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Effect of thermal treatments and γ -irradiation on volatile and non-volatile Egyptian anise essential oil and its antioxidant properties

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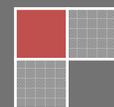
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ABSTRACT

Effect of thermal treatments; electric oven, microwave and γ -irradiation, at 6, 8, and 10 kGy, on composition of volatile and non-volatile anise essential oil was studied. Volatile profile of raw hydrodistilled anise oil mainly consists of *E*-anethole (79.68 %), hexahydrofarnesyl acetone (6.95 %), *p*-anisaldehyde (5.49 %), γ -himachalene (2.53 %) and estragole (0.76 %). Remarkable decrease of phenylpropanoids was observed in 6 and 8 kGy γ -irradiated samples. All samples revealed high antioxidant activities. Thermal and γ -irradiation showed high increase in total sesquiterpenes yield. Oxygenated compounds yield diminished, which significantly affected antioxidant activity. 8 kGy γ -Irradiated sample exhibited the strongest reducing effect on DPPH^{*} (84.57 %) and the highest inhibiting effect of linoleic acid (85.21 %) and subsequent bleaching of β -carotene, compared to butylated hydroxytoluene (98 %). 9 Phenolic compounds were identified using HPLC and C18 RP-HPLC techniques. The predominant components are *p*-quumaric acid (43.36 %) and ferulic acid (21.06 %).

KEYWORDS

Anise oil; Volatile and nonvolatile oils; Thermal treatment; γ -irradiation; antioxidant activity; GC-mass.



INTRODUCTION

Spice extracts have received exceptional attention due to their useful physiological functions and antimicrobial activity. Therefore, there is a need for more research work dealing with antimicrobial effects to food-related bacteria, such as; food spoilage bacteria and food borne bacterial pathogens^[24]. In food processing, lipid oxidation causes a loss in nutritional and gustative quality of foods, in addition it generates free radicals. Conventional artificial antioxidants, such as; butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), etc. were used to avoid or delay autoxidation process^[20]. These synthetic antioxidants had been suspected to promote negative health effects. So that, there is a growing interest in studies on natural additives as potential antioxidants. Various antioxidant aromatic and medicinal plants exhibited effective retardation of lipid peroxidation process, in oils and fatty foods^[17]. Anise (*Pimpinella anisum L.*) is known as one of the most widely used medicinal plant. Anise seeds are used as spices and their essential oil have several applications as aromatic agents in food and liquor industries, as well as in many folk medicinal systems. Some folk descriptions included use of anise seed as a medicinal plant, pharmaceutical, perfumery, cosmetics, and candy production industries^[19, 32]. Ionizing radiation preservation treatments have been accredited as tremendous preservation methods^[30]. Although the safety of irradiated food is well documented, a little is known about the effects of irradiation on the antioxidant activity of phytochemicals^[34]. It is known that, irradiation doses from 5 up to 10 kGy do not produce toxicological hazards and nutritional or microbiological problems in foods and are sufficient to reduce the population of microbes without changing essential quality and flavor attributes^[13]. Furthermore, spices are usually irradiated in prepackaged form to prevent post contamination, in which possible abnormal compounds from packaging materials may be created by irradiation and may migrate into the foods. Consequently, this migration may be potentially harmful and impact food flavor^[16]. It was proved that treatment with ionizing radiation is an effective process for destroying insects and micro-organisms^[1, 2]. Ionizing radiation process has some advantages such as: does not require additives, safe controllable technology, and cold treatment that preserve volatile components. Microwave utility in food processing industries afforded good tool for sterilizing, defrosting, blanching, dehydration, and cooking, with an improved product quality and reduced time of exposure to energy^[14, 1, 3]. Some spices are thermally processed, for their microbial stability and removal of extraneous matter. Roasting is an important phase in cooking process used to release characteristic flavour volatiles and undesirable constituents^[28]. Heat processing, such as cooking or roasting, changes the nature of many food and alters its physical, chemical and nutritional characteristics, and hence its flavour and bioavailability of proteins, carbohydrates, lipids and vitamins^[8]. Available information about the extent of destruction of bioactive principles of spices during food processing is insufficient^[27]. The present work deals with the evaluation of some thermal treatments; conventionally roasting by electric oven and microwave heating. Also present study describes comparison between these thermal treatments and cold sterilization using γ -irradiation at safe doses. This comparative study includes evaluation of phenolic content, anti-radical activities, volatile and non-volatile oils to choose an ideal treatment, improving quality of Egyptian anise.

MATERIALS AND METHODS

Plant materials and chemicals

Seeds of Anise (*Pimpinella anisum L.*) were purchased from local planted crops at Giza during 2013/2014.

