*Egypt. J. Chem.* **Vol. 67**, No. 8. pp. 239 - 250 (2024)

**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Effect Of Thermal Treatments And γ-Irradiation On The Volatile, Phenolic acids, and Antioxidant Activity of Egyptian Fennel Essential Oil

**Magda A. Abd El Mageed[1], Mostafa M. Ismail[2] , Mohamed Abass [2] , Gamil E. Ibrahim[1] and Engy. M. Mahmoud [1]***

[1] *Chemistry of Flavour & Aroma Dept National research center .NRC, Dokki, Giza 12622, Egypt.*

[2] *Department of Chemistry, Faculty of Education, Ain Shams University,Roxy, Cairo 11757, Egypt*

**Abstract**

The effect of various thermal treatments (electric oven, microwave) and γ-irradiation at three doses, (6,8 and 10 KGy) on the composition of volatile and nonvolatile of Fennel (*Feniculumvulgare* mill) essential oil and also their antioxidant properties were considered. The hydrodistilled oil (HD) of control and treated samples were subjected to gas chromatography—mass spectrometry (GC/MS) analysis. The volatile profile of raw HD oil of fennel consisted mainly of estragole (65.67%) followed by carbon (9.6%), limonene (6.77%), fenchone (6.71%), and trans-anethole (5.92%). Roasting caused a drastic increase in the total yield of phenylpropanoid (major compounds) in all treated samples by thermal or by y-irradiation ranging from (82.12%) in the electric oven roasted sample to (89.63%) in 10 KGy irradiated sample compared to (72.67%) in control one. This is due to the very high increase in estragole percentage in all treated samples, ranging between (79.7%) in the electric oven sample to (87.75%) in the 10 KGy irradiated sample compared to (65.67%) in the control one. Roasting caused a decrease in the total yield of monoterpenes in all treated samples except the electric oven-roasted sample, which gained a slight increase compared to the control sample. At the same time, roasting caused a drastic decrease in oxygenated terpenoids in all treated fennel samples in comparison to the row sample. i.e. Roasting cause a drastic decrease in all components of HD oil of all treated samples of fennel except estragole which increased to reach about 90% in 10 KGy irradiated sample. This compound is considered a flavouring agent and, by the European pharmacopeia limit, does not exceed 6.0% in essential oils. it has a negative effect on animal health and was deleted from the list of flavour and food stuffs. This indicated in antioxidant activity of HD fennel essential oil; the strongest effect for reduction of DPPH radical was by microwave heated sample, which exhibited (58.65%±0.36) followed by 1OKGy irradiated sample, which exhibited (49.27%±0.32) compared to BHT (98%) at the same concentration 30 mg/ml.

**Keyword:** Fennel oil, roasting, microwave, γ -irradiation, antioxidant, volatile, phenolic

## 1. Introduction

Spices have recently gained attention for their useful physiological functions and antimicrobial activity. While there are many reports on the antimicrobial activity of spice extracts and their essential oils, the available information is limited to a small group of microorganisms tested at high concentrations of no practical use. More research is required on the antimicrobial effects of food-related bacteria, such as food spoilage and food-borne bacterial pathogens (Roby et al., 2013).

Lipid oxidation in food processing can cause nutritional value and taste loss and create oxidized products and free radicals. Conventional artificial antioxidants have been used for over 50 years to prevent this, but they may have adverse health effects. Natural additives are being studied as alternatives (Li and Yi.,

*Corresponding author e-mail: engy_nermo@yahoo.com
Received date 10 December 2023; revised date 09 January 2024; accepted date 16 January 2024
DOI: 10.21608/EJCHEM.2024.253638.8974

2003). Aromatic and medicinal plants are effective in retarding lipid peroxidation in oils and fatty foods due to their antioxidant properties, gaining interest from research groups. This has increased the demand for these plants and their prices in industrialized and non-industrialized countries (Kulisic et al.., 2004).

Fennel is a member of the *Apiaceae* family, classified into two subspecies: *vulgare* and *piperitum*. Sweet fennel is widely used in culinary preparation and perfumery due to its medicinal and aromatic compound content. It is also considered a spice and can affect vegetative growth, edible bulb yield, and the quality of sweet fennel plants (Paschalina et al., 2006; Zaki et al., 2009). The use of herbs and spices for food preservation and nutrition has increased. Treatment with ionizing radiation is a safe and accredited preservation method for herbs, but its effects on the antioxidant activity of phytochemicals are mainly unknown. The Joint FAO/IAEA/WHO Expert Committee has confirmed the safety of irradiation up to 10 kGy, and dried foods like fennel powder can be irradiated at a maximum dose of 10 kGy (Šunić,et el., 2023). An irradiation dose of 5 to 10 kGy is sufficient to reduce the population of microbes while keeping the spice flavor intact. However, creating unsaturated hydrocarbons and migrating abnormal compounds from packaging materials during irradiation may harm food flavor (Krzymien et al., 2001; Afifi et al.,2021).

Microwave energy is commonly used for heating in food processing. It can also be adopted for pasteurization and sterilization of food at lower temperatures and shorter times than traditional methods (Fung & Cunningham, 1980). Spices and herbs can be effectively treated to destroy insects and microorganisms using ionizing radiation, a safe and controllable physical process that does not require additives. This treatment is advantageous because it is a cold process and does not cause any loss of volatile components. Microwave energy has been used for the past 40 years to pasteurize, sterilize, defrost, blanch, dehydrate, and cook food, and has opened new possibilities in food preparation (Abd-elmageed et al., 2011, 2012, and 2014). During microwave pasteurization, the material of high dielectric capacity absorbs electromagnetic energy, which leads to an increase in temperature. This process offers similar benefits to traditional methods but with improved product quality and reduced energy exposure time (Hashem&Alamri, 2010). The use of microwaves for processing spices is a relatively new technique. It has gained popularity due to its convenience and ease of handling (Abd-elmageed, 2007).

Cooking alters food components, changing their nutritional value and properties. Heat can impact the bioavailability of carbs, lipids, proteins, and vitamins. Research on how heat processing affects spice bioactive components is limited. It is important to assess spice availability in its original form after cooking. If significant losses occur, it raises questions about spice health benefits after conventional heat processing during domestic food preparation (Srinivasan, 2005). Spices are often processed to remove extraneous matter and ensure microbial stability. Roasting is crucial to releasing characteristic flavor volatiles and eliminating undesirable constituents, impacting flavor quality (Susheela, 2000).,(Mohammadi,et al,2023).

