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Cumin (*Cuminum cymium* L.) Oil Composition and Antimicrobial Activity Alteration in Response to Storage and Irradiation

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Abstract: This investigation was conducted to determine the effect of cumin fruits (*Cuminum cymium* L.) storage for 3 and 6 months at ambient temperature after irradiated with gamma irradiation at 10 and 20 kGy on essential oil percentage, components, chemical properties and its antimicrobial activity. Also, the possibility to store cumin fruits essential oil at 4°C and -20°C for 3 and 6 months without treating the fruits was studied. Results showed no clear effect of either gamma irradiation doses or storage periods of cumin fruits on essential oil components fractionated by GCMS. Concerning irradiation doses, results revealed that increasing gamma irradiation doses up to 20kGy significantly caused a gradual decline in the antioxidant activity (as DPPH) and total flavonoids. Contrarily, increasing gamma irradiation doses enhanced total phenolic compounds in stored irradiated fruits. Concerning cumin essential oil stored at different temperatures, results showed very slight significant decrease in the antioxidant activity due to storage at 4°C other than -20°C. The same trend was observed on both total phenolic compounds and total flavonoids. From another point, storage up to 6 months for irradiated fruits caused a significant decrement in the antioxidant activity (as DPPH) and total flavonoids of essential oil. While, it enhanced total phenolic compounds of irradiated fruits essential oil. Concerning antimicrobial activity of essential oil extracted from irradiated cumin fruits, the increase of gamma irradiation doses up to 20kGy, caused a gradual decline in inhibition zone on both *Bacillus subtilis* and *Escherichia coli*. While, storing cumin essential oil at both 4°C and -20°C for up to 6 months had no effect on *Bacillus subtilis* and *E. coli*. Economically, it could be concluded that storing essential oil at 4°C is the best treatment compared with storing irradiated cumin fruits at ambient temperature as it resulted in some changes in oil quality or stored essential oil at -20°C which caused a slight change compared to storage at 4°C.

Key words: Cumin · Gamma irradiation · Storage · Antioxidant activity · Phenolic compounds · Flavonoids · Antimicrobial activity

INTRODUCTION

Cumin (*Cuminum cyminum* L.) is an aromatic plant belongs to the Apiaceae family and is used as flavor foods, adding to fragrances and for medical preparations [1]. Cumin is used in vegetarian and non-vegetarian food preparations as an ingredient of garam masala. Cumin is known for its carminative, stimulant, diuretic, antispasmodic and astringent properties. It is also used in treatment of diarrhea, in dyspepsia and jaundice of Sultana *et al*. [2]. Cumin fruits contain volatile oil (2-5%) that imparts the characteristic of aroma to the fruits [3]. Essential oils are concentrated in liquids form of complex mixtures of volatile compounds and can be extracted from several plant organs. Essential oils are a good source of several bioactive compounds, which possess antioxidative and antimicrobial properties. In addition, some essential oils have been used as medicine. Furthermore, the uses of essential oils have been received an increase attention as the natural additives for the extension of food products shelf life, due to the risk in using synthetic preservatives [4]. The constituents

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of essential oils are terpenes (monoterpenes and sesquerpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative and so on) and terpenoids (isoprenoids) [5 - 6]. The major constituents of the essential oils of cumin were safranal, γ-terpinene, γ-terpinene-7-al, cuminaldehyde, β-pinene and *p*-cymene [7]. Cumin fruits essential oil has high antifungal, antibacterial and antioxidant activities. Therefore, it is also used as a fumigant or additive in the storage of foodstuffs [8]. Spices and herbs are highly susceptible to microbial contamination [9]. An appropriate preservation and sterilisation technique should be used in order to maintain shelf life, quality and safety of spices. Food irradiation is a process of exposing food to ionising radiation such as gamma rays emitted from the radioisotopes ^{60}Co and ^{137}Cs or high energy electrons and X-rays produced by machine sources. The use of ionizing radiation to destroy harmful biological organisms in food is considered a safe, well proven process that has found many applications. Depending on the absorbed dose of radiation, various effects can be achieved in reducing storage losses, Gamma-irradiation is one of the commercial methods used in sterilisation of spices. Gamma-irradiation of spices and herbs up to 10 kGy is approved for decontamination of spices and herbs in the European Union, whereas the maximum dose is 30 kGy in USA and Australia [10-12]. Extending shelf life and/or improving microbiological and parasitological safety of foods [13]. Gamma-irradiation is frequently used in sterilization of herbs and spices, but it may affect some of their quality attributes. The irradiation-induced changes in the quality depend on atmosphere (especially on O_2 level) during treatment. Thus, it can be controlled by modified atmosphere packaging [14].

The aim of this study was to investigate the effect of cumin fruits (*Cuminum cymium* L.) storage for 3 and 6 months at ambient temperature after irradiated with gamma irradiation at 10 and 20kGy on essential oil percentage, components, chemical properties and its antimicrobial activity. Also, the possibility to store cumin fruits essential oil at 4°C and -20°C for 3 and 6 months without treating the fruits was studied.

MATERIALS AND METHODS

This investigation was carried out in the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt and the Central Laboratory, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt during 2017 and 2018 seasons. The aim of this investigation was to study the effect of cumin fruits storage for 3 and 6 months at ambient temperature after irradiated with gamma irradiation at 10 and 20kGy on essential oil percentage, components, chemical properties and its antimicrobial activity. Also, the possibility to store cumin fruits essential oil at 4°C and -20°C for 3 and 6 months without treating the fruits was studied.