1,1-Diphenyl-2-picryl hydrazyl (DPPH[•]), butylated hydroxytoluene (BHT), β -carotene, gallic acid and Folin-Ciocalteu reagents and standard *n*-paraffin (C₈-C₂₂) were obtained from Sigma-Aldrich. Methanol and formic acid (HPL grade) were purchased from Sigma-Aldrich. All other solvents and chemicals are of Analytical Grade.

Thermal processing and irradiation of anise samples

Fresh three dry anise seed samples (100 g/sample) were roasted in a separately conventional electric oven at 120 °C and for 20 minutes. Similarly, three samples (100 g/sample) were separately subjected to microwave heating (Daewoo DE Microwave, Mod: KoG-181G, 200-240V 50Hz Korea). The samples were irradiated at microwave power equals 1400 W for 3 minutes. Also, Anise-seed samples were γ -irradiated at Radiation Research Centre, Cairo, Egypt. Samples were packaged in a sanitized brown glass capped bottles (1L) and by γ -cell, cobalt-60 γ -irradiator, at dose rate 1.29744 kGy/h. The applied doses in this study are 0, 6, 8 and 10 kGy. The actual doses were within 75.4 % of the target dose. The irradiation room temperature is 18 °C. The non-irradiated control sample was placed outside the irradiation chamber to have the same environmental temperature effect, in place of irradiated sample. The irradiated anise samples were transferred and kept in dry place. The raw and treated samples were separately ground in a spice mix grinder.

Isolation of essential oil

Portions (100 g) of each raw, heated and irradiated samples prepared plant material were hydrodistilled for 3 hours in a Clevenger type apparatus, according to the method recommended in the European pharmacopeia to isolate the essential oil. The obtained essential oils were dried over anhydrous sodium sulfate. The collected essential oils of raw and treated samples were immediately analyzed, using GC and GC-MS.

Gas chromatographic analysis (GC)

GC-analysis was performed, using Hewlett-Packard model 5890 equipped with a flame ionization detector (FID). A fused silica capillary column DB-5 (60 mm \times 0.32 mm. id) was used. The oven temperature was maintained initially at 50 °C, for 5 min. Then heating was 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 retention indices (Kovats index) of the separated volatile components were calculated using hydrocarbons (C₆-C₂₂). Aldrich products were used as references.

Gas chromatographic–mass spectrometric analysis (GC-MS)

The analysis was carried out, using a coupled gas chromatography Hewlett-Packard (model 5890)–mass spectrometry Hewlett-Packard MS (model 5970). The ionization voltage was 70 eV, mass range m/z 39–400 amu. 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, Cairo, Egypt) and compared with those of authentic compounds and published data^[4]. The quantitative determination was carried out based on peak area integration.

Antioxidant activity assay

Anise and their extracts used for the determination of the antioxidant activity assays, and total phenolic content (TPC) were prepared as follow: 1 gram of respective solid anise was suspended with 100 mL of water/methanol or ethanol (80 % v/v) solution. This suspension was shaken for 1 hour, using a laboratory shaker at 1000 rpm. The solid phase was separated, using filtration. This step was carried out in triplicate and the final extracts were stored, in closed vials in darkness at 4°C.

DPPH scavenging assay

Each extract (10, 20, 30 $\mu\text{g/mL}$), in methanol, was mixed with 3 mL of methanolic solution containing DPPH \cdot . The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance was then measured at λ 517 nm, using spectrophotometer (Shimadzu, UV-160-IPC), against a blank solvent sample.

β -Carotene / linoleic acid assay

The antioxidant activity of ethanolic and methanolic extracts of raw and all treated samples of anise was performed, using β -Carotene bleaching assay (BCBA) according to the method described in literature (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 °C under vacuum. To this mixture, oxygenated water (20 mL) was added. 4 mL Aliquots mixtures were pipetted into different test tubes containing, 10 μg of each extract (10, 20, 30 $\mu\text{g/mL}$), in ethanol. All determinations were carried out in triplicate.

