

Research

Effect of Gama Irradiation on quality and storage ability of Mentha (*Mentha viridis* L.)

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Abstract

The effects of low-doses γ -irradiation (0.25, 0.75 and 1.25kGy) during cold storage at 4°C and relative humidity (RH) of 98% on discarded%. Fresh weight loss, total phenols, total flavonoids, total antioxidant capacity, ascorbic acid, chlorophyll content (Chl a, Chl b), carotenoids, volatile oil % and its compositions of fresh mint (*Mentha viridis* L. inn) were studied. Irradiation at 0.75 K.Gy exhibited the lowest value of discarded *Mentha viridis* L. for the first and second seasons and reduced the rate of weight loss% more than 50% compared to the control. Significant decreases in flavonoids compounds and ascorbic acid content were observed by irradiation and increasing the period of storage at (4°C, 98% RH). The results indicated a significant increase in total phenols and total antioxidant capacity in irradiated *Mentha viridis* L. at 0.75 K.G compared to control. The results indicated that irradiation decreased all chlorophyll content and total carotenoids of fresh mint in the two seasons. In spite of chlorophyll content and total carotenoids increased significantly by day 3, they sharply decreased by day 6 and day 9 in the two seasons. Volatile oil extracted from *Mentha viridis* L. was analyzed by capillary GC-2010 plus Gas Chromatographs. The obtained results showed the presence of four compounds (Carvone, β -caryophyllene, carveol and Menthen-2-ol) that were identified before storage at (4°C, 98% RH). Despite leaves of *Mentha viridis* L. when exposed to cold storage provide new compounds such as monoterpene hydrocarbon (α -pinene, β -pinene and D- limonene) and oxygenated monoterpene (1,8 Cineole). These results demonstrate that irradiation of fresh mint affects quantitatively but not qualitatively the chemical composition of *Mentha viridis* L. volatile oil. These results suggest that it could be used as the base data for the effect of γ -irradiation on *Mentha viridis* L.

Keywords: Irradiation; Spearmint; Treatment ; Cold Storage; Volatile Oil

Introduction

Mentha viridis L. is an herbal plant that is found abundantly in the Mediterranean region. Its product, *Mentha viridis* oil. This oil is well known for its medicinal properties. In addition it is loaded with nutrients such as vitamin A and vitamin C, and has a sweet taste. The characteristic feature of this oil is its refreshing fragrance that provides the therapeutic benefits when inhaled. There are numerous health benefits of spearmint volatile oil such as it can reduce fever, provide relief from depression and asthma [1,2] on parsley indicated that hydro-cooling procedure reduced the loss of fresh weight from the leaves in the first 12 hours of storage and maintained the relative water content (RWC) at a high level even after seven days of storage at 5°C. Irradiation is a direct, simple and efficient one-time process. The genus *Mentha* belongs to the family Lamiaceae (Labiates), consisting of about 25–30 species [3]. This family is a rich source of polyphenolic compounds and hence could possess strong antioxidant properties. Members of the genus are characterized by their volatile oils that are of great economic importance, being used by the pharmaceutical, cosmetic, food, confectionery and liquor industries. Hence, they are cultivated as industrial crops in several countries. There are a few reports on

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the antioxidant property of Mentha [4,5,6]. For fresh culinary herbs, visual quality (no decay or yellowing) and good color and aroma are the most important quality characteristics [7,8,9,10]. It is the chlorophyll content in the tissue of leafy vegetables that determines their greenness [11]. Chlorophyll levels are also used to assess the senescence process and postharvest conservation [7]. Yellowing of leafy vegetables and fresh herbs occurs with the degradation of chlorophyll [12]. Irradiation is a direct, simple and efficient one-time process. In addition to, irradiation has great advantages in contrast to the conventional methods of preservation such as cold storage, fumigation, salting and drying as it does not lead to loss of flavor, odor, texture or quality.

Irradiation treatment combined with proper refrigeration for storage can prolong the shelf-life of these food items without affecting the flavor and texture [13]. Therefore, a balance between the required effect videos and tolerance of Mentha to irradiation has to be investigated under various storage temperatures. So, irradiation can be used in combination with low temperature for to assess their effects on reduction the physiological loss in weight and decay phenomena of Mentha. So we carried out this study and evaluate the effect of irradiation on total phenols, total flavonoids, total antioxidant capacity, ascorbic acid, chlorophyll a, chlorophyll b, carotenoids ,volatile oil % and its compositions.