The present work deals with the evaluation of some thermal treatments (conventionally roasting by electric oven and microwave heating) which were suggested to decontaminate the spices and to compare the results with g-irradiation doses (6,8 and 10 kGy) (so-called cold sterilization) were recommended for this purpose as standard work. The evaluations included phenolic content, antiradical activities, and volatile and phenolic compounds to choose the best results in this comparative study for improving the quality of Egyptian Fennel(Hassan,et al.,2019).

## 2. Materials and Methods

### 2.1. Plant materials

Fennel (*Foeniculumvulgare Miller*) was purchased from local market at Giza during 2021-2022.

### 2.2. Chemicals

All the solvents and chemicals used in the current study were purchased from Sigma-Aldrich, Saint Louis, MO, USA. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), butylatedhydroxytoluene (BHT), b-carotene, gallic acid, Folin–Ciocalteu reagents, and standard hydrocarbons (C8-C22) were of analytical grade, while methanol and formic acid were HPLC grade.

### 2.3. Thermal processing and irradiation of samples

Three fresh, dry samples weighing 100g each were roasted in a conventional electric oven at 120°C for 20 minutes. Similarly, another set of three samples weighing 100g each was heated using a microwave oven and roasted after 3 minutes of heating (Daewoo DE Microwave, Mod: KoG-181G, 200-240V 50Hz, input power of 1400 W, made in Korea).

The samples were then packaged in sanitized brown glass capped bottles (1L) and irradiated using γ-cell, cobalt-60 γ-irradiator at a dose rate of 1.29744 KGy/hour at the Radiation Research Centre in Cairo, Egypt. The doses applied in this study were 0, 6, 8, and 10 KGy. The doses were within 75.4% of the target dose (Choi et al., 2010). The irradiation was carried out at a temperature of 18°C. A non-irradiated control sample was placed outside the irradiation chamber to account for the effect of environmental temperature. The irradiated anise samples were then transferred and kept in a dry place. The raw and treated samples were separately ground in a spice mix grinder.

**2.4. Isolation of essential oil**

To isolate the essential oil from each sample, 100g portions of raw, heated, and irradiated plant material were hydrodistilled for 3 hours using a Clevenger-type apparatus. The method recommended in the European Pharmacopeia was followed. The essential oils obtained were then dried using anhydrous sodium sulfate. The essential oils collected from the raw and treated samples were analyzed immediately using GC and GC-MS.

**2.5. Gas chromatographic (GC) analysis**

The GC analysis was conducted using a Hewlett-Packard 5890 model from the USA, equipped with a flame ionization detector (FID). A fused silica capillary column DB-5 (60m 0.32 mm. id) was used for this purpose. Initially, the oven temperature was kept at 50°C for 5 minutes and was then increased at a rate of 4°C/minute from 50 to 250°C. The carrier gas used was Helium, which flowed at a 1.1 ml/min rate. The injector and detector temperatures were set at 220 and 250°C, respectively. To calculate the retention indices (Kovats index) of the separated volatile components, hydrocarbons ($C_6$-$C_{22}$) were used as references.

**2.5.1. Gas chromatographic-Mass spectrometry analysis**

The analysis was carried out using Hewlett-Packard model (5890) gas chromatography coupled with mass spectrometry Hewlett-Packard-MS (5970). The ionization energy was 70 eV, and the mass range was 39-400 amu. The GC condition was as mentioned above. The peaks were identified by matching with data from the mass spectra library (National Institute of Standards and Technology), and they were compared with those of authentic compounds and published data (Adams et al., 2007). The quantitative determination was carried out based on peak area integration.

**2.6. Antioxidant activity assay**

To prepare essential oil extracts for antioxidant activity assays and total phenolic content (TPC) determination, 1g of solid fennel essential oil was mixed with 100 mL of 80% (v/v) water/methanol or ethanol solution. Then, the suspension was for 1 hour at 1000 rpm, and filtered the solid phase thrice. The extracts were then stored in closed vials in darkness at 4°C. (Tănase, et al.,2022),(Naaz et al,2022)

**2.6.1. DPPH scavengingassay.**

The experiment measured the antioxidant activity of each extract at different concentrations. To do this, 10, 20, and 30 µg/mL of the extract were mixed with 3 ml of a methanol solution containing DPPH radicals. The mixture was vigorously shaken and kept in darkness for 30 min. At 517nm, the absorbance of the mixture was measured using a Shimadzu UV-160-IPC spectrophotometer against a blank (Najja et al., 2011). The result was calculated using the following formula: I%=[(ΔA517C-ΔA517S)/ΔA517S]

Here, ΔA is the average absorbance, C is the control, and S is the sample.

**2.6.2. β –Carotene scavenging activity assay**

The antioxidant activity of raw and treated standard samples was measured using β-carotene bleaching assay, as described by (Iqbal et al. 2007), with ethanolic and methanolic extracts. For the assay, 0.1 mg of β-carotene was dissolved in 0.2 mL chloroform, 10 mg of linoleic acid, and 100 mg of tween 40. After removing the solvent at 40°C under vacuum, 20 mL of oxygenated water was added to the resulting mixture. Aliquots of the mixture (4 mL) were pipetted into separate test tubes containing 10µg of each extract (10,20,30µg/mL) in ethanol. The bleaching inhibition was calculated using the following equation: %Inhibition=[(AB-AA)/AB]*100, where AB represents the blank sample absorption at t=0 min and AA represents the absorption of the sample solution at t=60 min. The results were expressed as the ability of the extracts to prevent the bleaching of β-carotene. All determinations were performed in triplicate. (Abdellaoui et al,2020).

**2.7. Determination of total phenolic content (TPC)**

The total phenolic content (TPC) of both raw and treated samples was determined using the FolinCiocalteu reagent assay by (Singleton ,1998). The gallic acid in methanol (50-2500mg/L) was used as an external standard. Samples, standards, and blanks were prepared in triplicate. The sample absorbance, indicative of polyphenols, was measured photometrically at 760nm. The results are expressed as milligrams of gallic acid equivalent per 100g of dry weight (mg GAE/100gDW).

**2.8. HPLC analysis**

**2.8.1. Extraction procedure**

The extraction parameters that were ultimately selected include a temperature of 25°C and the use of a water-methanol mixture (80:20). To extract the plant material, 250 milligrams of powdered material were sonicated with 25 mL of the solvent mixture in an ultrasonic bath for 20 minutes. After centrifugation at 7600×g for 10 minutes, the supernatant was adjusted to 25 mL. Blanks and standards with known concentrations were also placed between the samples to monitor the quantification.