Fruit Materials: Cumin fruits (*Cuminum cymium* L.) were collected from a private farm in Minia Governorate, Egypt during 2017 and 2018 seasons. The collected fruits were divided into two groups, the first group was subjected to gamma irradiation then storage treatments, while the other one was directly distilled to extract the essential oil. In this regard, 250g. of the collected fruits were allocated for each treatment.

Fruits Irradiation Treatments: Cumin fruits were packed in polyethylene packages then exposed to gamma irradiation at different doses i.e. 0 as control (without irradiation), 10 or 20kGy, the applied dose rates were 1.393 and 1.291kGy h^{-1} for 2017 and 2018 seasons, respectively. Fruits irradiation was performed in National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt using a self-contained dry-storage gamma irradiator (Indian gamma cell GE 4000A) that uses ^{60}Co as a radiation source.

Fruits Storage Treatments: Both irradiated and unirradiated fruit samples were stored at room temperature in a clean place for zero time (without storage), 3 and 6 months until oil extraction time.

Essential Oil Storage Conditions: In order to investigate the impacts of essential oil storage periods and temperatures, oils were directly extracted from untreated cumin fruits, then stored for 3 and 6 months at 4°C and -20°C temperatures.

Isolation of Essential Oil: Cumin fruits were crushed and the essential oil was isolated by water distillation method described by Guenther [15] as essential oil percentage, the fruits from each treatment were placed in a distillation flask and adequate quantity of extraction water was added. The extraction process was carried out with boiling water continuously for 3-4 hours then oil percentage was recorded. The distillated oils were dried by anhydrous sodium sulfate and poured in a dark bottles then kept in cold place.

Data Recorded: After the essential oil has been extracted from either treated and untreated cumin fruits, the following determinations were done:

Essential Oil Percentage: Essential oil percentage was calculated by the method described by Guenther [15].

Essential oil GCMS Fractionation: The pure essential oil for each sample was injected in Gas Chromatograph Mass Spectrometer ((GCMS) model Schimadzu QP-5000 equipped with DB-1 column (30m × 0.25mm × 0.250 Micron film thickness) and the compounds were detected. Moreover, to determine the exact effects of storage conditions on essential oil composition during the experiment period, the fresh extracted oil was analyzed immediately after extraction. Analysis of essential oil using Gas Chromatograph Mass Spectrometer ((GCMS) was performed.

Antioxidant Activity as (DPPH): The antioxidant activity was evaluated in oil samples by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method according to the procedure of Chen *et al.* [16] as g. TE/100g.

Total Phenols (g/100g.): Total phenols were analyzed in oil samples spectrophotometrically using the method described by Swain and Hillis [17]. Results were expressed as g.GAE/100g.

Total Flavonoids (mg/100g.): Total flavonoids content in oil samples was measured according to Zhuang *et al.* [18] as mg/100g.

Antimicrobial Inhibition Growth Measurement: Disc diffusion method was employed for the determination of antimicrobial activities of the essential oils. 0.1 ml from 10^8cfu/ml bacterial suspension was spread on the nutrient agar plates. Filter paper discs (0.6 cm in diameter) were impregnated with 10μl of the undiluted oil and were placed on the inoculated plates. These plates, after remaining at 4°C for 2h, were incubated at 37°C for 24h. All tests were performed in triplicate. The diameters of the inhibition zones of *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative) strains were measured in millimeters and represented as inhibition zone [19].

Experimental Design and Statistical Analysis: This experiment was designed as a randomized complete block design in a factorial experiment with two factors. All obtained data were subjected to statistical analysis as proposed by Gomez and Gomez [20] and means were compared by LSD at 5% level of probability.

RESULTS AND DISCUSSION

Effect of Gamma Irradiation Doses (kGy.) and Storage Periods on Essential Oil Yield (%): Results in Table (1) show the effect of gamma radiation doses (0, 10 and 20kGy), storage periods and their interaction on cumin essential oil yield. Regarding the effect of gamma radiation, data indicated that increasing gamma irradiation doses significantly decreased the essential oil in both seasons.

Concerning the effect of storage periods, a significant decline in essential oil yield was observed due to extending storage period up to 6 months.

As for the interaction between gamma radiation and storage periods, it is clear that the highest essential oil yield was recorded with unirradiated samples in addition to zero time storage (4.67 and 4.43%, in both seasons, respectively). However, the lowest value was obtained at 20kGy in addition to storage 6 months as recording 3.70 and 3.77%, in both seasons, respectively.

These results are in accordance with that obtained by Agrawal [21] who stated that cumin fruits contain 2.5% - 4.0% volatile oil. Also, Seoa *et al.* [22] reported that there were no differences between volatile oils yield extracted from non and irradiated (up to 10kGy) *Angelica gigas* samples. However, the yield from the irradiated sample at highest dose was slightly decreased. Also, Kirkin and Gunes [14] stated that the essential oil yield of thyme was increased by irradiation at 6 and 14kGy in the modified atmosphere packages (MAP), but the change was insignificant in the aerobic packages (AP) However, the irradiation and the packaging treatments did not affect essential oil yield of rosemary, black pepper and cumin. Contrarily, they explained the increment in the yield which is in disagreement with our results to that essential oils are placed in cellular contents protected by cell membranes and cell wall, which can be destroyed by irradiation resulting in extraction of the cellular components to a larger extent.

