene recorded 15.96% in control sample increased to 22.17%, 23.3% and 25.24% respectively after irradiation at the three doses (Table 1).

These results are in accordance with Abd Elmageed *et al*., 2011; Sadecka 2010; Chacko *et al*., 1996 and Emam *et al*., 1995. Due to the presence of sesquiterpenes in a higher content, which are responsible for desirable pepper flavour attributes, all irradiated sample (6,8,10 KGy) showed higher quality than control one.

The drastic increase in oxygenated terpenes against the decrease in monoterpens seems to be very interesting. Monoterpenes easily oxidized under irradiation conditions to generate oxygenated derivatives as

well as altering the flavour profile. These results proved that the volatile oils extracted from black pepper are radio sensitive. These changes can be attributed to the irradiation effects on terpenes, such as oxidation or hydroxylation of the aromatic ring of terpenes. A low moisture content (8-10%) in samples potentiates the direct and indirect effects of irradiation to increase the content of alcohols as explained by (Urbain 1986). On the other hand, terpenes found in most of the essential oils, have the same skeleton structure but with different side groups, i.e., OH, - CHO- or – COOH. Therefore, irradiation can induce configurational changes in the position of double bonds and the functional groups to produce new compounds. (Sadecka 2010).

The volatile profile of raw HD oil of white pepper consisted mainly of β-caryophyllene (21.17%) followed by δ-carene (20.23%), limonene (17.64%), β-pinene (14.02%), α-pinene (7.16%), myrecene (4.34%), δ-elemene (3.15%) and β-farnesene (2.16%) (Table 2). These results are in quite agreement with Abd Elmageed *et al*., 2011 and Plessi *et al*., 2002. The effect of γ-irradiation on white pepper is similar to that on black pepper (Fig 3). Monoterpenes decreased to 48.24%; 37.94% and 4.02% in three doses (6.8.10 KGy) samples, respectively in comparison to 66.26% in the raw one, whereas, sesquiterpenes increased to 39.78%; 47.32% and 40. 64% in the three irradiated samples in comparison to 30.27% in the control one.

Table 1: Volatile components isolated in the hydrodistilled oil of control and irradiated black pepper (BP) seeds at 6, 8, 10 KGy.

Peak No	K_I[a]	Compounds	Control (B.p.)	Irradiated samples of w.p.			Methods of Identification [b]
				6 KGy	8 KGy	10 KGy	
1	929	∝ -Thujene	0.19	1.79	1.89	2.15	MS,KI
2	939	∝ -Pinene	9.12	5.18	5.73	6.63	ST,MS,KI
3	953	Camphene	0.52	0.22	0.26	0.35	MS,KI
4	977	Sabinene	14.04	10.87	14.01	15.87	ST,MS, KI
5	982	β -Pinene	2.11	2.80	-	-	MS, KI
6	1009	∝ -Phellandrene	0.51	0.62	-	-	MS,KI
7	1013	δ -Carene	27.85	7.25	5.59	5.67	MS,KI
8	1033	Limonene	24.07	9.71	9.94	11.13	ST,MS,KI
9	1036	1,8 Cineole	0.12	0.17	0.15	0.17	MS, KI
10	1064	γ -Terpinene	0.42	0.83	0.90	1.02	MS, KI
11	1088	Terpinolene	-	0.12	0.20	0.19	MS, KI
12	1097	Linalool	0.30	1.02	1.23	1.35	ST, MS, RI
13	1180	Terpinene-4-ol	0.12	0.61	1.16	1.03	MS, KI
14	1197	∝ -Terpineol	0.22	2.20	2.79	2.96	MS,KI
15	1344	δ -Elemene	0.65	5.92	4.78	0.96	MS, KI
16	1388	β -Cubebene	0.32	0.28	0.25	0.16	MS,KI
17	1402	β -Elemene	0.33	1.01	1.90	2.17	MS,KI
18	1437	β -Caryophyllene	15.96	22.17	23.30	25.24	ST,MS,KI
19	1446	β -Farnesene	0.89	3.31	3.22	3.53	MS,KI
20	1454	∝ -Humulene	-	0.43	0.42	0.16	MS,KI
21	1469	∝ -Guiene	-	0.13	1.42	1.16	MS,KI
22	1471	Aromadendrene	-	1.44	1.17	1.04	MS,KI
23	1475	unknown	-	1.32	2.99	3.18	MS,KI
24	1480	Germacerene-D	0.81	3.18	0.34	-	MS,KI
25	1486	β -Selinene	0.52	1.36	1.84	2.15	MS,KI
26	1490	∝ -Selinene	0.24	5.15	3.32	3.37	MS,KI
27	1497	∝ -Muurolene	0.07	2.49	2.53	2.49	MS,KI
28	1507	β -Bisabolene	0.33	3.46	3.01	3.27	MS,KI
29	1530	δ -Gadinene	0.04	2.02	2.28	1.71	MS,KI
30	1533	β -Sesquiphellandrene	0.24	0.79	1.57	0.97	MS,KI
31	1560	Germacrene-B	-	0.41	0.25	0.45	MS,KI
32	1581	Carophyllene Oxide	-	1.73	1.55	1.58	MS,KI

Compounds listed according to their elution on DB5 column
-: not detected
a: Kovats index
b:Compounds identified by GC-MS(MS) and/or by comparison of MS and KI of standard compound run under similar conditions

Concerning the total yield of oxygenated terpenes their concentrations show drastic increased with increasing the dose of irradiation, recorded 12.49%, 14.12% and 52.55% for 6,8 and 10 KGy irradiated samples, respectively, compared to 3.37% for the control sample (Fig 3); it appears that irradiation dose 10 KGy of white pepper causing a very decrease in total concentration of monoterpenes 4.02% whereas causing a very high

increase in the total concentration of oxygenated terpenes 52.55% (Fig 3); this is due to the increase in terpinene-4-ol; 1.21%; carveol 2.94%, carvon 1.52%, caryophyllone oxide 40.71%, guaiol 1.34%; spathulenol 1.9% and α-muurolol 1.76% as compared with the control sample (Table 2). These results are in accordance with sadecka (2010) but at dose 30 KGy or high doses which can occur configurational changes including changes in the position of double bonds and the functional groups to produce different compounds.