**2.8.2. HPLC analysis instrumentation**

The analysis used an Agilent 100 series 1050 chromatograph equipped with an automatic injector, a vacuum degasser, and a DAD system. A Discovery HS C18 (250 mm × 4.8 mm, 5 um) column from Supelco (Bellefonte, PA, USA) was used for separation. A gradient was created to prepare the mobile phase from 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The mobile phase composition ranged from 10% B to 26% B within 40 minutes. The flow rate was 0.2 ml/min, while the injection volume was 50 µl. The UV was detected at 280 nm.(Saber et al,2019)

**2.9. Statistical analyses**

Data was analyzed using SPSS 16.0 for Windows. Student's t-test compared sample and control differences. One-way ANOVA tested differences within irradiated and corresponding non-irradiated samples. Pearson's correlation coefficient calculated correlations. Data presented as mean ± SD. The significance level is set at $P < 0.05$.

**Results and Discussion –**

- **3. Results and Discussion**
- **3.2. Fennel (*FeniculumVulgare mill*)**
- **3.2.1. Volatile components in HD oil of raw, conventionally roasted, microwave heated and roasted by γ– irradiation at 6,8 and10 KGy fennel seeds.**

- Fennel (*FeniculumVulgare mill*) mature fruit (commonly known as seeds) subjected to roasted by electric oven, microwave, and γ- irradiation at 6,8 and10 KGy in a $Co^{60}$ package irradiator. These samples were analyzed and compared with raw samples. The volatile oil of the raw sample recovered after 3h of hydrodistillation (HD) was 0.75%, whereas HD volatile oil increased in all treated sample comprising0.81% in the roasted electric oven sample, 1.04% in microwave roasted sample; 0.83%,0.93%,1.02% (W/W) respectively in three doses irradiated samples.
- Eighteen volatile compounds were identified in HD oil of fennel in all samples under investigation. These compounds are 4 phenylpropanoides, 8 monoterpene hydrocarbons, 5 oxygenated monoterpenes, and trace amounts of ethanol in all samples. All these compounds are listed with their area percentage in (**Table 1).** Identification of the volatile compounds was identified by KI values and MS spectra (Adams, 2007) The typical gas chromatograms of the volatiles in HD oil of raw, roasted samples by electric oven, microwave, and γ-irradiation at three doses of fennel are shown in (**Figs. 1,2**) The volatile profile of raw HD oil of fennel consisted mainly of estragole (65.67%) followed by carvon (9.6%), limonene (6.77%), fenchon (6.71%) and trans anethole (5.92%). These results are in accordance with many authors **(**Miraldi 1999; Damjanovic*et al* 2005; Diaz-Marot*et al* 2006; Gulfraz*et al* 2008; Anwar *et al* 2009; Shahat*et al* 2011; Gori *et a*l 2012; Senatore*et al* 2013**)**. It is well known that the major components in the HD oil of anise and fennel seeds (=fruits) are phenylpropanoid fraction which yield (81-86%) (**Fig.3**) and trans–anethole was (72-79.7%) dominated in HD anise essential oil **(Table 1)**, whereas it comprised (72.67%-89.63%) (**Fig. 1**) and estragole was dominated since comprised (65.76%-87.75%) in HD fennel oil (**Table 1**). These results are in agreement with **(**Miguel et al**., 2010),** who found that the total yield from phenylpropanoid fraction (80-89%) and estragole (79-88%) dominated the fennel fruit oil. Roasted cause a drastic increase in the total yield of phenylpropanoid in all samples under investigation which comprise 82.12%; 88.45%; 86.12%; 87.98% and 89.63% in electric oven, microwave and three irradiated samples respectively compared to 72.67% in control sample (**Fig. 1).** This is due to the very high increase in estragole percentage in all treated samples which comprised 79.71%; 86.42%; 83.58%; 85.87% and 87.75% in roasted electric oven, microwave and roasted by irradiation at three doses respectively compared to (65.67%) in control one (**Table 1).** Also, roasted by different treatments caused a remarkable decrease in the percentage of trans and cisanethole and p-anisaldehyde (**Table 1).** Roasting causeda decrease in the total yield of monoterpenes in all samples under investigation except roasting by electric oven sample compared to

the control sample (**Fig. 1).** Limonene is the major monoterpenecomprising8.77%; 5.31%; 6.66%; 5.86% and 5.46% in electric oven, microwave and three irradiated samples respectively compared to 6.77% in control one (**Table 1).** Also, roasted causeda drastic decrease in oxygenated terpinoides in all samples which comprised 7.05%; 4.96%; 5.83%; 4.59% and 3.79% in electric oven, microwave and three irradiated samples respectively compared to 17.83% in control sample (**Fig. 1**).

From previous results it is found that all samples under investigation contain approximately the same concentration of all compounds excluding *trans*-anethole and estragole; besides in HD oil of anise which was trans-anethole was major compound in roasted samples showed higher antioxidant activity in all samples (**Figs. 4,5)**

In a study conducted by **(**Shahat et al.,2011**)**, it was confirmed that the chemical composition of three different fennel cultivars - *Foeniculumvulgare* var. azoricum, var. dulce, and var. vulgare - varied significantly.

The HD oil of *azoricum* and dulce cultivars contained 61% and 46% trans-anethole, respectively, while the oil of the vulgare cultivar contained only 5% of this compound.

On the other hand, the oil of the vulgare cultivar had a concentration of 58% estragole, compared to 12% and 6% in the HD oil of azoricum and dulce cultivars, respectively.

Furthermore, the antioxidant activity of the azoricum and dulce cultivars was significantly higher than that of the vulgare cultivar. This is because the oils of these two cultivars contained higher concentrations of trans-anethole, the main compound responsible for the antioxidant activity.

Anethole and estragole have different chemical structures, with anethole having a conjugated double bond on its propenyl side chain, while estragole has a nonconjugated double bond.

Anethole easily forms a conjugated radical cation, making it an excellent radical scavenger (Yamaoket al., 2009). Estragole is a good alkylation agent, which can easily alkylate DNA molecules and is a suspected carcinogen (Mcdonald, 1999). This observation may explain the differences in the antioxidant activity between the tested essential oils. The European Pharmacopeia limits the amount of estragole in essential oil to 6.0%, as it is considered to have adverse effects on animal and human health and has been removed from the list of flavors in foodstuffs (Burt, 2004).