GCMS Fractionation of Cumin Essential Oil from Stored Fruits and Oil Stored up to 6 Months at Different Temperatures: Table (2) illustrated the GCMS fractionation of cumin essential oil either extracted from stored fruits or stored oil. Concerning the oil components at zero time, the major compounds were Cuminaldehyde

Table 1: Effect of gamma irradiation doses (kGy.) and storage period on essential oil yield (%) during 2017 and 2018 seasons

Treatments	2017 Season				2018 Season			
	Storage periods by month (B)				Storage periods by month (B)			
Doses (kGy.) (A)	0	3	6	Mean	0	3	6	Mean
0	4.67	4.13	4.03	4.28	4.43	3.97	3.90	4.10
10	4.23	4.07	4.03	4.11	4.17	3.83	3.87	3.96
20	4.17	3.90	3.70	3.92	4.07	3.90	3.77	3.91
Mean	4.36	4.03	3.92		4.22	3.90	3.84	
LSD at 0.05	A=0.144 B=0.100 A×B=0.073				A=0.147 B=0.092 A×B=0.159			

Table 2: GCMS fractionation of cumin essential oil from stored fruits and oil stored up to 6 months at different temperatures

	Cumin fruits peak area %									Cumin oil peak area %			
	Zero time			3 months			6 months			3 months		6 months	
Components	0 kGy.	10 kGy.	20 kGy.	0 kGy.	10 kGy.	20 kGy.	0 kGy.	10 kGy.	20 kGy.	-20°C	4°C	-20°C	4°C
α - Pinene	0.69	0.44	0.77	0.25	0.30	0.54	0.44	0.41	0.47	0.57	0.35	0.65	0.45
Sabinene	0.92	0.88	0.73	0.76	0.78	0.66	0.81	0.78	0.67	0.89	0.86	0.88	0.83
β - Pinene	16.32	16.01	16.96	15.37	15.71	15.13	15.55	16.02	16.35	16.94	16.64	16.99	16.62
β - Myrcene	1.14	1.27	1.53	1.39	1.24	1.50	1.58	1.60	1.79	1.12	1.05	1.28	1.11
α - Phellanderene	0.78	0.82	1.73	1.38	0.64	1.58	1.18	1.55	2.28	1.02	0.60	1.08	0.63
Carene	0.66	0.81	0.81	0.39	0.68	0.63	0.37	0.82	0.55	0.79	0.71	0.58	0.77
P - Cymene	19.02	18.15	17.72	16.97	17.78	17.44	18.00	17.39	17.04	17.23	17.44	18.44	18.87
Limonene	2.15	2.11	2.75	2.22	2.20	2.24	2.23	2.92	1.67	1.56	1.73	1.90	1.95
γ -Terpinene	15.56	15.24	15.51	14.12	13.15	13.33	14.21	14.01	13.07	13.53	14.48	13.60	13.56
trans-Carveol	3.66	3.11	3.55	3.41	3.45	3.47	3.01	3.20	3.18	3.09	3.16	3.10	3.02
Cumin aldhyde	24.24	24.86	25.71	24.92	24.90	25.80	26.61	25.89	26.32	25.57	25.33	25.95	25.65
Cumin alcohol	6.10	6.71	6.05	5.82	5.77	5.52	6.63	5.74	5.20	5.70	5.07	5.46	5.08
Perillaldhyde	4.31	5.29	5.06	4.96	5.50	4.94	4.53	4.74	4.51	5.01	5.19	5.49	5.16
Others	4.45	4.30	1.12	8.04	7.90	7.22	4.85	4.93	6.90	6.98	7.39	5.60	6.30

(24.24%), followed by *p*- Cymene (19.02%), then β-Pinene (16.32%), γ-Terpinene (15.56 %), Cumin alcohol (6.10 %) and Perillaldhyde (4.31 %).

Gamma radiation up to 20kGy dose did not affect the essential oil components as it fluctuated up and down due to gamma irradiation doses.

As for storing essential oil of cumin at 4°C or -20°C, there was no effect of storage temperatures on essential components as the values were nearly close to each other.

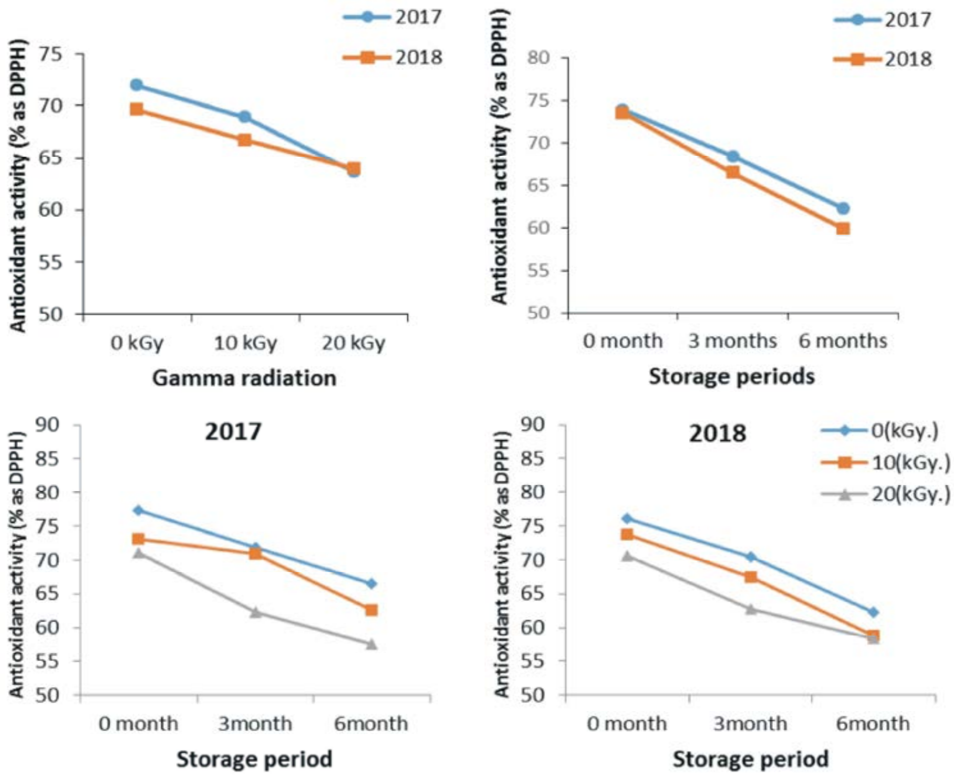
Results of GC-MS analysis of the essential oil of *C. cyminum* L. fruits showed that cumin aldehyde is the main ingredient. Other studies reported that c-terpinene and *p*-mentha-1, 4-dien-7-al are the main constituents of the essential oil [23, 24]. In addition, these results are in agreement with that reported by Pajohi *et al.* [25] who concluded that essential oil composition of *Cuminum cyminum* L. fruit identified by GC-MS recorded β-pinene (7.72%), *p*-cymene (8.55%), gamma terpinene (12.94), α-Terpinen-7-al (20.70%) and cumin aldehyde (29.02%) as main components. Also, Hussein *et al.* [26] found that cuminaldehyde, *p*-mentha 1,4-diene-7-al, pmentha 1,3-diene-7-al, γ-terpinene and *p*- cymene were the major compounds in cumin essential oil (Eos).