Therefore, the irradiated samples should be expected to give better reproduction of natural aroma than the control sample, additionally, oxygenated terpenes exhibited a higher antioxidant power in comparison with the other identified classes (Radonic and Milos 2003).

Table 2: Volatile components isolated in the hydrodistilled oil of control and irradiated white pepper (WP) seeds at 6, 8, 10 KGy.(*Values expressed as relative area percentages to total identified components).

Peak No	K_I[a]	Compounds	Control (W.p.)	Irradiated samples of W.p.			Methods of Identification [b]
				6 KGy	8 KGy	10 KGy	
1	929	∝ -Thujene	*0.92	8.88	4.18	0.08	MS,KI
2	939	∝ -Pinene	7.16	0.32	0.18	-	ST,MS,KI
3	982	β -Pinene	14.02	9.54	7.73	0.37	MS,KI
4	992	Myrecene	4.34	0.23	0.52	0.94	ST,MS, KI
5	1013	δ – Carene	20.23	14.96	14.17	-	MS, KI
6	1033	Limonene	17.64	12.73	10.56	2.41	MS,KI
7	1064	γ -Terpinene	0.38	0.67	0.26	0.15	MS,KI
8	1088	Terpinolene	1.57	0.91	0.34	0.07	ST,MS,KI
9	1097	Linalool	1.15	2.52	1.33	0.89	MS, KI
10	1180	Terpinene-4-ol	0.40	0.93	1.20	1.21	MS, KI
11	1197	∞-Terpineol	0.30	0.67	0.87	0.28	MS, KI
12	1202	Carveol	0.30	0.44	1.40	2.94	ST, MS, RI
13	1212	unknown	0.10	0.33	0.61	2.78	MS, KI
14	1247	Carvon	0.21	6.62	2.19	1.52	MS,KI
15	1344	δ-Elemene	3.15	0.07	0.13	3.65	MS, KI
16	1376	∞-Copaene	0.76	1.35	0.24	6.28	MS,KI
17	1388	β -Cubebene	0.87	0.14	0.08	0.19	MS,KI
18	1437	β -Caryophyllene	21.17	27.10	31.00	13.23	ST,MS,KI
19	1446	β -Farnesene	2.16	4.41	5.33	0.50	MS,KI
20	1454	∝ -Humulene	0.48	0.27	0.79	0.71	MS,KI
21	1480	Germacrene-D	0.34	1.72	2.66	0.51	MS,KI
22	1486	β -Selinene	0.08	1.70	2.76	0.14	MS,KI
23	1490	∞-Selinene	0.05	0.85	1.50	1.48	MS,KI
24	1497	∞-Muurolene	0.11	0.15	0.32	0.16	MS,KI
25	1507	β -Bisabolene	0.34	0.91	1.84	7.82	MS,KI
26	1530	δ -Cadinene	0.34	0.06	0.22	1.66	MS,KI
27	1533	β -Sesquiphellandrene	0.09	0.08	0.19	1.33	MS,KI
28	1560	Germacrene-B	0.33	0.12	6.26	2.98	MS,KI
29	1581	Caryophyllene oxide	0.77	0.87	6.27	40.71	MS,KI
30	1589	Guaiol	0.09	0.18	0.36	1.34	MS,KI
31	1619	Spathulenol	0.07	0.14	0.26	1.90	MS,KI
32	1635	∝ -Muurolol	0.08	0.12	0.24	1.76	MS,KI

Compounds listed according to their elution on DB5 column
-: not detected
a: Kovats index
b:Compounds identified by GC-MS(MS) and/or by comparison of MS and KI of standard compound run under similar conditions

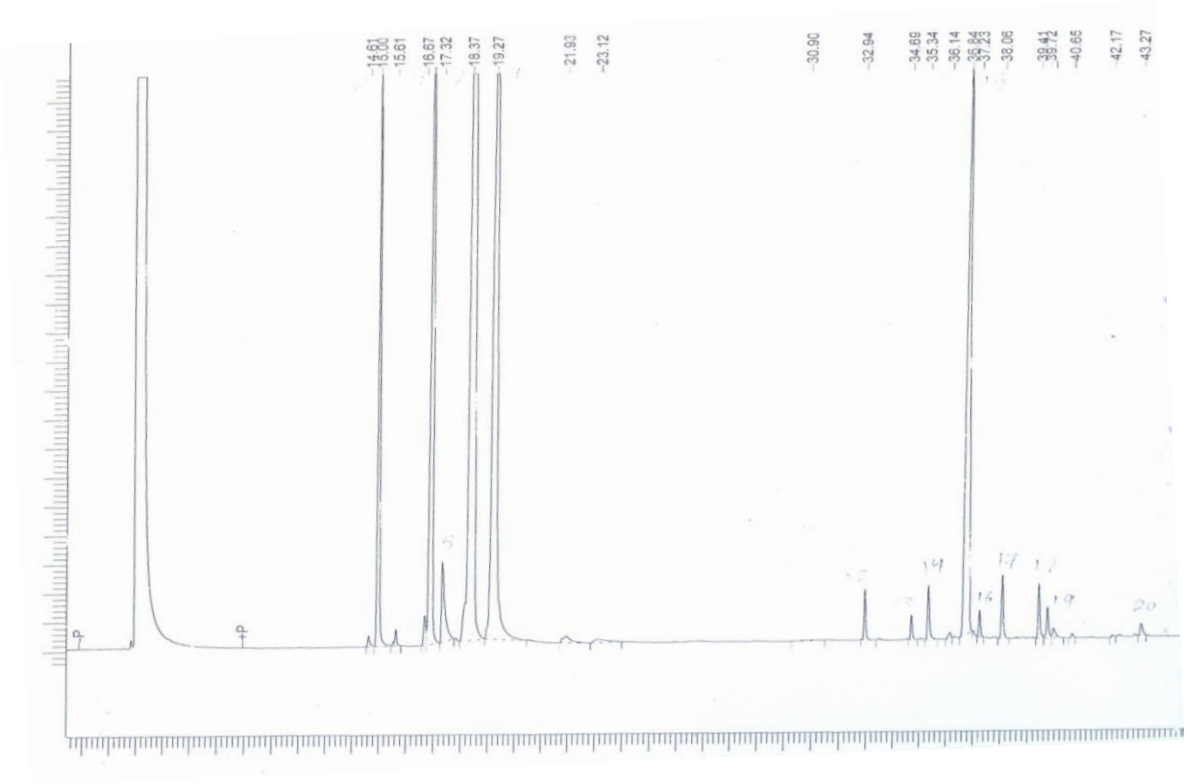
Antioxidant activity assays:

DPPH scavenging assay:

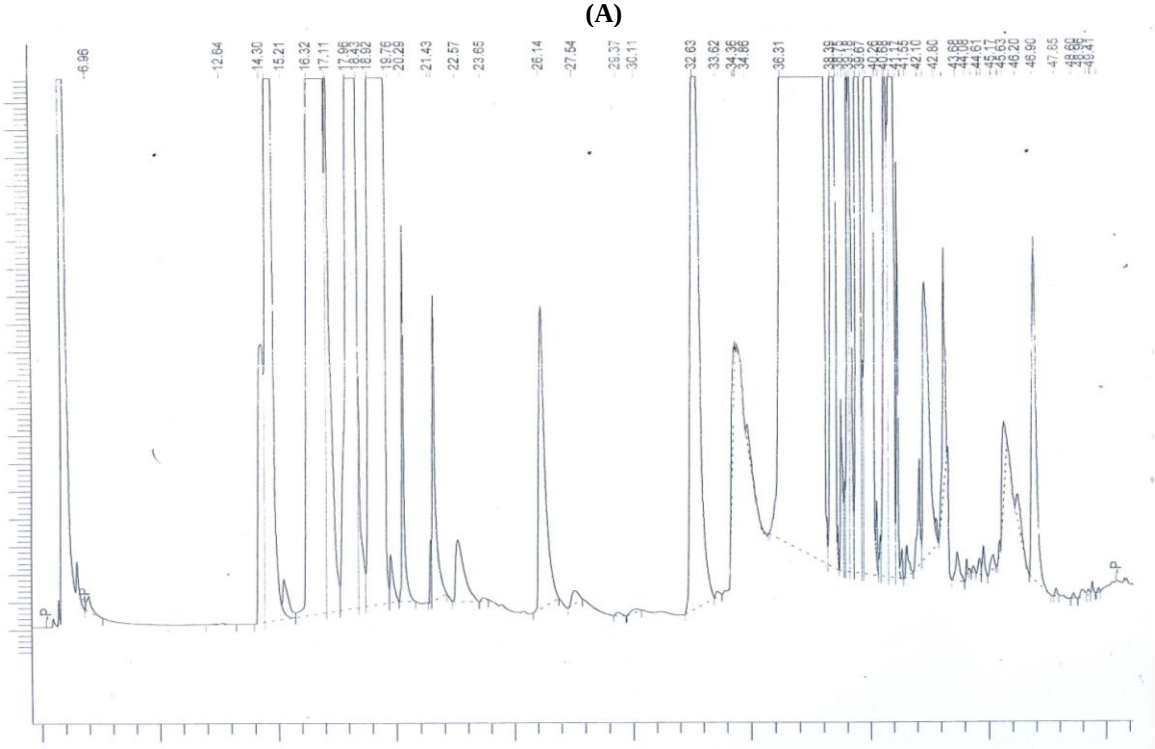
Irradiation resulted in a slight increase in the DPPH radical-scavenging ability of both essential oils (EO), ethanol and methanol extracts at 6, 8 and 10 kGy which was found to be non-significant when compared to non-irradiated sample (Fig.2). Ethanolic extract of black pepper showed a higher antioxidant activity in comparison with non-irradiated and irradiated samples with 72.4%. The gradual increase in the DPPH scavenging activity at 10 kGy may be due to the presence of nutrients such as carbohydrates and proteins in the white and black pepper (Jae *et al*., 2009). Oh *et al*. (2006) found that irradiation of foods in presence of carbohydrates and proteins accelerate the formation of Maillard reaction products (MRPs). Also, MRPs scavenge the formed free radicals in both DPPH and superoxide methods. Irradiated anise, cinnamon, ginger, licorice, mint, nutmeg, and

vanilla, with respect to the non- irradiated samples, water extracts of the irradiated spices at 1, 3, 5, and 10 kGy did not show significant differences of the antioxidant activity in the radical-scavenging assays (Murcia *et al*., 2004).