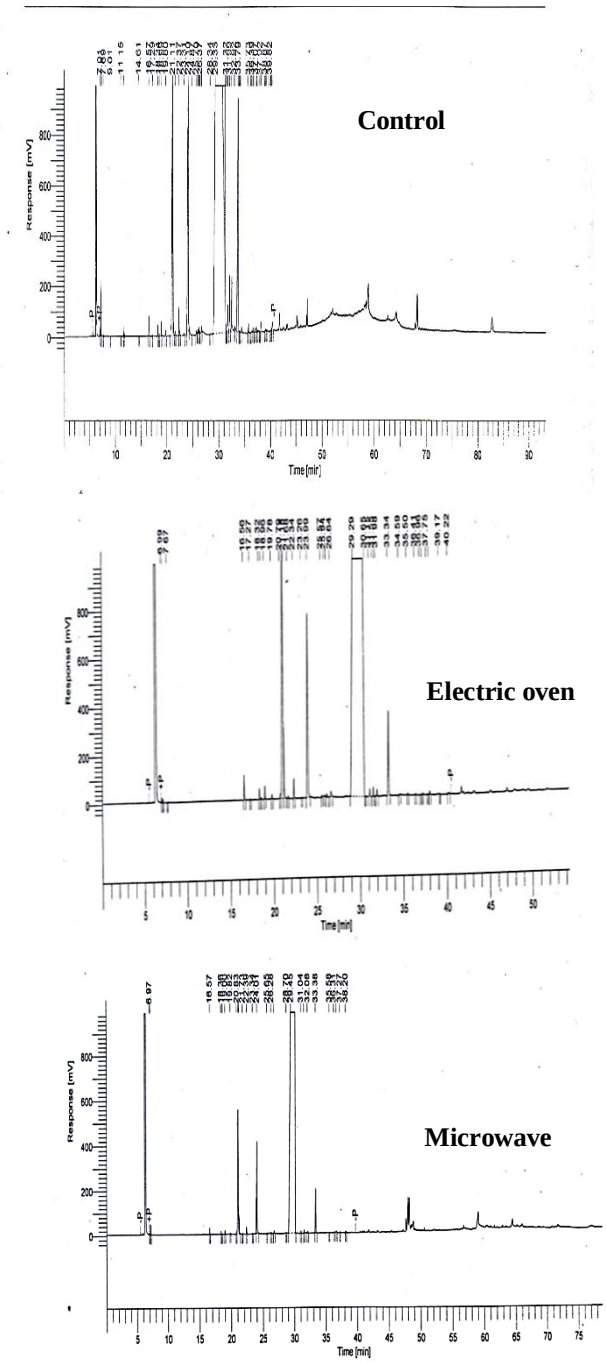
### 3.2.2. Antioxidant activity of the HD fennel seed essential oils

The profile of scavenging activity on DPPH radical as well as the evaluated antioxidant activity using β-carotene/linoleic acid assay are shown in (**Figs. 4,5)** for raw, conventionally roasted, microwave heated, and γ-irradiation 6,8 and10 KGy fennel seeds essential oil.

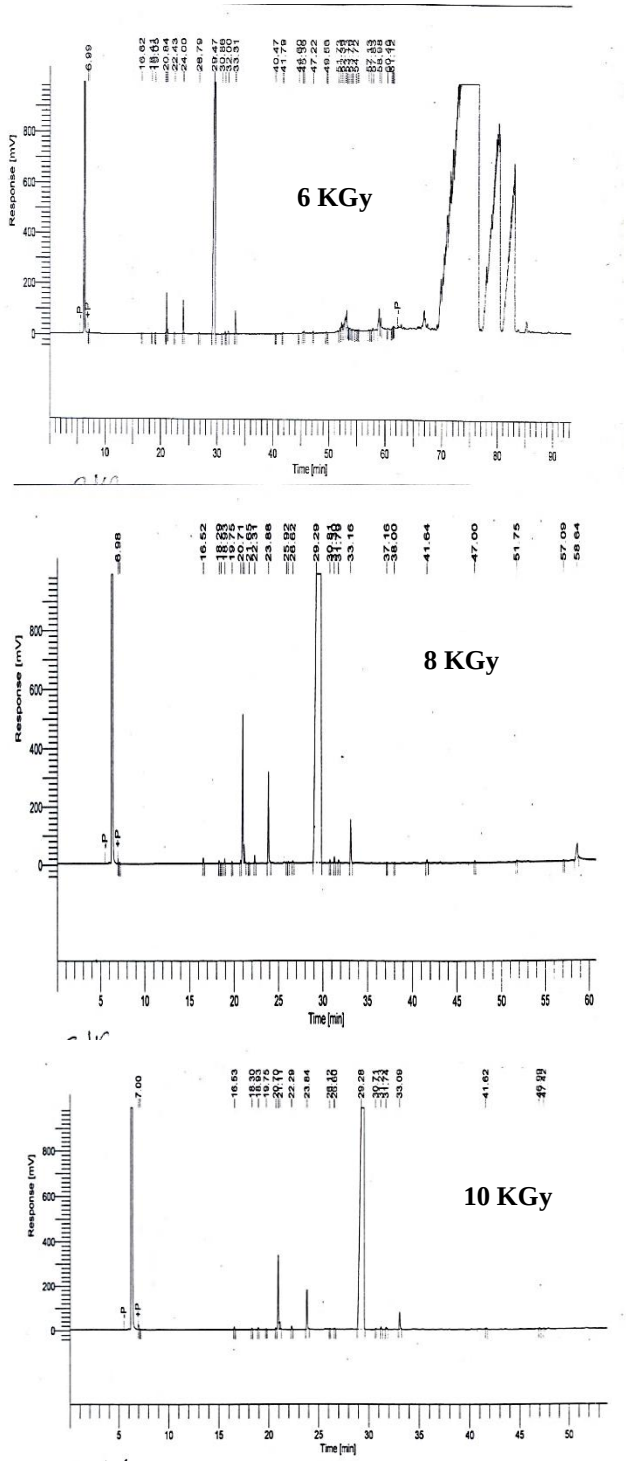
The results mentioned above confirmed that *trans*-anethole is good radical scavenging, *i.e.*, a good antioxidant, while estragole (which is the major component in HD fennel seed oil) is a carcinogenic agent (Shahat et al., 2011; Mcdonald,1999).

This is indicated by our results, which showed the most potent effect for reduction of DPPH radical was by microwave heated sample, which exhibited (58.65%±0.36) followed by 10KGy irradiated sample, which exhibited (49.27%±0.32) compared to the BHT (98%) at the same concentration 30 µg/ml (**Fig. 4)** the same behavior was shown in (**Fig. 5)** the highest inhibiting effect for oxidation of linoleic acid and then subsequent bleaching of β-carotene also was by microwave roasted sample followed by 10KGy irradiated sample which comprised (61.13%±0.49, 47.57%±0.52) compared to synthetic antioxidant BHT(98%).