Effect of gamma irradiation doses (kGy.) and storage periods on antioxidant activity (% as DPPH) in cumin fruits oil

Data in Figure (1) show the effect of gamma radiation doses, storage periods and their interaction on antioxidant activity (%) of cumin fruits oil during 2017 and 2018 seasons. Results revealed that the increasing gamma radiation doses up to 20kGy significantly had a gradual decline in the antioxidant activity (% as DPPH).

As for the effect of storage period, it was observed that extending storage periods up to 6 months significantly decrease the antioxidant activity.

Concerning the interaction between gamma radiation and storage period, results show that the highest value of antioxidant activity of stored cumin fruits oil was given by



LSD at 0.05: A=0.593 B=0.851 A×B=1.473 LSD at 0.05 : A=0.493 B=0.747 A×B=1.297

Fig. 1: Effect of gamma radiation doses (kGy.) and storage periods on antioxidant activity (% as DPPH) of stored cumin fruits oil during 2017 and 2018 seasons.

unirradiated treatment in addition to zero time storage (77.30 and 76.06%, in both seasons, respectively). However, the lowest value of antioxidant activity of stored cumin fruits oil was obtained by 20kGy in addition to storage up to 6 months as recorded 57.57 and 58.43%, in both seasons respectively.

These results are in disagreement with that obtained by Variyar *et al.* [27] who stated that stress caused by low doses of gamma irradiation could induce antioxidant activities in plant material.

While, Bag and Chattopadhyay [28] stated that cumin fruit oil exhibit synergistic antioxidant activity and may be used as a potential source of safe and potent natural antioxidant agent in pharmaceutical and food industries. Its synergistic interactions may increase its antioxidant efficacy at sufficiently low concentration which may reduce its adverse side effects and facilitate the use in food preservation system. Chemical analysis revealed that *p*-coumaric acid from cumin fruit oil was the bioactive compound responsible for both synergistic antibacterial and antioxidant activities. These results agreed with those reported by Foti *et al.* [29], Muhammad *et al.* [30] and Meullemiestre *et al.* [31].

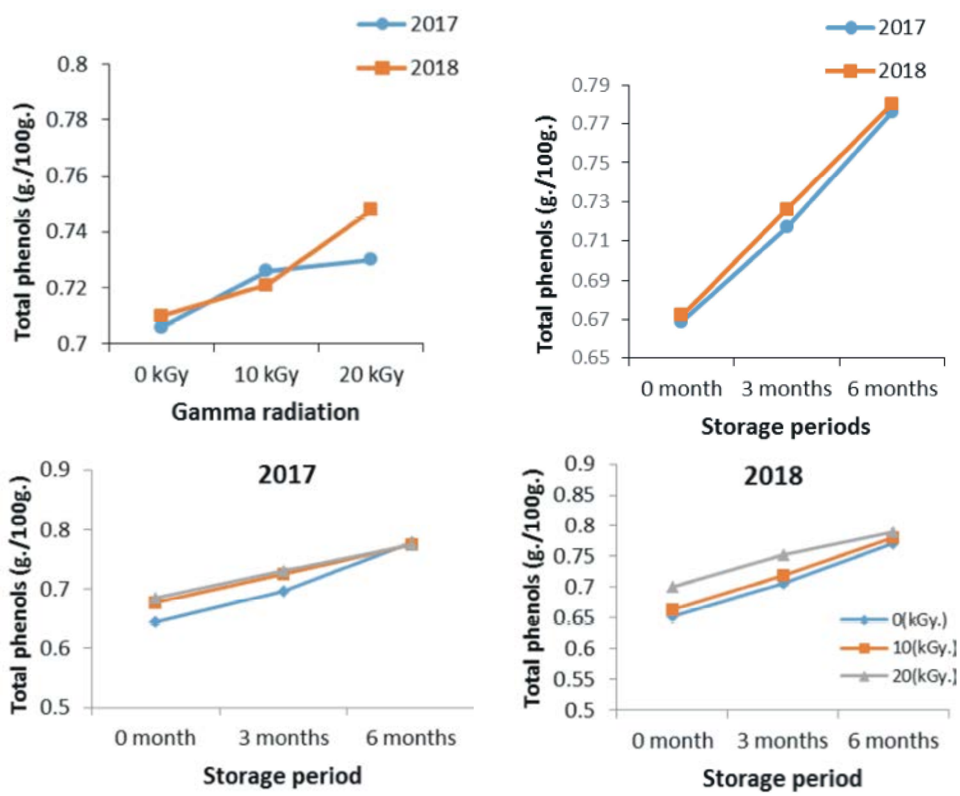
Increasing the dose to 10 kGy in both cultivars significantly decreased the DPPH % to 61.20% and 55.61%, respectively [32]. Gamma irradiation is suitable for breaking the chemical bonds of polyphenols and consequently discharging soluble phenolics of small molecular weights prompt the development of phenolics rich in antioxidants. Moreover, Kirkin and Gunes [14] stated that irradiation of cumin (14kGy) decreased the DPPH scavenging activity in its extract compared to the nonirradiated control.