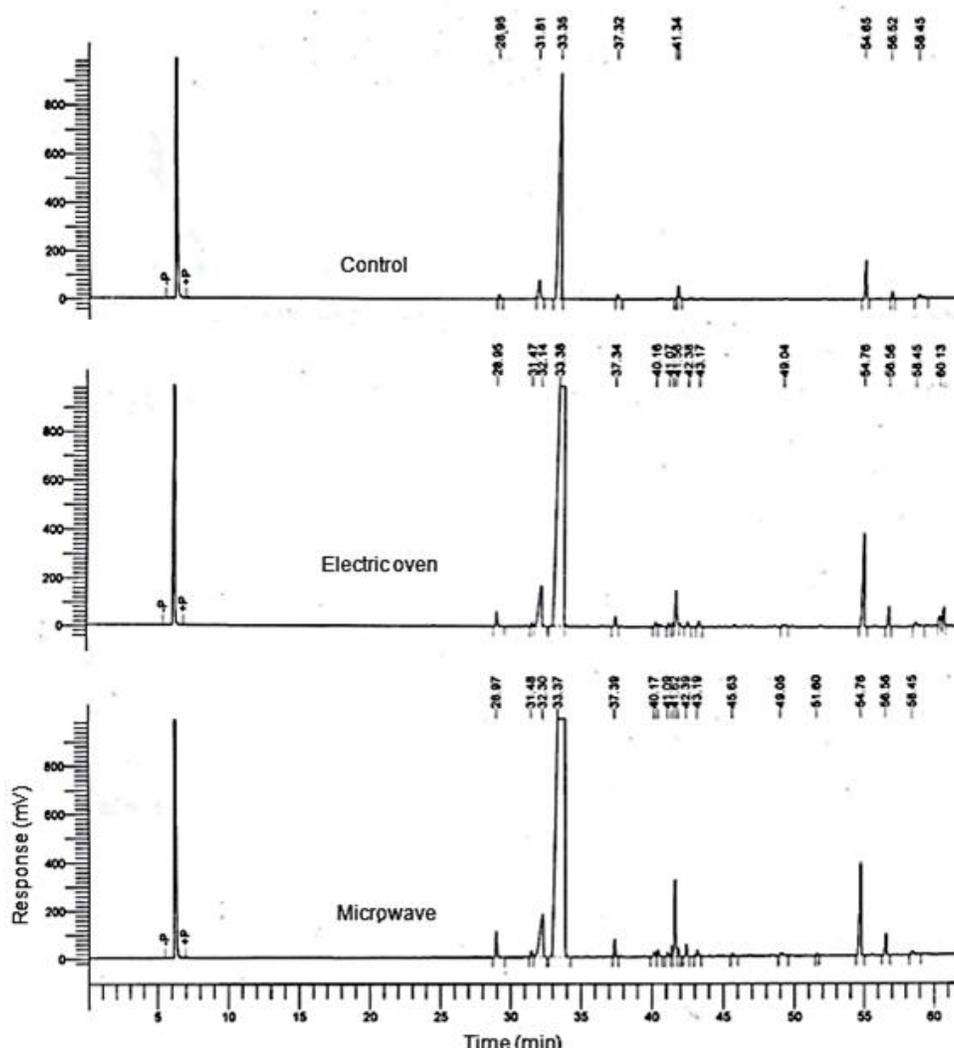


Figure 1 : Gas chromatograms of volatiles in HD oil of raw (control), thermally roasted (electric oven) and microwave heated anise seeds