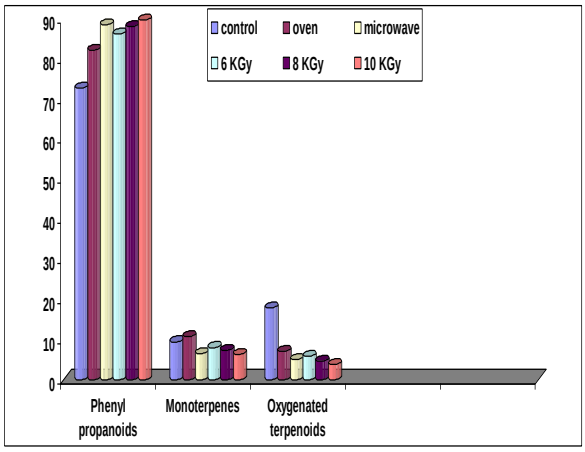
These results were confirmed by the total phenolic content of all samples under investigation since they comprised very low concentrations ranging between 25.03% in the 8 KGy irradiated sample to 35.61% in the electric oven roasted sample (**Fig. 4)**.




(Fig. 1): Gas Chromatograms of volatiles in HD oil of raw (control), thermally roasted (Electric oven), and microwave heated fennel seeds.

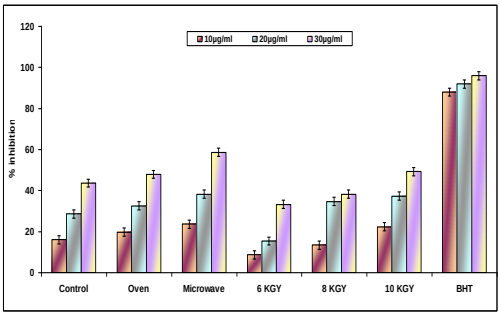



(Fig. 2) Gas Chromatograms of fennel essential oil treated with different g-irradiation doses (6,8 and 10 KGy).

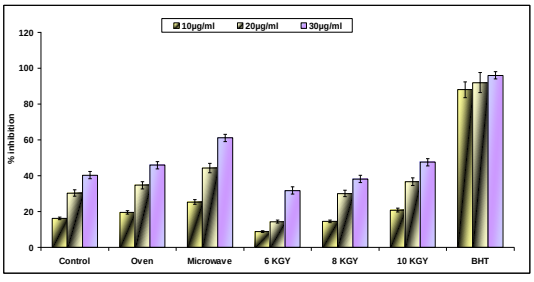



(Fig. 3)The total area percentages of the main chemical classes in HD fennel oil treated with thermal treatments (electric oven, microwave) and γ-irradiation at three doses.

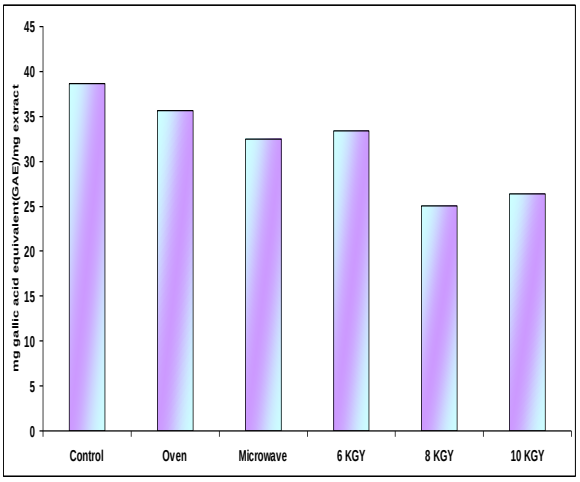
These results due to the high percentage of estragole in all samples which ranged (65.67% to 87.75%) (**Table 1)**. These results are by **(**Zangouras *et al.*, 1981; Diaz-Maroto *et al.*, 2006; Shahat *et al.*, 2011).




(Fig. 4) Scavenging effect of fennel volatile oil on DPPH radicals with different concentrations compared to BHT




(Fig. 5) Scavenging effect of fennel volatile oil on β-carotene linoleic acid bleaching with different concentrations compared to BHT




(Fig. 6) Total Phenolic content of fennel extract

### 3.2.3. HPLC analysis of selected samples (microwave treatment)

A total of 14 compounds were identified with the help of HPLC. As per Table 4, 3-caffeoylquinic acid and 4-caffeoylquinic acid are the primary phenolic substances, accounting for 45.69% and 6.83%, respectively. The discovery made in this study confirms the presence of several flavonoids that were previously described by (Bilia et al,2000), and it also provides evidence of substantial amounts of ferulic acid, caffeic acid, and caffeoylquinic acid derivative, as reported by (Guillen et al, 1996).

Regarding the other substances detected in the irradiated sample after microwave treatment, 5-caffeoylquinic acid was identified, which accounted for 6.65% of the sample.

This substance is a common metabolite found in higher plants and is a known component of fennel fruit (Kunzemann & Herrmann, 1977). It is worth noting that coumarins, characteristic metabolites of fennel and the Apiaceae family (Murray et al., 1982), were not found in the sample. This is significant from a drug use safety perspective, mainly since fennel fruits are commercially used for medicinal purposes.

**Table (1) Volatile components isolated in the hydrodistillation oil of fennel raw, electric oven, microwave and γ -irradiation at 6,8 and 10 KGy. (*** Values expressed as relative area parentages to total identified compounds)

| Peak No. | KI[a] | Compound | Control (Raw) | Roasted | | γ-Irradiation (KGY) | | | Type [b] | Identification method [c] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Electric oven | Microwave | 6 | 8 | 10 | | |
| 1 | 620 | Ethanol | *0.05 | 0.06 | 0.02 | 0.03 | 0.08 | 0.27 | LOC | MS and KI |
| 2 | 955 | Camphene | 0.48 | 0.42 | 0.18 | 0.15 | 0.15 | 0.17 | M | MS and KI |
| 3 | 977 | Sabinene | 0.31 | 0.18 | 0.09 | 0.23 | 0.19 | 0.09 | M | MS and KI |
| 4 | 992 | Myrecene | 0.37 | 0.27 | 0.12 | 0.26 | 0.23 | 0.13 | M | MS and KI |
| 5 | 1009 | α-Phelandrene | 0.42 | 0.15 | 0.25 | 0.27 | 0.20 | 0.12 | M | MS and KI |
| 6 | 1031 | *P*-Cymene | - | 0.15 | 0.12 | 0.16 | 0.12 | 0.12 | M | MS and KI |
| 7 | 1035 | Limonene | 6.77 | 8.77 | 5.31 | 6.66 | 5.68 | 5.46 | M | MS, KI, and STD |
| 8 | 1038 | 1,8-Cineol | 1.07 | 1.10 | 0.81 | 1.84 | 0.79 | 0.77 | LOC | MS, KI, and STD |
| 9 | 1050 | (E)-β-Ocimene | 0.55 | 0.46 | 0.25 | - | 0.53 | - | M | MS and KI |
| 10 | 1064 | γ-Terpinene | 0.54 | 0.36 | 0.24 | 0.28 | 0.27 | 0.21 | M | MS and KI |
| 11 | 1098 | Fenchone | 6.71 | 5.55 | 3.81 | 3.58 | 3.27 | 2.80 | LOC | MS, KI, and STD |
| 12 | 1142 | Camphor | 0.19 | 0.09 | 0.09 | - | 0.19 | 0.06 | LOC | MS and KI |
| 13 | 1156 | Borneol | 0.26 | 0.15 | 0.14 | 0.26 | 0.23 | 0.08 | LOC | MS and KI |
| 14 | 1202 | Estragole | 65.67 | 79.71 | 86.42 | 83.58 | 85.87 | 87.75 | Ph.Pro.Der | MS, KI, and STD |
| 15 | 1246 | Carvone | 9.60 | 0.16 | 0.11 | 0.15 | 0.11 | 0.08 | LOC | MS and KI |
| 16 | 1258 | (Z)-Anethole | 0.32 | 0.15 | 0.10 | 0.18 | 0.19 | 0.18 | Ph.Pro.Der | MS and KI |
| 17 | 1271 | P-Anisaldehyde | 0.76 | 0.14 | 0.10 | 0.30 | 0.13 | 0.20 | Ph.Pro.Der | MS and KI |
| 18 | 1299 | (E)-Anethole | 5.92 | 2.12 | 1.83 | 2.06 | 1.79 | 1.50 | Ph.Pro.Der | MS, KI, and STD |