Effect of Storage Periods and Temperature on Antioxidant Activity (%as DPPH) of Stored Cumin Essential Oil: Data in Table (3) show the effect of essential oil storage periods, temperatures and their interaction on antioxidant activity during 2017 and 2018 seasons. Results revealed that extending storage periods up to 6 months resulted in a slight decline on antioxidant activity of stored cumin essential oil when compared with zero time stored oil, in both seasons.

Concerning storage temperatures, it is clear that there was a significant decrement in antioxidant activity of cumin essential oil when stored at 4°C compared to

Table 3: Effect of storage periods, temperature and its interaction on antioxidant activity (%) of stored cumin essential oil during 2017 and 2018 seasons

	2017 Season			2018 Season		
	Temperature (B)			Temperature (B)		
Treatments Storage periods by month (A)	-20°C	4°C	Mean	-20°C	4°C	Mean
0	75.17	75.17	75.17	75.18	75.16	75.17
3	71.67	69.27	70.47	71.31	69.50	70.40
6	71.56	70.28	70.92	71.16	69.98	70.57
Mean	72.80	71.57		72.55	71.55	
LSD at 0.05 for	A=1.304 B=0.344 A×B=0.595		A=0.619 B=0.494 A×B=0.855			



LSD at 0.05 : A=0.016 B=0.010 A×B=0.018 LSD at 0.05: A=0.019 B=0.022 A×B=N.S

Fig. 2: Effect of gamma radiation doses (kGy) and storage periods on total phenols content (g./100g.) of stored cumin fruits during 2017 and 2018 seasons.

storage at -20°C, the obtained results were 71.57 and 71.55% for at 4°C and 72.80 and 72.55% for -20°C in the first and second seasons, respectively.

Regarding the interaction between storage periods and temperature, the obtained data show that the highest antioxidant activity of cumin essential oil was obtained without storage as recorded 75.17 and 75.16% in the first and second seasons, respectively. Contrarily, the lowest antioxidant activity of cumin essential oil was obtained from storing oil for 3 months at 4°C (69.27 and 69.50 in both seasons, respectively.

Effect of Gamma Irradiation Doses (kGy) and Storage Periods on Total Phenols Content: Data in Figure (2) show the effect of gamma irradiation and storage periods on total phenols (g/100g.) of essential oil extracted from treated cumin fruits during 2017 and 2018 seasons. Results revealed that increasing gamma irradiation doses up to 20kGy gradually enhanced the total phenols.

As for the effect of storage periods, it could be observed that extending storage period up to 6 months enhanced the total phenols content (g/100g.).

Table 4: Effect of storage periods, temperature and its interaction on total phenols content (g./100g.) of stored cumin essential oil

Treatments Storage periods by month (A)	2017 Season Temperature (B) -20°C	4°C	Mean	2018 Season Temperature (B) -20°C	4°C	Mean
0	0.644	0.644	0.644	0.652	0.652	0.652
3	0.662	0.670	0.666	0.655	0.670	0.663
6	0.682	0.690	0.686	0.676	0.689	0.683
Mean	0.663	0.668		0.661	0.670	
LSD at 0.05 for	A=0.004 B=0.001 A×B=0.002			A=0.005 B=0.002 A×B=0.004		

Concerning the interaction between gamma irradiation doses and storage periods, results show that the highest value of total phenols of cumin essential oil was obtained by unirradiated cumin fruits after 3 months of storage (0.696g/100g.) for 2017 season, while the highest value was obtained by 20kGy after 6 months of storage as recorded 0.790g/100g. in 2018 season. Contrarily, the lowest value of total phenols of cumin essential oil was obtained by unirradiated cumin fruits at zero time of storage as recorded 0.644 and 0.652g/100g. for 2017 and 2018 seasons, respectively.

Results coincide with the fact that polyphenol stability under different conditions is a very important aspect that must be taken into account to ensure that phenolic compounds possess the desired properties and maintain their activity under different storage conditions, which can involve high temperatures and light [33]. Within this context, when extracts were exposure to 50°C and to light, degradation was faster, presenting a maximum of 60% degradation at the end of the experiment. The three extracts were very similarly susceptible to polyphenol degradation under these conditions. High temperature and light could change or degrade the structure of the polyphenols, resulting in marked changes in their affinity [34-35]. However, this effect was not observed at low temperatures, under which they maintained high stability (up of 97%) during 60 days.