Materials and Methods

The experiment was carried out during two seasons of 2016 and 2017. The samples of fresh *Mentha viridis* L. were purchased from a local farm in El Kanater El Khairia, Egypt and transported to National Center for Radiation Research and Technology (NCRRT) located in Nasr City, Cairo, Egypt.

Then, the *Mentha viridis* L. were washed and dried well by exposing them to air for 4 hours and divided into four groups; each group was 500g (4 treatments X 3 replicates X 2 group X 500 g for each group = 12 Kg). Four treatments were carried out as follows:-

Treatment 1: control (untreated).

Treatment 2: irradiation at 0.25kG.

Treatment 3: irradiation at 0.75 kG.

Treatment 4: irradiation at 1.25 kG.

Each replicate consisted of two subgroups, one group for studying physical properties and another one for determining chemical constituents.

The experiment was carried out in a split plot design with three replicates; the main treatments were the time of storage, while the irradiation represented the sub-main treatments.

Time Of Storage (Main Treatments)

The *Mentha viridis* L. were stored at 4°C, 98 % RH (relative humidity) for 0,3,6 and 9 days

Irradiation (Sub-Maintreatments)

The *Mentha viridis* L were carried out at room temperature using the Co-60 source at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The irradiation facility used was an Egypt's Mega Gamma- 1, of the type J-6500 supplied by the Atomic Energy of Canada Limited. The applied doses were 0.0, 0.25, 0.75 and 1.25 kG for *Mentha viridis* L. The dose rate delivered during the experimental duration was 1.774kGy/hr., as monitored by radio chromic film [14]. After irradiation the experimental materials(*Mentha viridis* L.) were transferred into a cold storage room adjusted at 4°C, 98% RH.

The Following Measurements Were Done

Physical Properties

Discarded %

Mentha viridis L. showed any sign of decay or visual disorders during the cold storage time were counted and discarded every 3 days, then the percentage of discarded was calculated according to [15] as follow:-

$$\text{Discarded \%} = \frac{\text{Decayed Weight}}{\text{Total sample weight}} \times 100$$

Any treatment was terminated in case of having 50% discarded *Mentha viridis* L. was terminated

Weight Loss (WL %)

Percentage of *Mentha viridi* L. weight loss was calculated the beginning of experiment at zero time of storage and recorded the initial weight of *Mentha viridis* L. The weight loss of all treatments was calculated by weighing every 3 days [15]. as in the following equation:

$$\text{WL \%} = \frac{\text{herb initial weight} - \text{herb weight at each sampling date}}{\text{initial weight}} \times 100$$

Chemical Constituents

Sample preparation and extraction For methanolic, the extraction process was carried out by grounding (2 g) green leaves in a pestle with 20 ml of 80% methanol. The homogenate was filtered to obtain methanolic extraction colorless.

Spectrophotometric measurements

The spectrophotometric measurements were performed using an ultraviolet-visible spectrophotometer (model MA9523-SPEKOL 211, ISKRA, Horjul, Slovenia).

Total Flavonoids

Total flavonoids were estimated using method of [16] using Aluminum chloride, the absorbance was measured at 420 nm.

Total Phenols

The total phenolics content of methanolic extract was determined according to the method described by [17] using folin-ciocalteu reagent. The absorbance was recorded at 725nm.

Total antioxidant Capacity

The total antioxidant capacity of the green leaves extracts was evaluated by thephosphomolybdenum method by [18]. The absorbance of the solution was measured at 695 nm with a spectrophotometer against methanol as the blank. Ascorbic acid (AA) was used as the standard.

Ascorbic acid Content

Ascorbic acid was determined according to the method of [19]. The absorbance was recorded at 515nm.

Chlorophyll (A, B) and Carotenoids S Determination

The protocol devised by [20] was followed to determine chlorophyll a, b and carotenoids s contents. 0.2 gram green leaves sample was ground in 10 ml of 80% acetone and filtered through Whatman No. 1 filter papers. The filtered extract was transferred in cuvette and absorbance was noted at 663, 645, 505 and 453 nm by using UV-spectrophotometer.