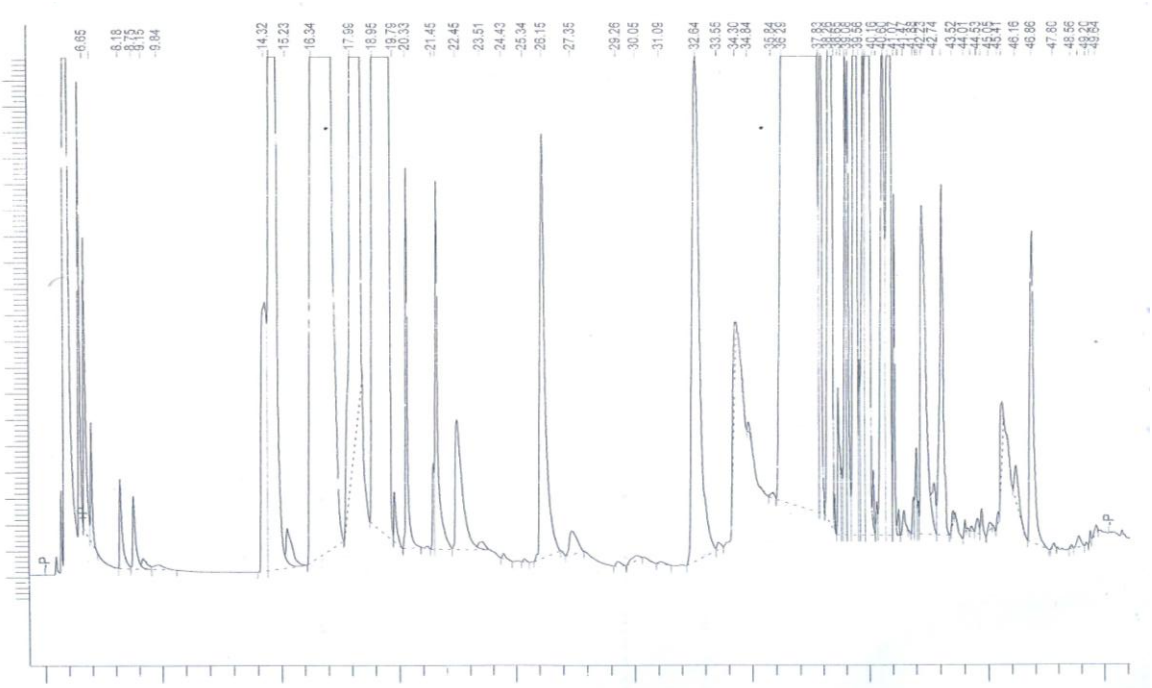
The radical-scavenging activity of ethanol and water extracts of non-irradiated Korean medicinal herb was, however, indistinguishable from that of samples treated with a dose of 10 kGy gamma irradiation (Byun *et al*., 1999).



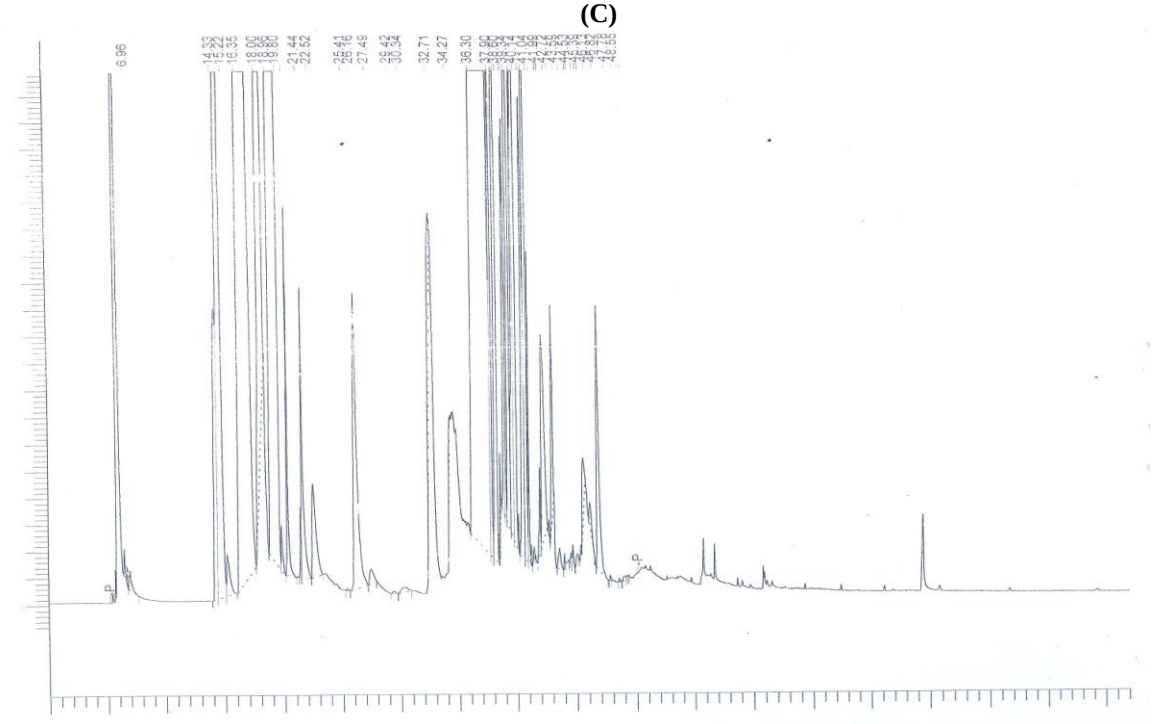
(A)



(B)



(C)



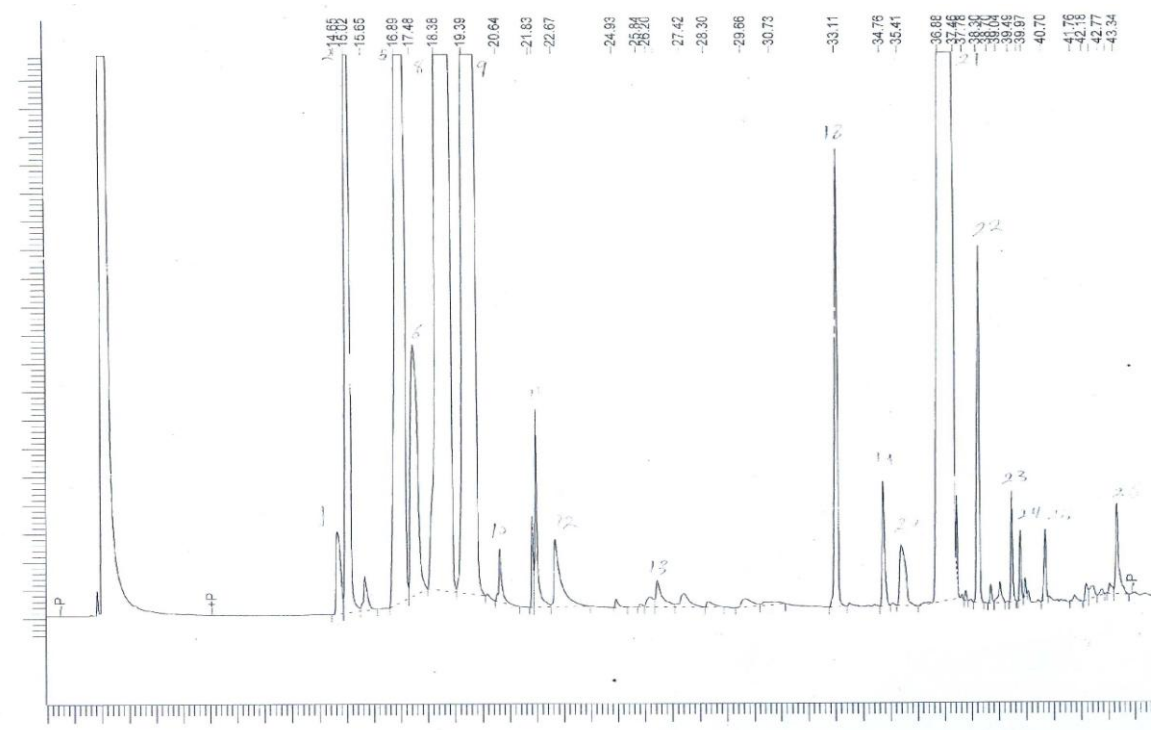
(D)