These results are in agreement with those concerning that phenolic content in radiation processed samples was increased with increasing the irradiation dose. Similarly, Variyar *et al.* [27] found an increase in amounts of phenolic acids in irradiated cloves and nutmeg. The adverse result was observed by Campos *et al.* [36] who found that the total phenolic content of *Maytenus aquifolium* leaves was shown to be unaffected by any of the doses of gamma rays investigated. Also, Campos *et al.* [36] stated that phenolic compounds are responsible for antioxidant activity. Similar results were reported by Kim *et al.* [37] in unripe and ripe mango peel extract.

These results agreed with those reported by El-Beltagi *et al.* [32] concluded that γ- irradiation enhanced the natural polyphenolic compounds, total phenolic contents of Egyptian date palm fruits.

However, results are in disagreement with those obtained by Aly and Elfaramawy [38] who mentioned that on *Vicia faba* L. seeds, increasing the irradiation dose significantly decreased the total contents of phenolic compounds as compared with control.

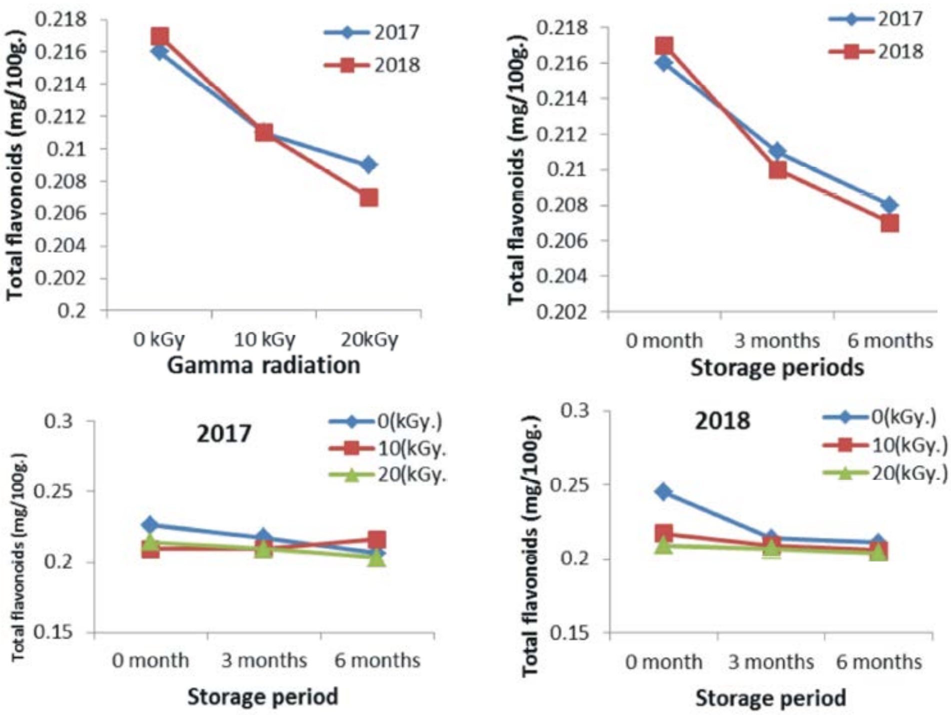
Effect of Storage Periods and Temperature on Total Phenols Content of Stored Cumin Essential Oil: Data in Table (4) illustrate the effect of storage periods, temperature and their interaction on total phenols content of stored cumin essential oil during 2017 and 2018 seasons.

As for the effect of storage period, data showed that extending storage periods up to 6 months significantly enhanced the phenols content of the essential oil as recorded 0.686 and 0.683g/100g. in the first and second seasons, respectively.

Concerning the effect of storage temperatures, it is clear that temperatures had a significant effect on total phenols, storage cumin essential oil at 4°C other recorded the highest values (0.668 and 0.670g/100g.) in the first and second seasons, respectively.

As for the interaction between storage periods and temperature, the highest value of total phenols content resulted from storage for 6 months at 4°C recording 0.690 and 0.689g/100g. during 2017 and 2018 seasons, respectively.

Effect of Gamma Irradiation Doses (kGy) and Storage Periods on Total Flavonoids Content: Results in Figure (3) illustrate the effect of gamma radiation doses and storage periods on total flavonoids content (mg/100g.) in essential oil extracted from stored cumin fruits during 2017 and 2018 seasons. Concerning the effect of gamma radiation, increasing doses up to 20kGy had a gradual decline in total flavonoids.



LSD at 0.05 : A=N.S B=N.S A×B=N.S   LSD at 0.05: A=0.009 B=0.013 A×B=N.S

Fig. 3: Effect of gamma radiation doses (kGy) and storage periods on total flavonoids content (mg/100g.) in stored cumin fruits during 2017 and 2018 seasons

Concerning the effect of storage period, it is clear that extending storage period up to 6 months decreased the total flavonoids content (mg/100g.).

Regarding the interaction between gamma radiation doses and storage periods, results show that the highest values of total flavonoids (mg/100g.) was given with unirradiated samples in addition to zero time storage as recorded 0.226 and 0.225mg/100g during 2017 and 2018 seasons, respectively. While, 20kGy in addition to storage for 6 months had the lowest values amounting 0.203 and 0.204mg/100g. during 2017 and 2018 seasons, respectively.

These are in agreement with those obtained by Aly and Elfaramawy [38] who found that there was a significant decrease in falvonoids of irradiated *Vicia faba* L. seeds at dose levels (2.5, 5.0, 10 and 20 kGy). Moreover, by increasing the dose level to 10 kGy flavonoid contents were decreased on date fruit genotypes [32].

These results are in disagreement with that reported by Volf *et al.* [33] who stated that flavonoids are phenolic compounds; hence, the amount of total phenols includes flavonoids. Consequently, flavonoids exhibited the same behavior as total phenols. In DPPH, the antioxidant activity of the plant extracts could be related with their phenolic content.