Following formulae were used to calculate chlorophyll a, chlorophyll b and carotenoids s contents.

$$\text{Chlorophyll a} = 0.999 A_{663} - 0.0989 A_{645}$$

$$\text{Chlorophyll b} = -0.328 A_{663} + 1.77 A_{645}$$

$$\text{Carotenoids} = (0.216 A_{663} - 1.22 A_{645}) - (0.304 A_{505} + 0.452 A_{453}).$$

Volatile oil Study

Volatile oil percentage determined in leaves according to the method described in the [21]. Analysis of the volatile oil by using capillary GC-2010 plus Gas Chromatographs (Shimadzu Corp., Japan), coupled with a Shimadzu FID 2010 Plus detector (Flame Ionization Detector). The GC system was equipped with a Stabilwax column (30 m x 0.25 mm i.d., 0.25 μ m film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min at a split ratio of 1:10 and the following temperature program: 40° C for 1 min; rising at 4.0° C/min to 150° C and held for 6 min; rising at 4° C/min to 210° C and held for 1min. The injector and detector were held at 210° and 250° C, respectively. Diluted samples (1:10 hexane, v/v) of 0.2 μ L of the mixtures were always injected. Most of the compounds were identified using GC standards.

Statistical analysis of Data

The data were subjected to analysis of variance (ANOVA) with one-way classification. Linear regression analysis was utilized to define the relationship between different parameters and irradiation dose (kGy). All analyses were conducted using the general linear model procedure of the Statistical Analysis System Institute, Inc [22], where appropriate treatment means was separated using the Duncan's Multiple Range Test [23]. The α - level for significance was $P \leq 0.05$.

Results and Discussion

Effect of Irradiation on Physical Properties Of “*Menthaviridis*!”

Discarded %

Data tabulated in Table (1) show that radiation treatments during cold storage reduced the discarded *Mentha viridis* L. % as compared to the control (untreated). Concerning to cold storage the highest value of discarded were (52.420 and 52.893%) for control in the first and second seasons, respectively after 9 days. After 6 days of cold storage the discard *Mentha viridis* L. % were recorded that 11.483 against 8.183% for control with the use of 0.75 K.Gy dose. On the other hand, after 9 days of cold storage, 0.75 K.G dose was exhibit the lowest value of discarded *Mentha viridis* L (23.830 and 24.050%) for the first and second seasons, respectively. But control and 0.25 KG dose exhibited the highest value of discard (52.42 and 39.657) for the first seasons. The same trends were observed in the second season.

Table 1: Effect of γ -irradiation on discarded (%) of "*Mentha viridis* L. during cold storage at (4°C, 98% RH) during 2016 and 2017 seasons .

treatments kGy	Storage period in days				
	2016 season				
	0 time	3 days	6 days	9 days	Mean
control	0.000 C	2.267 C	8.183 B	52.420 A	15.718
0.25	0.000 C	3.067 C	17.883 B	39.657 A	15.152
0.75	0.000 C	0.133 C	11.483 B	23.830 A	8.862
1.25	0.000 D	1.867 C	25.136 B	33.390 A	15.098
Mean	0.000	1.834	15.671	37.324	
2017 season					
control	0.000 C	3.067 BC	8.253 B	52.893 A	16.053
0.25	0.000 C	3.867 C	18.033 B	40.063 A	15.491
0.75	0.000 C	0.933 C	11.573 B	24.050 A	9.139
1.25	0.000 D	2.667 C	25.343 B	33.757 A	15.442
Mean	0.000	2.634	15.801	37.691	

Weight Loss (WL %)

Data in Table (2) show the effect of irradiation 0.25,0.75, 1.25 kGy doses and the cold storage at 4°C, 98% RH on fresh weight loss % of *Mentha viridis* L. the fresh weight loss % increased by increasing the storage periods. However, there was a significant effect of 0.75 and 1.25 irradiation doses and cold storage on reducing the rate of weight loss% compared to the control (untreated)*Mentha viridis* L. after 9 days of cold storage the least weight loss% was recorded 6.370 by irradiated 0.75 kGy doses in the first season. on the contrary, there was a sharp increase in *Mentha viridis* L. weight loss % by irradiation 0.25 kGy dose and untreated (control) which recorded 33.937% and 18.250% after 9 days respectively in the first season. The same trend of