Fig. 1: Gas chromatograms of volatiles in the hydrodistilled oil of raw **(A)** and irradiated black pepper seeds at 6, 8, 10 KGy **(B)**, **(C)**, and **(D)** respectively.

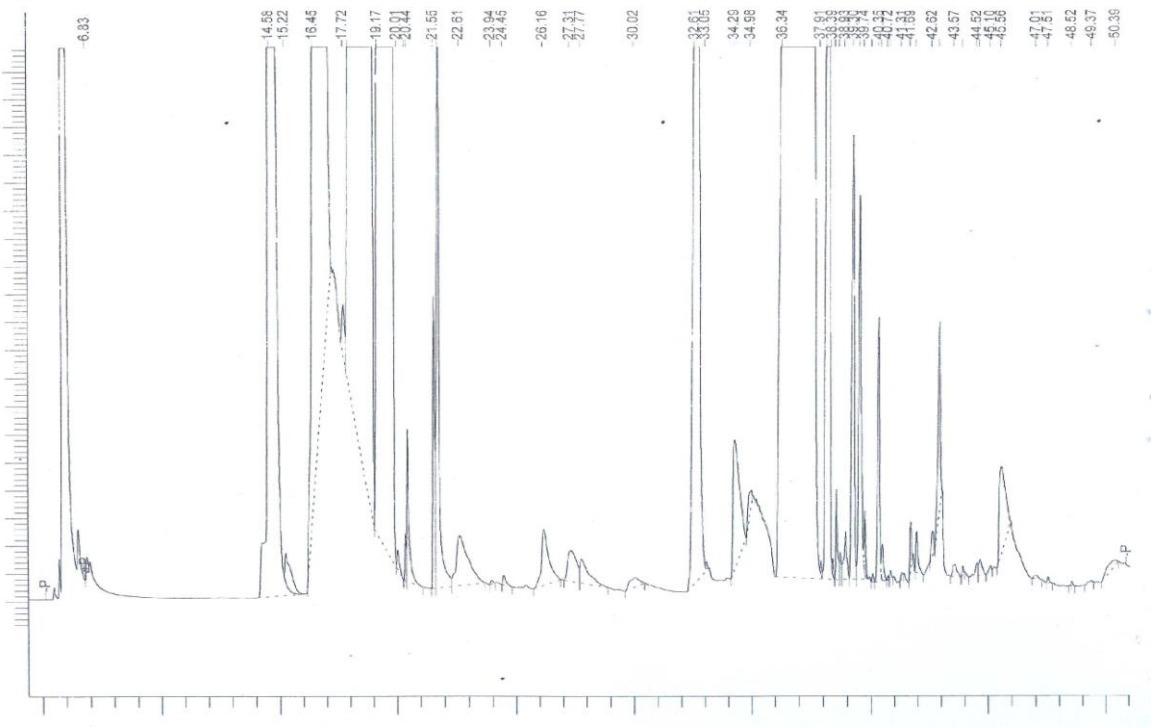
β-Carotene linoleic acid bleaching assay:

Figure 3 shows the inhibition % based on the β-carotene bleaching assay of 0, 6, 8 and 10kGy of white and black pepper EO and their extracts. Irradiation resulted in the non-significant increase in the 8 and 10kGy irradiated white and black pepper EO and extracts, however there was a non-significant increase in the 8 and 10kGy irradiated white and black pepper EOs and their extracts when compared to the non-irradiated samples. The basic principle of β-carotene bleaching is a free radical- mediated phenomenon, resulting in the formation of hydroperoxides from linoleic acid. In this model system, β-carotene undergoes rapid discoloration due to attack of free radicals formed upon abstraction of a hydrogen atom from the diallylic methylene group of linoleic acid (Jae *et al*., 2009). Wettasinghe and Shahidi (1999) reported that antioxidant present in the reaction