Effect of Storage Periods and Temperature on Total Flavonoids Content in Stored Essential Oil of Cumin: Results in Table (5) show the effect of storage periods, temperature and their interaction on total flavonoids in stored essential oil of untreated cumin fruits during 2017 and 2018 seasons. Concerning the effect of storage periods, results revealed that extending storage period up to 6 months significantly reduced total flavonoids of stored essential oil of cumin fruits.

Taking temperature into consideration, it had no effect on total flavonoids as it recorded nearly the same values in both seasons.

Concerning the interaction between storage periods and temperature, results show that the highest value of total flavonoids in essential oil resulted from zero time at both temperatures in both seasons. While, the lowest total flavonoids content in essential oil was obtained for 6 months at 4°C recording 0.210 and 0.209 mg/100g during 2017 and 2018 seasons, respectively.

Effect of Gamma Radiation Doses (kGy) and Storage Periods on Diameter of Inhibition Zone (Mm) of Cumin Fruits Essential Oil in (*Bacillus subtilis*): Data in Table (6) show the effect of gamma radiation doses, storage periods and their interaction on diameter of

Table 5: Effect of storage periods and temperature on total flavonoids content (mg/100g.) in stored essential oil of cumin during 2017 and 2018 seasons

	2017 Season			2018 Season		
	Temperature (B)			Temperature (B)		
Treatments Storage periods by month (A)	-20°C	4°C	Mean	-20°C	4°C	Mean
0	0.217	0.217	0.217	0.217	0.217	0.217
3	0.215	0.213	0.214	0.214	0.211	0.213
6	0.211	0.210	0.210	0.210	0.209	0.209
Mean	0.214	0.213		0.213	0.212	
LSD at 0.05 for	A=0.005 B=N.S A×B=0.004			A=0.005 B=N.S A×B=0.005		

Table 6: Effect of gamma radiation doses (kGy) and storage periods on diameter of inhibition zone (mm) of cumin fruits essential oil in (*Bacillus subtilis*) during 2017 and 2018 seasons

	2017 Season				2018 Season			
	Storage periods by month (B)				Storage periods by month (B)			
Treatments Doses (kGy) (A)	0	3	6	Mean	0	3	6	Mean
0	12.33	10.00	10.2	10.84	11.33	9.67	8.67	9.89
10	10.67	8.33	7.33	8.78	10.33	8.33	7.67	8.78
20	10.33	8.00	7.67	8.67	9.67	8.33	7.33	8.44
Mean	11.11	8.78	8.40		10.44	8.78	7.89	
LSD at 0.05	A=1.47 B=0.88 A×B=1.53				A=0.67 B=0.54 A×B=0.94			

Table 7: Diameter of inhibition zone of (*B. subtilis*) resulted from application from cumin oil under cold storage.

	2017 Season			2018 Season		
	Temperature (B)			Temperature (B)		
Treatments *S.P (m) (A)	-20°C	4°C	Mean (A)	-20°C	4°C	Mean (A)
0	10.00	9.50	9.75	10.33	9.50	9.92
3	10.33	9.33	9.83	9.67	9.33	9.50
6	10.00	9.17	9.59	9.50	9.67	9.59
Mean (B)	10.11	9.33		9.83	9.50	
LSD at 0.05	A=N.S B=N.S A×B=N.S			A=N.S B=N.S A×B=N.S		

*S.P (m) = Storage periods (month).

inhibition zone (mm) of cumin essential oil in (*Bacillus subtilis*) during 2017 and 2018 seasons.

As for the effect of gamma irradiation, increasing doses led to decrease the microbiological activity. Concerning of the effect of storage periods, extending periods up to 6 months, resulted in a gradual decrease in the microbial activity of the essential oil against *Bacillus subtilis*.

Respecting the interaction between gamma irradiation doses and storage period of cumin essential oil, results revealed that the highest activity of *Bacillus subtilis* was given with unirradiated samples in addition to zero time storage as recorded 12.33 and 11.33 mm during 2017 and 2018 seasons, respectively. However, the lowest activity of *Bacillus subtilis* was given by 10kGy after 6 months as recorded 7.33 mm in the first season, while 10kGy after 3 months had the lowest activity amounting 8.0 mm in the second one.

These results agree with that obtained by Bag and Chattopadhyay [28] who revealed that cumin fruit oil exhibit both synergistic antibacterial activity and may be used as a potential source of safe and potent natural antibacterial agents in pharmaceutical and food industries. Its synergistic interactions may increase its antibacterial efficacy at sufficiently low concentration which may reduce its adverse side effects and facilitate the use in food preservation system. Chemical analysis revealed that *p*-coumaric acid from cumin fruit oil was the bioactive compound responsible for both synergistic antibacterial and antioxidant activities.

Diameter of Inhibition Zone of *B. subtilis* Resulted from Application from Cumin Oil under Cold Storage: Data in Table (7) demonstrate the effect of cumin essential oil storage periods and temperatures on the diameter of inhibition zone of *Bacillus subtilis* during 2017 and 2018 seasons.

Table 8: Effect of gamma radiation doses (kGy) and storage periods on diameter of inhibition zone (mm) of cumin fruits essential oil against (*Escherichia coli*) during 2017 and 2018 seasons

Treatments	2017 Season				2018 Season			
	Storage periods by month (B)				Storage periods by month (B)			
Doses (kGy) (A)	0	3	6	Mean	0	3	6	Mean
0	20.67	18.33	16.67	18.56	20.33	19.33	17.67	19.11
10	17.67	16.67	16.33	16.89	18.00	17.33	17.33	17.55
20	17.33	16.33	15.67	16.44	17.67	16.67	16.33	16.89
Mean	18.56	17.11	16.22		18.67	17.78	17.11	
LSD at 0.05	A=0.99 B=0.92 A×B=1.59				A=1.11 B=0.79 A×B=1.37			

Table 9: Diameter of inhibition zone of (*E. coli*) resulted from application from cumin oil under cold storage.