[a]KI is Linear Kovat indices based on elution using DB-5 column. [b] Ph.Pro.Der.: Phenylpropanoids derivatives, M: Monoterpene hydrocarbons, and LOC: light oxygenated compounds. [c] compounds identified by NIST library, Kovat index, and standard compounds run under similar GCMS conditions

**Table (2)** Phenolic compounds in the selected sample of fennel (Microwave).

| Phenolic compounds | Concentration |
|---|---|
| *p*-hydroxybenzoic acid-*O*-glucoside | *1.29 |
| 3-caffeoylquinic acid | 45.69 |
| 5-caffeoylquinic acid | 6.65 |
| 4-caffeoylquinic acid | 6.83 |
| quercetin-3-*O*-rutinoside | 6.76 |
| eriodictyol-7-*O*-rutinoside | 5.03 |
| luteolin-7-*O*-rutinoside | 4.34 |
| rosmarinic acid | 4.51 |
| kaempferol | 3.36 |
| Naringenin | 2.87 |
| Gallic acid | 1.13 |
| Caffeic acid | 2.87 |
| Hesperidin | 5.52 |
| Cinnamic acid | 0.77 |

*: Values are expressed as relative area percentage.

Additionally, this study reports for the first time the presence of quercetin-3-O-rutinoside, dicaffeoylquinic acids, and rosmarinic acid in fennel fruits (Table 4). These compounds contribute to the antioxidant activity of the fruits. Furthermore, it was observed that distilled fennel contained more antioxidant phenolic compounds than non-distilled fennel. This is because many volatile substances are eliminated during the distillation process. Overall, the sum of the phenolic compounds in fennel showed that the three caffeoylquinic acid derivatives and rosmarinic acid exhibited the best antioxidant properties (Parejo et al., 2004).

**Conclusion**

The volatile profile of raw HD oil of fennel Eighteen volatile compounds were identified in HD oil of fennel. These compounds are 4 phenylpropanoides, 8 monoterpene hydrocarbons, 5 oxygenated monoterpenes and traces amount of ethanol.The volatile profile of raw HD oil of fennel consisted mainly of estragole (65.67%) followed by carvon (9.6%), limonene (6.77%), fenchon (6.71%) and trans- anethole (5.92%). Roasting causes a drastic increase in the total yield of phenylpropanoid (major compounds) in all treated samples by thermal or by γ-irradiation ranging from (82.12%) in electric oven roasted sample to (89.63%) in 10 KGy irradiated sample compared to (72.67%) in control one. This is due to the very high increase in estragole percentage in all treated samples ranging between (79.7%) in electric oven sample to (87.75%) in 10 KGy irradiated sample compared to (65.67%) in control one.Also Roasting causes a decrease in the total yield of monoterpenes in all treated samples except electric oven roasted sample which gained slight increase compared to control sample. At the same time roasting causes a drastic decrease in oxygenated terpenoids in all treated fennel samples in comparison to row sample. i.e. Roasting causes a drastic decrease in all components of HD oil of all treated samples of fennel except estragole which increased to reach about 90% in 10 KGy irradiated sample. This compound is considered a flavouring agent and by European pharmacopeia limit not exceed than 6.0% inen esstial oils and it has negative effect on animal health and deleted from the list of flavour and food stuffs, This indicated the antioxidant activity of HD fennel essential oil, the strongest effect of reduction of DPPH radical by microwave heated sample which exhibits (58.65%±0.36) followed by 10KGy irradiated sample which exhibits (49.27%±0.32) compared to BHT (98%) at the same concentration 30 µg/ml.These results were confirmed by total phenolic contents of all samples under investigation since comprised very law concentration ranged between 25.03% in 8 KGy irradiated sample to 35.61% in electric oven roasted sample.HPLC analysis for selected sample (microwave treatment) A total 14 polyphenolic compounds were identified 3-caffeoylquinic more predominant (45.69%) followed by 4-caffeoylquinic (6.83 %) and 5-caffeoylquinic acid (6.65%,) are common metabolite constituents of fennel fruit, quercetin-3-Orutinoside and rosmarinic acid which may contribute antioxidant activity in fennel fruits, and this reported for the first time in our study.

## References


1. Abd El Mageed, M.A. ;H.A. Shaaban,; Ibrahim, G.E.; Mahmoud, K.A; and F. Osman. (2014). Impact of γ-irradiation on the Aroma Volatiles, Antioxidant and Antimicrobial Activities of Black and White Pepper (*Piper nigrum* L.). Middle East Journal of Applied Sciences, 4(2): 262-276.
2. Abd El Mageed, M.A.; Farouk, A.; El-Massry, K.F.; Ramadan, M.M. and Shahin, M.S. (2011). Effect of Microwaves on Volatile Compounds of White and Black Peper (*Piper nigrum* L.) and their Antioxidant activities. J. Essential Oil-Bearing Plants 14 (2):214 – 223.
3. Abd El Mageed, M.A; Mansour, A.F.; ElMasry,K.F.;Ramadan, M.M; Shabeen, M.S; And Shaaban, H,A. (2012). Effect of microwaves on essential oils of coriander and cumin seeds and their antioxidant and antimicrobial activities. Jeobp15 (4):614-627.
4. Abd El Mageed; M.A.(2007).Effect of microwave of heating and conventional roasting on volatile compounds in oregano leaves, coriander and cumin seeds. J.Agric.Sci Mansoura university, 32(2):1521-1535.
5. Abdellaoui, M., Derouich, M., & El-Rhaffari, L. (2020). Essential oil and chemical composition of wild and cultivated fennel (Foeniculum vulgare Mill.): a comparative study. *South African Journal of Botany*, *135*, 93-100.
6. Acha1ina, S.C., Koutsos, V.T., Katsiotis, S.T., (2006). Study of nitrogen fertilization rate on fennel cultivars for essential oil yield and composition. Journal of Vegetation Science 12, 85—93.
7. Adams, R. (2007). Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry. Carol Steam, IL: Allured Publishing.
8. Afifi, S. M., El-Mahis, A., Heiss, A. G., & Farag, M. A. (2021). Gas chromatography–mass spectrometry-based classification of 12 fennel (Foeniculum vulgare Miller) varieties

based on their aroma profiles and estragole levels as analyzed using chemometric tools. ACS omega, 6(8), 5775-5785