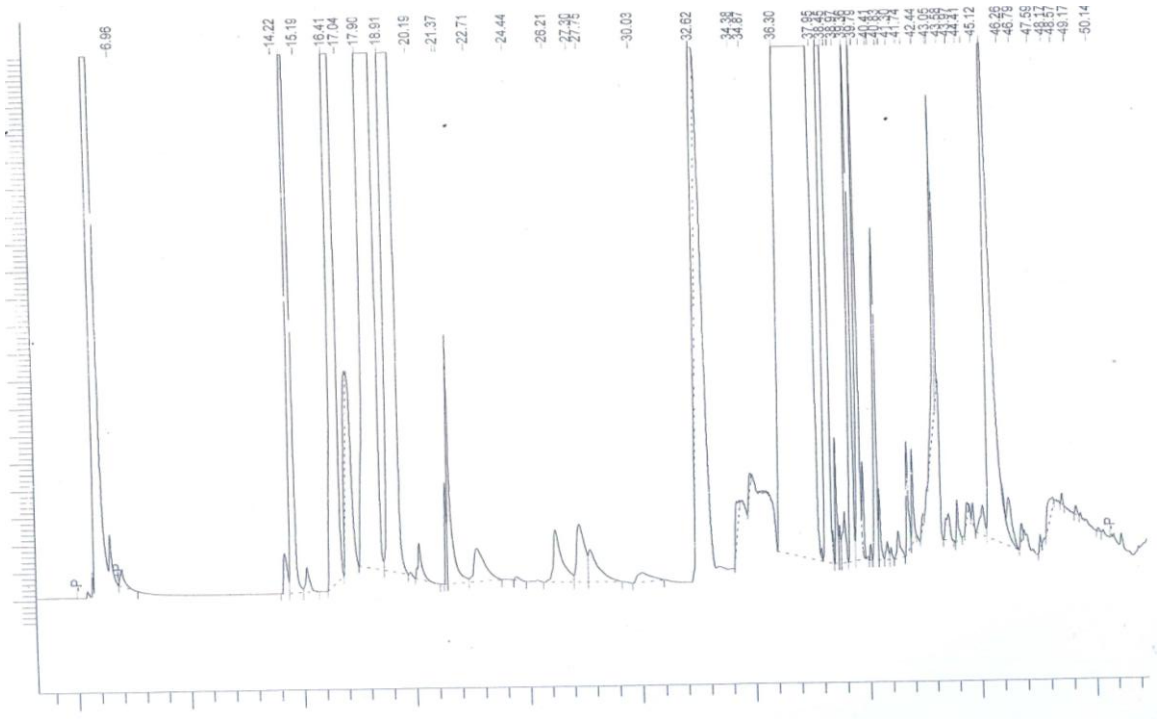
mixture inhibits the rate of bleaching by quenching the free radicals produced in the system during incubation at 50 0C. Behera et al. (2004) observed that β-carotene bleaching was significantly inhibited in the presence of irradiated sugar/ amino acid solution, whereas no protection was offered by non-irradiated solution. Our results were found in agreement with the published data by Chawla *et al*. (2007). In our previous studies, the strongest effect for reduction of DPPH radical was by white pepper microwave heated sample which exhibit 78.2% and 76.2% in β-carotene assay compared to TBHQ with 98.2% (Abd El-mageed, 2011).



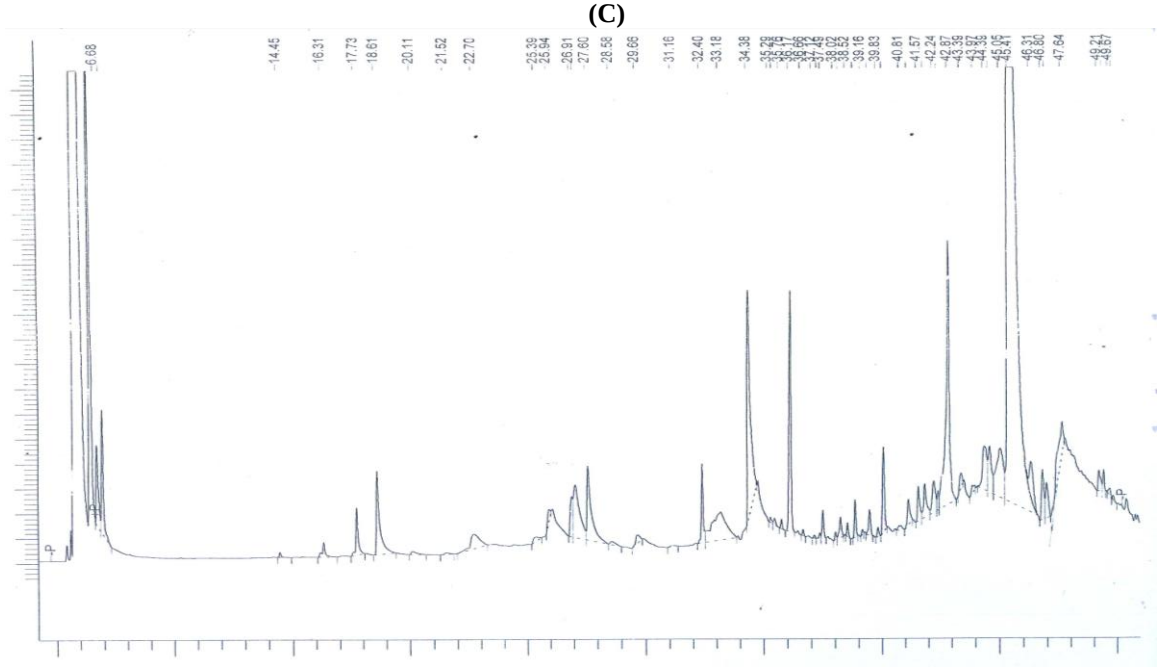
(A)



(B)



(C)



(D)

Fig. 2: Gas chromatograms of volatiles in the hydrodistilled oil of raw **(A)** and irradiated white pepper seeds at 6,8, 10 KGy **(B)**, **(C)**, and **(D)** respectively.

Total phenolic contents:

As the irradiation dose increased, higher total phenolic contents (TPC) were found, significant only in 6 and 10 kGy in comparison with non-irradiated samples (fig. 4). Total phenolic contents (TPC) increased with increased irradiation dose. From our knowledges, there are a few studies on the TPC in black and white pepper. Ethanolic extract of black pepper showed higher TPC with 182.5 mg GAE/ gm DW in comparison with other extracts irradiated and non-irradiated. Harrison & Were (2007) suggested TPC may be due to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by gamma irradiation. Irradiation exerts its effects as direct and indirect mechanisms, in case of indirect mechanism, radiolysis of water results in the production of radicals such as hydrated electrons, hydroxyl radicals, and hydrogen atoms (Fan & Mastovska, 2006). These radicals may break the glycosidic bonds that are

present in pepper seeds, leading to the formation of free phenols that may increase the total phenolic content in irradiated extracts when compared with non-irradiated extracts.