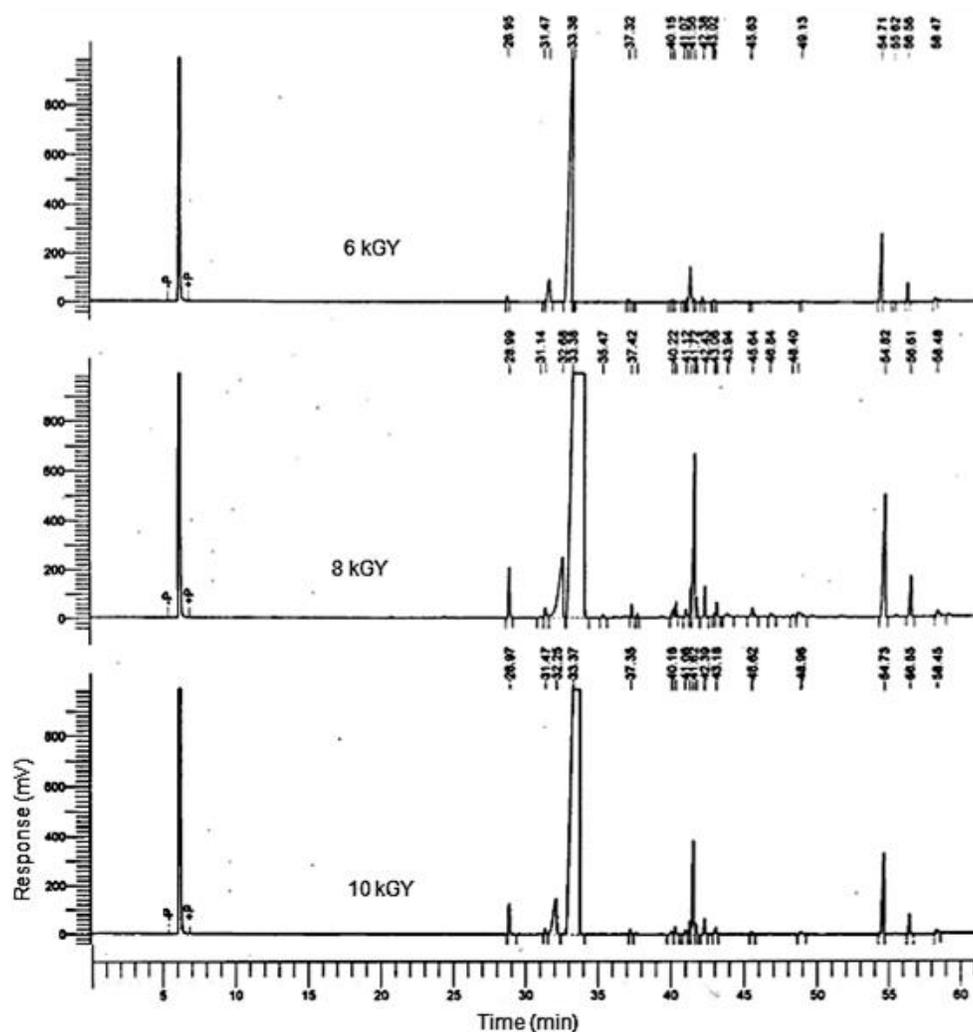


Figure 2 : Gas chromatograms of anise essential oil treated with different γ -irradiation dosed (6, 8 and 10 kGy)

Determination of total phenolic content (TPC)

Spectrophotometric determination of total phenolic content (TPC) in both raw and treated anise samples was carried out, using gallic acid in methanol (50-2500 mg/L) as an external standard, by Folin-Ciocalteu reagent assay, according to Singleton (1998). Samples, standards, and blanks were made in triplicate. The sample absorbance (indicative for polyphenols) was photometrically determined, at λ 760 nm. Results are expressed as milligrams of gallic acid equivalent per 100 g DW (mg GAE/100 g DW).

HPLC analysis

Extraction procedure

According to the results obtained in the previous experiments, the optimum extraction parameters are: most favorable temperature is 25 °C and appropriate solvent is a mixture of water: methanol (80: 20). 250 mg Powdered plant material were sonicated with 25 mL of the solvent mixture in an ultrasonic bath, for 20 min. After centrifugation, at 7600 \times g for 10 min, the supernatant was adjusted to 25 mL in a measuring flask. Samples were quantified immediately after extraction in order to avoid possible chemical alterations. Blanks and standards containing known concentrations were placed between the samples to monitor the quantification.

HPLC analysis instrumentation

Analyses were carried out, in an Agilent 100 series 1050 chromatograph equipped with an automatic injector, vacuum degasser and a DAD system. A Discovery® HS C₁₈ column (250 mm \times 4.8 mm, 5 μ m), Supelco, Bellefonte, PA, USA, was used for all the separations. The mobile phase was a gradient prepared from: (A) 0.1 % formic acid in water and (B) 0.1% formic acid in acetonitrile. The composition ranged from 10 % B to 26 % B in 40 min. The flow rate was 0.2 mL/min, and the injection volume 50 μ L. UV detection was performed at λ 280 nm.

Statistical analyses

Statistical analyses were performed, using SPSS version 16.0 for Windows. Student's t-test analyzed differences between samples and controls. One-way ANOVA tested differences within the irradiated and corresponding non-irradiated samples. Correlations were calculated, using Pearson's correlation coefficient. All data are presented as mean \pm SD. The significance level α was set at $p < 0.05$.

RESULTS AND DISCUSSION

Volatile components in hydrodistillation oil (HD) of raw, conventionally roasted, microwave heated, and γ -irradiated at 6, 8, and 10 kGy anise seeds

Seeds of anise (*P. anisum*) were subjected to conventionally roasting and microwave heating, as well as, exposed to γ -irradiation at 6, 8 and 10 kGy, in a Cobalt-60 package irradiator. These samples were analyzed and a comparison was done between these treatments and raw sample. In the case of raw sample, the volatile oil recovered after 3 h of hydrodistillation (HD) was 1.06 %. HD volatile oil increased in all treated samples. Thus, in electric oven roasted sample yield was 1.15 %; microwave heated sample yield was 1.5 %; irradiated samples yields were 1.08 %; 1.16 % and 1.31 % (w/w) in 6, 8 and 10 kGy, respectively. Identification of the volatile compounds was identified by KI values and MS spectra^[4].