9. Akgul NB; Basaran P; Rasco BA (2008). The effect of temperature (−5 to 130 °C) and fiber direction on the dielectric properties of beef Semitendinosus at radio frequency and microwave frequencies. J. Food Sci. (73):243-249.
10. Anwar ,F.; Ali ,M.; Hussain ,A.; and Shahid,M.(2009). Antioxidant and antimicrobial activities of essential oil fennel (*Foeniculum Vulgare Mill*.)seeds from Pakistan. Flavour Fragr.*J*, 24:170-176 .
11. Atta-Aly, M.A., (2001). Fennel swollen base yield and qua ity as affected by variety and source of nitrogen fertilizer. Science Horticulture 88 3), 19 1—202.
12. Basaran P. and Akhan, U. (2010). Microwave irradiation of hazelnuts for the control of aflatoxin producing Aspergillus parasiticus. Innov. Food Sci. Emerg. (11):113-117.
13. Belitz, H.D. and Grosch, W., (1987). Food Chemistry. Springer, Berlin.
14. Bendini. A; Gallina-Toschi T; Lercker G. (1998). Influence of -irradiation and microwaves on the linear unsaturated hydrocarbon fraction in spices. Z Lebensm U Forsch A (207):214–218.
15. Bilia, A. R.; Fumarola, M.; Gallori, S.; Mazzi, G.; Vincieri, F. F.( 2000). Identification by HPLC-DAD and HPLC-MS analyses and quantification of constituents of fennel teas and decoctions. J. Agric. Food Chem., *48*, 4734-4738.
16. Blumenthal, M., Goldberg, A., Brinckmann, J., (2000). Herbal Medicine American Botanical Council, Publ. Integrative Medicine Communications, Newton, MA, USA.
17. Choi, J. ; Kim, H.J.; Chun, B.S.; Ahn, D.H.; Kim, G. and Lee, J.W. (2010). Changes in colour and antioxidant activities of Hizikia fusiformis cooking drips by gamma irradiation –LWT-Food Sci. Tech. 43, 1074-1078.
18. Damjanovic,B.; Lepojevic,L.; Vladimir Z ivkovic,V.and Tolic,A.(2005). Extraction of fennel (*Foeniculum vulgare* Mill.) seeds with supercritical CO2: Comparison with hydrodistillation Food Chemistry 92 :143–149.
19. Diaz-Maroto,M.;Perez-Coello,M.; Esteban,J. and Sanz,J.(2006). Comparison of the volatile composition of wild fennel samples (*Foeniculm Vulgare Mill*)from center spain.*J*.Agric Food Chem 54:6814-6818
20. Farkas, J. (1985). Radiation processing of dry food ingredients—a review. Rad Phys Chem (25):271–280.
21. Fung, D.Y. and Cunningham, F.E. (1980) Effect of microwaves on micro organisms in foods. J Food Prot; (43):641-650.
22. Gori,L.; Gallo,E.; Mascherini,V.;,Mugelli,A.; Vannacci,A.; and Firenzuoli,F.(2012). can estragole in fennel seed decoctions really be considered a danger for human health? A Fennel Safety Update .Evidence-Based complementary and alternative medicine 2012, Article ID 860542, 10 pages doi:10.1155/2012/860542.
23. Guillen, M. D.; Manzanos, M. J. (1996). A study of several parts of the plant *Foeniculum* V*ulgare* as a source of compounds with industrial interest. *Food Re*V. *Int.*, *29*, 85-88.
24. Gulfraz,M.; Mehmood,S.; Minhas,N.; Jabeen,N.; Kausar,R.;Jabeen,K and Arshad, G. (2008). Composition and antimicrobial properties of essential oil of *Foeniculum vulgare*. African Journal of Biotechnology 7 (24), pp. 4364-4368, 17.
25. Hashem, M. and Alamri, S. (2010). Contamination of common spices in Saudi Arabia markets with potential mycotoxin-producing fungi. Saudi J. Biol. Sci. 17:167-175.
26. Hassan, A. B., Ahmed, S. M., Sir Elkhatim, K. A., Abdelhalim, T. S., Fawale, S. O., Adiamo, O. Q., & Mohamed Ahmed, I. A. (2019). Effect of gamma irradiation and microwave heating treatments on microbial load and antioxidant potentials in cinnamon, fennel and hot pepper. *Journal of Food Measurement and Characterization*, *13*, 1130-1138.
27. Iqbal, S., M.I. Bhange R,F. Anwar, (2007) antioxidant properties and components of bran extracts from selected wheat varieties commercially available in Pakistan. LWT-Food science & technology,40(2):361-367.
28. Iqbal,S.,M.I. Bhange R, F. Anwar, (2007). Antioxidant properties and components of bran extracts from selected wheat varieties