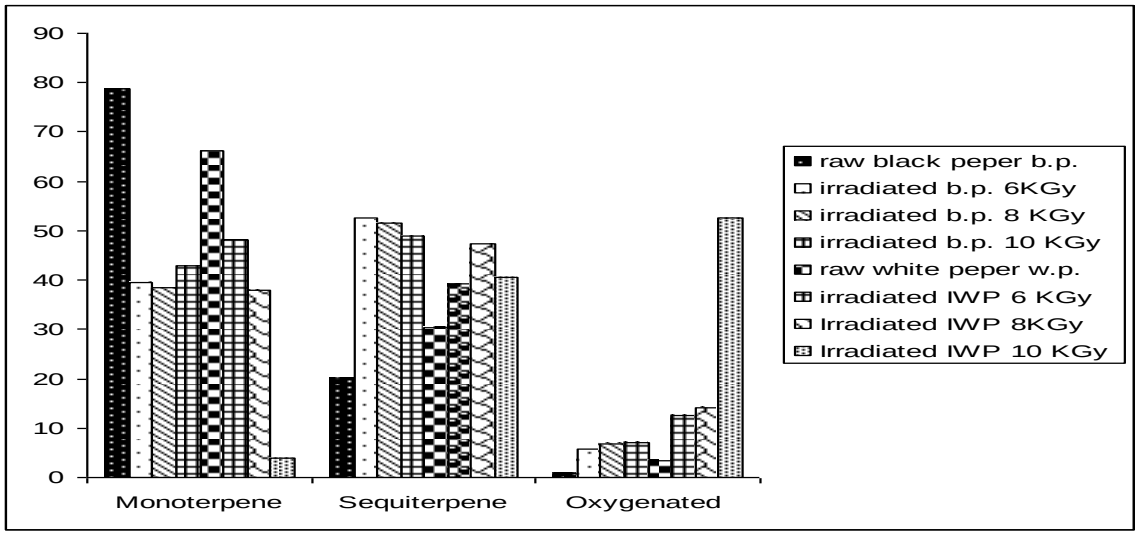


Fig. 3: The total area percentages of the main chemical classes of the essential oils of raw, irradiated black and white pepper at 6, 8, 10 KGy.

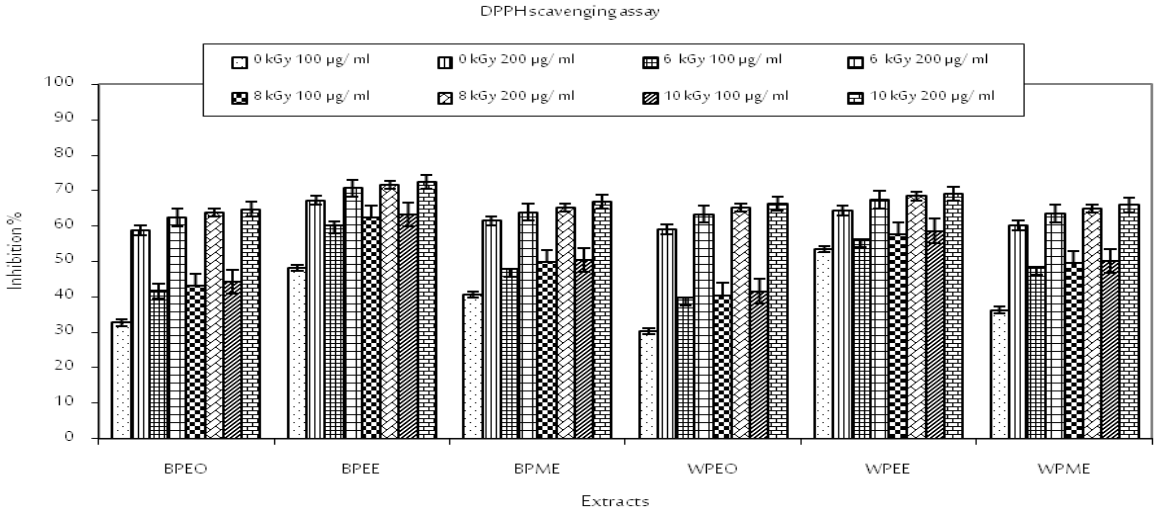


Fig. 2: DPPH scavenging assay of black and white pepper EOs and their extracts at 0, 6, 8 and 10 kGy.
BPEE: black pepper ethanol extract; BPME: black pepper methanol extract; WPEE: white pepper ethanol extract and WPME: white pepper methanol extract.

Antimicrobial activity:

The zone of inhibition was measured for both black and white pepper essential oils and the results depicted in Table (3).It was found that G+ bacteria were more susceptible than G- bacteria.

The black and white pepper essential oils have antimicrobial effects on and among all tested microorganisms. Inhibition zone was highest in *Staphylococcus aureus*, it was lowest in *Escherichia coli*, but the black pepper oil was effective (Inhibition zone was found 10-20 mm) in comparison to white pepper oil (Inhibition zone was found 8-16 mm). Also, the results in (Table 3) showed that the γ-irradiation doses (6, 8 and10 kGy) no significant effect on the antimicrobial activity of the essential oil of black and white pepper.

The MIC values of black and white pepper essential oils are presented in Table (4). Each essential oil has shown very high susceptibility against bacterial strains, as MIC values obtained were very low. The MIC value of black pepper oil was the lowest against *Staphylococcus aureus* and *Streptococcus faecalis* (0.25µl/ml), while it was the highest (0.75µl/ml) against *Listeria monocytogens* and the MIC ranged between (0.25 – 0.75µl/ml).The MIC value of white pepper oil was the lowest against *Staphylococcus aureus* (0.3µl/ml), while it

was the highest (1.0µl/ml) against *Listeria monocytogens* and the MIC ranged between (0.3 – 1.0µl/ml). Also, the results in Table (4) showed that the γ-irradiation doses (6, 8 and10 kGy) revealed no significant effect on the MIC of the essential oil of black and white pepper.