TABLE 1 listed the eighteen volatile compounds which were identified in HD oil of anise, of all investigated samples, along with their area percentages. The typical gas chromatograms of the volatiles in HD oil of raw, roasted samples by electric oven and microwave heated as well as irradiated samples, at different doses 6, 8 and 10 kGy, from anise seeds, are shown in Figures 1 and 2. The total area percentages of the main chemical classes of all previous samples are shown in Figure 3. The volatile profile of raw HD oil of anise consisted mainly of *E*-anethole (79.68 %), hexahydrofarnesyl acetone (6.95 %), *p*-anisaldehyde (5.49 %), γ -himachalene (2.53 %), and estragole (0.76 %) (TABLE 1). These results are in accordance with literature reported data about major components of HD oil of anise; phenylpropanoid derivatives; *E*-anethole, *p*-anisaldehyde, *Z*-anethole and estragole (methylchavicol)^[33]. Roasting using electric oven and/or microwave (MW) heating did not lead to significant change in the total area percentage of these compounds. Thus, roasted and MW heated samples comprised phenylpropanoids 85.64 % and 84.80 %, respectively, compared to control sample which comprised 85.93 %. Also, it is found that γ -irradiation caused decrease in HD oil of irradiated samples at 6 and 8 kGy which comprised 80.69 % and 80.64 %, whereas at 10 kGy increased to reach 85.32 %, comparable to control sample; 85.93 % (Figure 3). These compounds are the main constituents in oils which are responsible for their antioxidant activities^[6]. The total percentage of these compounds ranged between 80.64 % and 85.64 %, for 8 kGy irradiated HD oil and electric oven-roasted anise seed samples, respectively. High content percentage of these compounds, especially; *E*-anethol and *p*-anisaldehyde, in all samples under investigation, advocated that they should have high antioxidant capacity^[31]. Benzene derivatives are represented, in HD of anise oil, in high amount 97.6 %.

Interestingly, estragole, the flavoring agent is considered to have negative effect on animal and human health and was deleted from the list of flavorings in food stuffs^[11]. The European pharmacopeia limit of estragole in essential oil of anise, 0.5–6.0 %, is not exceeded in present investigated samples. In this study estragole was found ranged between 0.66 % and 1.49 %, in 6 and 8 kGy irradiated HD oil samples, respectively (TABLE 1).

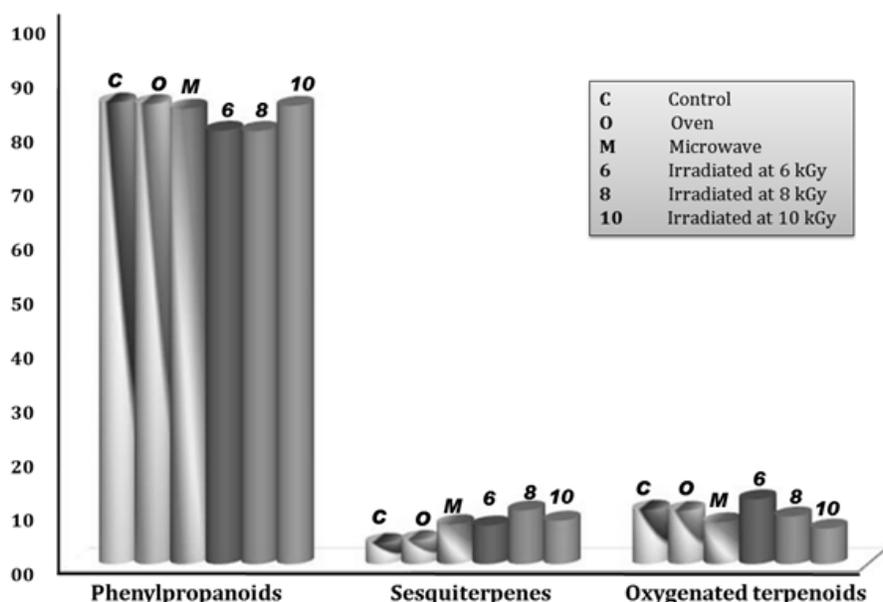


Figure 3 : The total area percentages of the main chemical classes in HD anise oil treated by thermal treatments using (electric oven, microwave) as well as γ -irradiated at three doses

TABLE 1 : Volatile components isolated in the hydrodistillation anise seed oil of raw, electric oven, microwave and γ -irradiation at 6, 8, and 10 kGy, Values are expressed as relative area parentages to total identified compounds