Treatments	2017 Season			2018 Season		
	Temperature (B)			Temperature (B)		
*S.P (m) (A)	-20°C	4°C	Mean (A)	-20°C	4°C	Mean (A)
0	18.33	17.17	17.75	18.63	16.57	17.60
3	18.17	16.50	17.34	18.33	16.50	17.42
6	17.67	16.17	16.92	17.50	16.33	16.92
Mean (B)	18.06	16.61		18.15	16.47	
LSD at 0.05	A=0.44 B=N.S A×B=N.S			A=0.94 B=0.21 A×B=N.S		

*S.P (m) = Storage periods (month).

Concerning the effect of storage periods, extending periods, data indicate that extending storage period up to 6 months had no effect on inhibition zone of *Bacillus subtilis*.

As for the effect of storage temperature, it is clear that storing essential oil at -20°C give very close results in comparison to those stored at 4°C.

Effect of Gamma Radiation Doses (kGy) and Storage Periods on Diameter of Inhibition Zone (Mm) of Cumin Fruits Essential Oil Against (*Escherichia coli*) During 2017 and 2018 Seasons: Results in Table (8) show the effects of gamma irradiation doses, storage periods and their interaction on diameter of inhibition zone (mm) of cumin essential oil against (*E. coli*) during 2017 and 2018 seasons.

Concerning the effect of gamma irradiation, increasing irradiation doses led to decrease the microbial activity. Also, for the effect of extending storage period up to six months, results show a gradual decrease in the diameter of inhibition zone (mm) of cumin essential oil against (*E. coli*).

Taking into consideration the interaction between gamma irradiation dose and storage period up to six months of cumin essential oil against the diameter of inhibition zone (mm) of cumin essential oil against (*E. coli*), results revealed that the highest inhibition zone of *E. coli* was given at unirradiated samples at zero time recording 20.67 and 20.33 mm during 2017 and 2018 seasons, respectively. However, the lowest inhibition zone of *E. coli* was given by 20kGy after six months recording 15.67 and 16.33mm during 2017 and 2018 seasons, respectively.

These results are In accordance with those reported by Radfar *et al.* [39] declared that increased phenolic contents can result in increased antimicrobial activity of the date extracts.

Diameter of Inhibition Zone of (*E. coli*) Resulted from Application from Cumin Oil under Cold Storage: Results presented in Table (9) show the effect of storage periods and temperature on the diameter of inhibition zone of stored cumin essential oil in *E. coli* during 2017 and 2018 seasons.

As for the effect of storage periods, results show that extending storage periods up to six months decreased the diameter of inhibition zone in both seasons.

Concerning the effect of storage temperature on diameter of inhibition zone, results indicate that no effect was noticed between storing essential oil at 4°C or at -20°C on inhibition zone diameter.

Wanner *et al.* [40] concluded that cumin essential oil and cuminic aldehyde, were tested against different Gram-positive and Gram-negative bacteria as well as three different Candida albicans. Cumin oil and cuminic aldehyde exhibited a considerable inhibitory effect against all the tested organisms.

Also, Bameri *et al.* [41] reported that Ethanol extracts of fruit of *Cyminum cuminum* were tested for antimicrobial activity *in vitro* by the microdilution method. Ethanol extract of fruit exhibited antimicrobial activity against biofilm *Escherichia coli*.

In addition, Chaudhary *et al.* [42] found that the volatile oil of *Cuminum cyminum* was active against Staphylococcus, *Bacillus cereus, Escherichia coli, Salmonella typhi*. The antimicrobial activity induced by methanolic, hydroalcoholic and aqueous extracts was less that that produced by volatile oils.

The antimicrobial activity increased in the irradiated samples for gram negative bacteria and did not change for gram + bacteria [43].

Moreover, Al-Snafi [44] cited that phytochemical analysis showed that *Cuminum cyminum* contained: flavonoid, steroid. The previous pharmacological studies revealed that *Cuminum cyminum* exerted antimicrobial, insecticidal, anti-inflammatory and antioxidant effects.

The antimicrobial activity of the essential oils is attributed to those known main components and the resulting synergistic or antagonistic action. However, minor components may also contribute to the biological activity [45]. The antibacterial activity of C. cyminum essential oil is perhaps attributable to the high level of cumin aldehyde (16.1%), a compound with known antimicrobial properties [46 and 47] and to β-pinene, the other main component (11.4%) of *C. cyminum* essential oil which inhibited the growth of bacteria [48 - 49].

CONCLUSION

This study revealed a negative effect of treating cumin fruits with gamma irradiation and storage periods on the subsequent extracted essential oil properties i.e. yield, antioxidant activity and total flavonoids content, while a positive effect was observed in case of total phenols content. The same trend was obtained, to some extent with less sharply, when the essential oil was stored at 4°C. While the ability of the stored essential oil on the inhibition of the two studied bacterial strains was not affected. Concerning essential oil components, no clear effect was observed when the essential oil was stored either with or without treating of cumin fruits before oil extraction. Finally and according to this study the extraction of cumin fruits essential oil directly after harvest then stored for 6 months at 4°C was favorable when compared to other treatments.

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