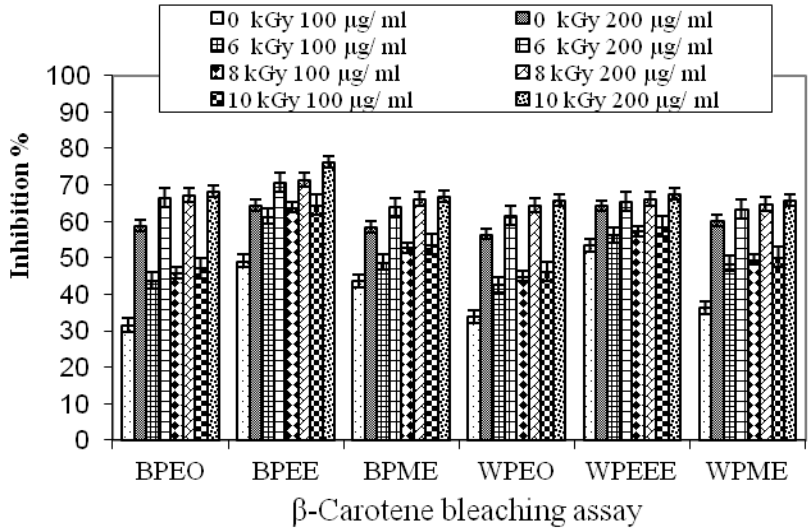


Fig. 3: β-Carotene linoleic acid bleaching assay of black and white pepper EOs and their extracts at 0, 6, 8 and 10 kGy.
BPEO: black pepper essential oil; WPEO: white pepper essential oil; BPEE: black pepper ethanol extract; BPME: black pepper methanol extract; WPEE: white pepper ethanol extract and WPME: white pepper methanol extract.

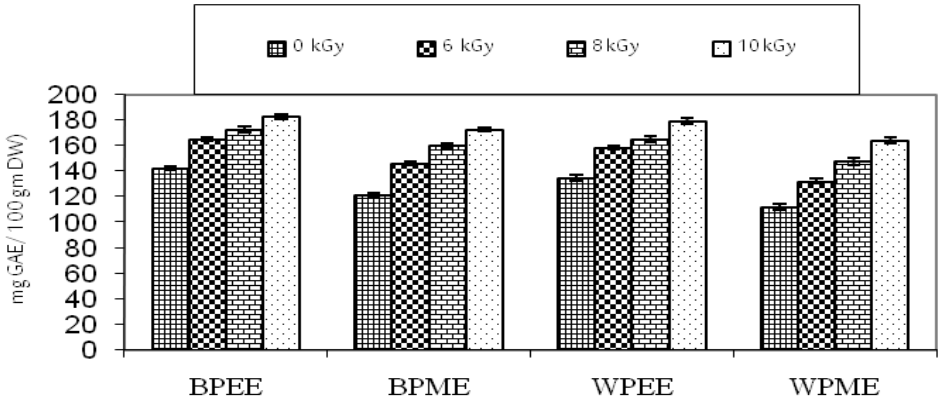


Fig. 4: Total phenolic contents of black and white pepper extracts at 0, 6, 8 and 10 kGy.
BPEE: black pepper ethanol extract; BPME: black pepper methanol extract; WPEE: white pepper ethanol extract and WPME: white pepper
methanol extract.

Table 3: Antimicrobial activities of black and white pepper essential oil against pathogenic bacteria before and after radiation.

Radiation dose (kGy)	Inhibition zones (mm)											
	S. aureus		*S. faecalis*		*B. cereus*		*Salmonella typhimurium*		*Listeria monocytogens*		*E. coli*	
	B	W	B	W	B	W	B	W	B	W	B	W
0=control	20	16	18	14	15	13	14	11	12	10	10	8
6	20	16	18	14	15	13	14	11	12	10	10	8
8	19	16	18	14	15	13	14	11	12	10	10	8
10	19	15	17	13	15	12	14	10	12	9	10	8

B: Black pepper essential oil and W: White pepper essential oil

Table 4: Determination of MIC value (µl/ml) of black and white pepper essential oils against pathogenic bacteria before and after radiation.

Radiation dose (kGy)	MIC Essential oil in (µl /ml)											
	S. aureus		*S. faecalis*		*B. cereus*		*Salmonella typhimurium*		*Listeria monocytogens*		*E. coli*	
	B	W	B	W	B	W	B	W	B	W	B	W
0=control	0.25	0.3	0.25	0.4	0.5	0.75	0.5	0.6	0.75	1.0	0.6	0.75
6	0.25	0.3	0.25	0.4	0.5	0.75	0.5	0.6	0.75	1.0	0.6	0.75
8	0.25	0.3	0.25	0.4	0.5	0.75	0.5	0.6	0.75	1.0	0.6	0.75
10	0.25	0.3	0.25	0.4	0.5	0.75	0.5	0.6	0.75	1.0	0.6	0.75

B: Black pepper essential oil and W: White pepper essential oil

Shiva *et al*., (2013) and Adolf *et al*., (2011) reported that essential oils extracted from black and white pepper showed antibacterial activity against different bacteria. The black pepper extracted with ethyl acetate solvent gave the best inhibition. These results confirmed our findings.

Previous works have reported the antimicrobial properties of black and white pepper essential oils extract (Yona *et al*., 2013; Shanmugapriya *et al.,* 2012*;* Jana, 2010). Some spices and herbs also, showed antimicrobial effects and the treatment of γ-irradiation (10 kGy) showed no significant effect on the antimicrobial activity of the essential oils in these studies (Hanan *et al*., (2008).

Conclusions:

The irradiated black and white pepper EOs has excellent aroma volatiles in comparison with un-irradiated samples. The results showed a slight increase in the DPPH radical-scavenging ability and β-carotene assay at 100 and 200 µg/ of both essential oils (EO), ethanol and methanol extracts at 6, 8 and 10 kGy. Also, TPC in black and white extracts were increased with γ-irradiation increased significant only in 6 and 10 kGy. EOs of black and white pepper have good antibacterial activity, but when compared to Gram negative bacteria, Gram positive bacteria are more susceptible to the pepper essential oils. The γ- irradiation at 6, 8 and 10 kGy did not affect the antimicrobial activity of the black and white pepper essential oil.

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