Peak No.	KI ^a	Compound ^b	Control (Raw)	Roasted		γ -Irradiation (kGy)			Type ^c
				Electric oven	Microwave	6	8	10	
1	1202	Estragole	0.76	0.83	1.17	0.66	1.49	1.41	PpD
2	1264	Z-Anethole	<i>nd</i>	0.25	0.37	0.18	0.44	0.36	PpD
3	1271	p-Anisaldehyde	5.49	6.04	5.60	4.79	6.32	4.72	PpD
4	1299	E-Anethole	79.68	78.52	77.66	75.06	72.39	78.83	PpD
5	1389	β -Elemene	1.10	0.68	0.86	0.41	0.51	0.29	SqH
6	1457	β -Caryophyllene	<i>nd</i>	0.34	0.31	0.27	0.30	0.27	SqH
7	1463	γ -Elemene	<i>nd</i>	<i>nd</i>	0.36	0.24	0.36	0.39	SqH
8	1480	γ -Curcumene	<i>nd</i>	0.24	0.28	0.18	0.33	0.29	SqH
9	1486	Aromadredrene	0.30	0.16	0.42	0.41	0.63	0.51	SqH
10	1491	γ -Himachalene	2.53	2.29	3.90	4.40	5.83	4.84	SqH
11	1498	α -Muurolene	<i>nd</i>	<i>nd</i>	0.34	0.37	0.63	0.43	SqH
12	1512	γ -Cadiene	<i>nd</i>	0.33	0.53	0.56	0.86	0.70	SqH
13	1532	α -Zingiberene	<i>nd</i>	0.43	0.43	0.41	0.55	0.36	SqH
14	1594	β -Caryophyllene oxide	<i>nd</i>	0.36	0.16	0.11	0.43	0.15	HOC
15	1687	α -Cadinol	<i>nd</i>	0.34	0.40	0.07	0.57	0.30	HOC
16	1845	Hexahydrofarnesylacetone	6.95	7.42	5.73	9.04	6.09	4.88	HOC
17	1902	E-Phytol	1.44	1.20	1.05	2.32	1.34	0.95	HOC
18	1963	E-Phytol acetate	1.73	0.58	0.42	0.52	0.44	0.32	HOC

^aKI: Linear Kovat Indexes: Compounds are listed according to their elution on DB-5 column, ^bAll compounds identified by GC-MS and/or Kovat Index on DB5 (KI) and/or by comparison of MS and KI of standard compounds, ^cPpD: Phenylpropanoids Derivatives, SqH: Sesquiterpene Hydrocarbons, HOC: Heavy Oxygenated Compounds, *nd*: not detected.

Conventional roasting caused increase in the total yield of sesquiterpenes (4.47 %), whereas microwave heating and γ -irradiation, at 6, 8 and 10 kGy, caused drastic increase in sesquiterpenes, which comprised 7.43 %, 7.18 %, 10 % and 8.18 %, respectively, compared to control one 3.93 % (Figure 3). This is attributable to the increase of β -caryophellene, γ -elemene; γ -curcumene; α -muurolene; γ -cadinene and α -zingiberene, in microwave as well as γ -irradiated samples. Meanwhile, these compounds were not detected in control one (TABLE 1). Besides increase in the percentage of aromadendrene and remarkable increase in the major sesquiterpene γ -himachalene which comprised in microwave and γ -irradiated samples 3.90 %, 4.40 %, 5.83 %, and 4.84 %, respectively, compared to control one 2.53 % (TABLE 1). These results are in accordance with findings reported in literature^[37, 2, 3].

The effect of roasted (electric oven or microwave heating) and γ -irradiation on the yield of oxygenated terpenoids of HD anise seeds oil, showed a remarkable decrease in all samples under investigation in comparison with their percentage in control (unprocessed) sample (Figure 3). Exceptionally, 6 kGy γ -irradiated sample revealed a remarkable increase in the total yield of heavy oxygenated compounds, comprising 12.06 % compared to their concentrations (10.12 %) in control one (Figure 3). This is owing to a remarkable increase in the percentage of terpenyl ketone (hexahydro-farnesylacetone), 9.04 % (TABLE 1). Higher percentages of phenolic content and oxygenated compounds strongly contribute to the fragrance and antioxidant activity^[23, 22].

Antioxidant activity of the HD anise seeds essential oils

The profile of scavenging activity on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH[•]) as well as the evaluated antioxidant activity, using β -carotene/linoleate assays, for raw, conventionally roasted, microwave and γ -irradiated 6, 8 and 10 kGy anise seeds essential oil, is shown in (Figures 4 and 5). The radical scavenging activity of essential oil on DPPH increased with increasing concentration of the oil. The strongest effect for reduction of DPPH radical was by 8 kGy irradiated sample (83.33 % \pm 0.08) and 6 kGy irradiated sample (83.33 % \pm 0.07), compared to TBHQ (98.8 % \pm 2.1), at the same concentration 300 μ g/mL (Figure 4).