commercially available in Pakistan. LWT-Food science & technology,40(2):361-367.
29. Krzymien ME, Carlsson DJ, Deschênes L, Mercier M. (2001). Analysis of volatile transformation products from additives in □-irradiated polyethylene packaging. Food Addit Contam (18):739–749.
30. Kulisic, T.; Radonic, A.; Katalinic, V.; Milos, M., (2004). Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem. (85):633–640.
31. Kunzemann, J. and Herrmann, K. (1977). Isolation and identification of flavon(ol)-O-glycosides in caraway (*Carum carvi* L.), fennel (*Foeniculum vulgare* M.), anise (Pimpinella anisum L.), and coriander (Coriandrum sativum L.) and of flavonol-Cglycosides in anise. I Phenolics of spices. Z. Lebensm.- Unters.- Forsch., 164, 194-200.
32. Li, Y.Y. and Yi, Z.Y. (2003). Present situation and development of food antistaling agent and preservatives. J. Beij. Inst. Petrochem. Technol. (11): 18–23.
33. McDonald, T.A. (1999). Evidence on the carcinogenicity of estragole. Reproductive and Cancer Hazard Assessment Section Office of Environmental Health Hazard Assessment California Environmental Protection Agency: Sacramento, California, USA,
34. Meyer, S.; Koch, R.; Metzger, J.O. (2003),Investigation of reactive intermediates of chemical reactions in solution by electrospray ionization mass spectrometry: Radical cation chain reactions. *Angew. Chem. Int. Ed. 42*, 4700-4703.
35. Mohammadi, M., Azizpour, H., & Bahmanyar, H. (2023). Extraction of Fennel Essential Oil Constituents Using a New Modified Rotating Disc Contactor. *Iran. J. Chem. Chem. Eng. Research Article Vol*, *42*(5).
36. Miraldi , E.(1999). Comparison of the essential oils from ten *Foeniculm vulgare* miller samples of fruits of different origin. Flavour&fragr. *J*.,14:279-282.
37. Murray, R. D. H.; Mendez, J.; and Brown, S. A. (1982). In The Natural Coumarins. Occurrence, Chemistry, Biochemistry Wiley-Interscience, John Wiley & Sons Ltd.: Chichester,; p 560.
38. Naaz, S., Ahmad, N., Qureshi, M. I., Hashmi, N., Akhtar, M. S., & Khan, M. M. A. (2022). Antimicrobial and antioxidant activities of fennel oil. *Bioinformation*, *18*(9), 795-800.
39. Najja,H.,Zerria, K.,Fattouch, S.,Ammar, E., and Neffati,M. (2011). Antioxidant and antimicrobial activities of *Allium Roseum.*"lazoul", a wild edible endemic spices in north Africa . International journal of food properties, (14):371-380.
40. Olson DG. 1998. Irradiation of food. Food Technol ;52(1):56–62.
41. Parejo, I., Francesc, V., Jaume, B., Carles. C. (2004). Development and validation of a high-performance liquid chromatographic method for the analysis of antioxidative phenolic compounds in fennel using a narrow bore reversed phase C18 column. Analytica Chimica Acta 512: 271–280
42. Rahman , F and Saleh, M. (2011). chemical composition, antimicrobial and Antioxidant activities of essential oils from organically cultivated Fennel Cultivars. *Molecules*, *16*, 1366-1377.
43. Roby, M.H.; Mohamed, A. S.; Khaled, A.;Selim, K. and Ibrahim, K. (2013). Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare L.*) and chamomile (*Matricaria chamomilla* L.). Industrial Crops and Products (44 ):437–445.
44. Russo, M.; Galleli, G.C. ;Bochini,P. and Carnacini,A.(1998). Essential oil chemical composition of wild population of Italian oregano spice (*origanum vulgare ssp. Hirtum*(link) letswaart): A preliminary evaluation of their use in chemotaxonomy by cluster analysis .1. inflorescences. J. of agric food chem. 46:3741-3746.
45. Saber, J. I., & Eshra, D. H. (2019). Using fennel seeds and their oil as a preservative and functional food to produce some food and drink products to alleviate cough symptoms. *Alexandria Science Exchange Journal*, *40*(July-September), 406-414.
46. Senatore,F.;Oliviero,F.; Scandolera,E.;Taglialatela-Scafati,O.; Roscigno,G.; Zaccardelli,M.and De Falco,E.(2013). chemical composition, antimicrobial and antioxidant activities of anethole-rich oil from leaves of selected varieties of fennel [Foeniculum vulgare Mill.

ssp. vulgare var. azoricum (Mill.) Thell].Pitoterapia 90:214-219.

47. Shahat ,A.; Ibrahim,A,; Hendawy ,S.; Omer,E.; Faiza Hammouda , F.; Abdel-
Singleton, V.L., (1998). Wine polyphenols. In: Linskens, H.F., Jackson, J.F. (Eds.), Modern Methods of Plant Analysis. New Series, vol. 6. Springer-Verlag, Berlin,pp. 173–219.
48. Srinivasan, K., (2005). Role of spices beyond food flavouring: Nutraceuticals with multiple health effects. Food Reviews International (21): 167–188.
49. Susheela, R. U. (2000). Handbook of spices, seasonings and flavourings (pp. 47–48). Lancaster, USA: Technomic Publishing.
50. Tapsell, L.C., Hemphill, I., Cobiac, L., Patch, C.S., Sullivan, D.R., Fenech, M., Roodenrys,S., Keogh, J.B., Clifton, P.M., Williams, P.G., Fazio, V.A., Inge, K.E., 2006. Healthbenefits of herbs and spices: the past, the present, the future. The Medical Journal of Australia (185): S4–S24.
51. Šunić, L., Ilić, Z. S., Stanojević, L., Milenković, L., Stanojević, J., Kovač, R., ... & Cvetković, D. (2023). Comparison of the Essential Oil Content, Constituents and Antioxidant Activity from Different Plant Parts during Development Stages of Wild Fennel (Foeniculum vulgare Mill.). *Horticulturae*, *9*(3), 364.
52. Tănase, L. A., Nistor, O. V., Mocanu, G. D., Andronoiu, D. G., Cîrciumaru, A., & Botez, E. (2022). Effects of Heat Treatments on Various Characteristics of Ready-to-Eat Zucchini Purees Enriched with Anise or Fennel. *Molecules*, *27*(22), 7964.
53. Thostenson, E.T. and Chou, T.W. (1999). Microwave processing: Fundamentals and applications. Composites: Part A, (30):1055-1071.
54. WHO (1981): Wholesomeness of irradiated food. Report a joint FAO/IAEA/WHO expert Committee. Technical Report Series 659. World Health Organization, Geneva: 7–34.
55. WHO (1999): High-dose irradiation: Wholesomeness of food irradiated with doses above 10 kGy. Report of a joint FAO/IAEA/WHO study group. WHO Technical Report Series 890. World Health Organization, Geneva.
56. Yamaok, R.; Tsujino, T.S.; Kimura, Y.; Ohta, M. (2009). Detection of organic free radicals in irradiated *Foeniculi fructus* by electron spin resonance spectroscopy, *J. Nat. Med*., *63*, 28-31.
57. Zaki, M.F., Abdelhafez, A.A.M., Camilia, Y., El-dewiny, (2009). Influence of biofertilization and nitrogen sources on growth, yield and quality of broccoli (Brassicaoleracea Var. Italica). Egyptian Journal of Applied Sciences 24, 86—111.
58. Zdsman, Y.A.H., (2009). Comparative study of some agricultural treatments effects on plant growth, yield and chemical constituents of some fennel varieties under Sinai conditions. Research Journal of Agriculture and Biological Science 5 (4), 541—554.