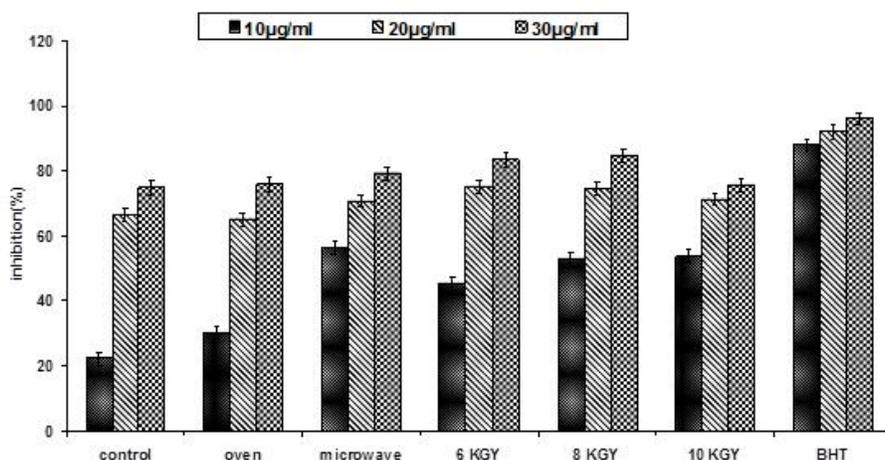


Figure 4 : DPPH scavenging assay of anise hydro-distilled oil control, electric oven, microwave and irradiated anise at 6, 8, and 10 kGy

As shown in Figure 5, the highest inhibiting effect, for oxidation of linoleic acid and the subsequent bleaching of β -carotene, was shown by 8 kGy γ -irradiated sample (85.21 %) and 6 kGy γ -irradiated sample (82.16 %), compared to TBHQ (87 %). These results are confirmed in Figure 6, which showed the total phenolic content of γ -irradiated samples.

Scavenging of the stable DPPH \cdot is an easy assay used to evaluate scavenging activity of antioxidants, since the radical compound is stable and does not have to be generated. DPPH \cdot accepts an electron or hydrogen radical to become a stable diamagnetic molecule and the anti-radical activity of an antioxidant is measured by the decrease in absorbance of DPPH \cdot . From figure 4, it is clear that DPPH \cdot percent scavenging activities of the extracts are dose-dependent.

Even though, it seems that DPPH \cdot scavenging activity of plant extracts might be attributed to individual phenolic acids, the overall antioxidant potential exhibited by these extracts were found liable to the synergistic effects of the mixture of total phenolic acids, by means of several phenolic compounds and other antioxidant components which are present in plant extracts. Polyphenols are thermally labile molecules; they get easily degraded upon heat treatment^[25]. In case of roasted samples, at very high temperature (160 °C), Maillard reactions might also contribute to the reduction of polyphenol levels^[21]. β -Carotene bleaching test is commonly used for determination of antioxidant activity of natural compounds because it is carried out in an emulsion, a condition frequently seen in foods. In this model system, reduction in the orange color of β -carotene backs to the abstraction of a hydrogen atom from one of its methylene groups is assessed. It can be clearly inferred that antioxidant activity coefficient (AAC) of the extracts is concentration-dependent (Figure 5).

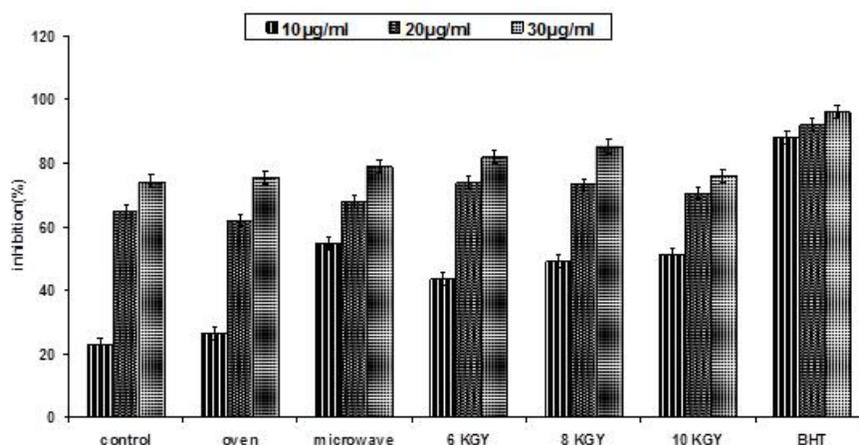


Figure 5 : β -Carotene linoleic acid bleaching assay of anise control, electric oven, microwave and irradiated anise at 6, 8, and 10 kGy

HPLC analysis of γ -irradiated selected sample and total phenolic

Figure 6 depicts the phenolic content for the different treatments in mg gallic acid equivalent /g extract. In general, significant differences in total phenolic content backs to various reasons such as; climate, location, temperature, choice of parts tested, time of taking samples and determination methods.

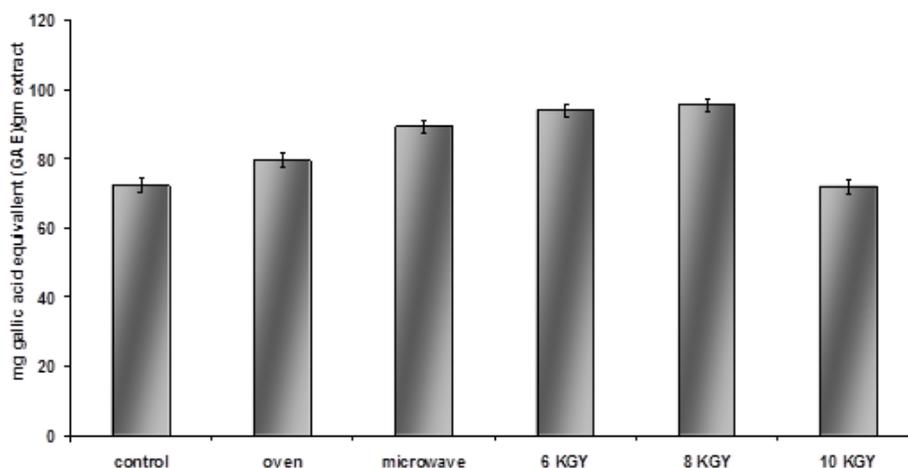


Figure 6 : Total Phenolic content of anise hydro-distilled oil in control, electric oven, microwave and irradiated doses at 6, 8, and 10 kGy

Identification of phenolic compounds was carried out for the selected treated essential oil. The most effective treatment, at 10 kGy, was preliminary performed, using HPLC-UV, *via* comparing the relative retention times and UV spectra of which with these of standard solutions. The obtained data for comparing HPLC peak areas, concentration, of phenolic compounds in the essential oil with those of external standard are listed in (TABLE 2). A total of nine phenolic compounds were identified in anise essential oil after γ -irradiation, at 10 kGy. These compounds are: gallic acid, catechin, ferulic acid, benzoic acid, cinnamic acid, rutin, quercetin, *p*-qumaric acid and kampferol. The obtained results showed that *p*-qumaric acid was the predominant phenolic compound (43.36 %), followed by ferulic acid (21.06 %), and benzoic acid (15.73 %).

TABLE 2 : Effect of γ -irradiation on phenolic compound of anise essential oil (treated with 10 kGy). Values are expressed ad relative area percentage

Phenolic compound	Concentration
Catechin	4.72
<i>P</i> -Qumaric acid	43.36
Ferulic acid	21.06
Benzoic acid	15.73
Cinnamic acid	6.07
Rurin	2.53
Quercetin	0.77
Gallic acid	2.33
Kampferol	0.86

CONCLUSIONS

Comparing effect of thermal treatments, using different tools such as electric oven, microwave and γ -irradiation, afforded interesting information about constitution of volatile and non-volatile essential oil of Egyptian anise. Volatile profile of raw hydrodistilled (HD) anise oil mainly consists of *E*-anethole (79.68 %), hexahydrofarnesyl acetone (6.95 %), *p*-anisaldehyde (5.49 %); γ -himachalene (2.53 %) and estragole (0.76 %). Both roasting and γ -irradiation, at 10 kGy, has no significant effect on total yield of phenylpropanoids. γ -Irradiations at 6 or 8 kGy lead to a remarkable decrease of phenylpropanoids. All samples revealed high antioxidant activities. Both thermal and γ -irradiation showed the same high increase in total yield of sesquiterpenes, although yield of oxygenated compounds diminished. This significantly affected the antioxidant activity of the treated samples. 8 kGy γ -Irradiated sample, exhibited the strongest reducing effect of DPPH radical (84.57 % \pm 1.43), as well as, the highest inhibiting effect of linoleic acid (85.21 % \pm 0.12) and subsequent bleaching of β -carotene, compared to bulylated hydroxytoluene (98 %). Nine phenolic compounds were identified using HPLC and C18 RP-HPLC techniques. The predominant compounds were *p*-qumaric acid (43.36 %) and ferulic acid (21.06 %).

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