Table 2: Effect of γ -irradiation on weight loss % of "*Mentha viridis* L. "during cold storage at (4°C, 98% RH), during 2016 and 2017 seasons

Treatments kGy	Storage period in days				
	2016 season				
	0 time	3 days	6 days	9 days	Mean
control	0.000D	1.867C	14.950 B	18.250A	8.767
0.25	0.000 D	5.067C	9.273 B	33.937A	12.069
0.75	0.000 B	5.200A	4.787 A	6.370 A	4.089
1.25	0.000 C	1.467C	3.387 B	13.033A	4.472
Mean	0.000	3.400	8.099	17.898	
2017 season					
control	0.000 D	2.267C	15.007 B	18.310A	8.896
0.25	0.000 D	5.733C	8.917 B	32.497A	11.787
0.75	0.000 B	6.933A	3.407 AB	6.400 A	4.185
1.25	0.000C	1.867BC	3.403 B	13.090A	4.59
Mean	0.000	16.8	30.734	70.297	

results was also found during of the experiments in the second season of study.

Our results are in agree with [24] who assessed weight loss and quality (appearance, aroma and color) in fresh coriander (8 g packages) stored at 4,10 and 25 o C for 2,4,6 and 8 days. They found that quality parameters decreased as storage temperature increased and storage at 4°C is recommended for preserving quality for one week, although weight loss after 8 days at 4°C was approximately 50 %.

Effect of Irradiation on Chemical Constituents of "*Menthaviridis*l."

Total Flavonoids

The variation in total flavonoids, total phenols and total antioxidant capacity of control and irradiated samples is depicted in (Table 3). Flavonoids content significantly decreased ($p < 0.05$) with increasing radiation dose at 0.25 to 1.25kG. Concerning storing at (4°C, 98% RH), it increased after 3 days and then decreased by increasing the period of storage in the two seasons. The results found that storing at (4°C, 98% RH); it recorded (8.180 and 8.583 mg/100g) in the first and second seasons respectively at the highest irradiation treatments of 1.25kGy after 9 days. Whereas, total flavonoids content of un irradiated sample was (24.067 and 25.210 mg/100g) in the first and second seasons respectively and reached its minimum to (10.530 and 11.303 mg/100g) in the first and second seasons respectively before storage. These results may indicate degradation or in solubilization of flavonoids compounds when they are exposed to gamma irradiation. However, studies need to be conducted with more samples to confirm the results. In contrast, an increase of the amounts of total flavonoids due to irradiation has also been reported by many authors. A study by [25] revealed that the total flavonoids content increased significantly with increasing dose of radiation in bitter gourd as compared to control sample. The increase in total flavonoid can be attributed to the phenylalanine ammonialyase activity, which is one of the key enzymes in the synthesis of phenolic compounds in plant tissue. our results found that total flavonoids content of control was (24.067 and 25.210mg/100g) in the first and second seasons respectively and reached to (30.587 and 32.273 mg/100g)in the first and second seasons respectively after stored at (4°C, 98% RH) during 3 days, while it cut back on until 9days. The protective effect of flavonoids is due to several mechanisms such as free radicals trapping, enzymes inhibition and metallic ions chelation. These properties depend on the structure of the flavonoids and the degree of substitution and saturation. Fruits and vegetables are rich source of flavonoids [26].

Table 3: Effect of γ -irradiation on total flavonoids, total phenols and total antioxidant (mg/100g) in leaves of "*Mentha viridis* L." during different cold storage periodat (4°C, 98% RH) during 2016 and 2017 seasons:

treatments	Total flavonoids					Total phenols					Total antioxidant capacity				
	Storage period in days														
	First season														
	0 time	3 days	6 days	9 days	Mean	0 time	3 days	6 days	9 days	Mean	0 time	3 days	6 days	9 days	Mean
control	24.067 C	30.587 A	28.060 B	18.900 D	25.404	180.633 D	226.487 A	216.317 B	207.423C	207.715	342.557 D	654.39 C	682.083 B	821.947A	625.244
0.25	19.827 A	16.457 B	13.577 C	13.517 C	15.845	184.187 C	227.673 A	227.533 A	227.120B	216.628	943.947 D	1851.527 C	1906.22 B	1996.137 A	1674.458
0.75	11.907 A	11.907 A	11.763 A	10.117 B	11.424	226.070 A	225.443 A	222.703 B	221.633B	223.962	1642.417 D	1774.333 C	2781.14 A	2114.86 B	2078.188
1.25	10.530 A	9.590 B	9.123 C	8.180 D	9.356	212.750 B	221.817 A	221.593 A	208.433C	216.148	814.39 D	1548.083 C	1636.333 B	2070.277A	1517.271
Mean	16.583	17.135	15.631	12.679		200.91	225.355	222.037	216.152		935.828	1457.083	1751.444	1750.805	
Second season															
control	25.210 C	32.273 A	28.927 B	20.350 D	26.69	170.540 A	228.240 A	216.783A	208.730A	206.073	312.777 C	654.337 BB	680.28 B	822.233A	617.407
0.25	20.253 A	17.763 B	13.900 C	14.263 C	16.545	185.520 C	228.557 B	228.503 B	229.343 A	217.981	944.143 D	1851.307 C	1906.05 B	1993.21 A	1673.68
0.75	12.507 A	12.697 A	11.353AB	8.380 B	11.234	226.780A	226.340 A	224.413 B	222.553 C	225.022	1644.143 D	1773.493 C	2773.567A	2115.24 B	2076.611
1.25	11.303 A	10.273 B	9.547 C	8.583 D	9.927	213.287B	222.467 A	222.333 A	209.200 C	216.822	814.99 D	1549.703 C	1637.297 B	2079.71 A	1520.425
Mean	17.318	18.252	15.932	12.894		199.032	226.401	223.008	217.457		929.013	1457.21	1749.299	1752.598	

Total Phenols

The quality and quantity of phenolic compounds are widely used as a quality indicator of plant material after any treatment, especially irradiation techniques. The data indicates a significant increase in total phenols and total antioxidant capacity in irradiated *Mentha viridis* L as compared to control. The results showed that the amount of total phenolic contents increased from 180.633mg/100g to around 226.487 mg/100g of the *Mentha viridis* L at (4°C, 98% RH) in the first season and from 170.540mg/100g to 228.240mg /100g in the second season after 3 days and then decreased by increasing the period of storage in the two seasons. Total phenols content of control were (180.633 and 170.540 mg/100g) in the first and second seasons respectively and reached to (226.070 and 226.780 mg/g) at dose 0.75 kG. Increasing in total phenols in irradiated plants has also been reported by [27]. Such increase in total phenols is due to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by gamma irradiation as suggested by [28]. This increase is probably related to the effect of the irradiation, which breaks down the polyphenol chemical bonds and consequently induces the release of low molecular weight soluble phenols. Similar observations have been reported for different plant material treated with different doses of gamma-irradiation. Indeed, [29] and [30] reported an increase in the total phenolic content in *Nelumbonucifera* rhizomes and cumin seeds, respectively, after applying gamma irradiation at doses lower than 6 kG. Lower doses were also reported to induce such an increase as observed [31,32] when they subjected mushrooms to gamma irradiation at doses between 0.5 and 2.5kGy. [28] used also 4 kGy dose irradiation to treat almond skin and

observed slight increase in the yield of the phenolic extract. [33] have explained that such an increase observed in very low dose irradiated Citrus Clementine peels was due to the enhanced enzymes activity especially the phenylalanine ammonia-lyase (PAL) after irradiation.

Total antioxidant Capacity

The results of correlation of antioxidants capacity in *Mentha viridis* L upon irradiation (0.25, 0.75 and 1.25 kG) and storage at (4°C, 98% RH) for (3, 6 and 9 days) are presented in (Table 3).

The results showed increasing in total antioxidant capacity with storing at (4°C, 98% RH) until 9 days in control and all irradiated samples in the two seasons. Also, total antioxidant capacity of control was (342.557 and 312.777mg/100g) in the first and the second seasons respectively and reached its maximum to (1642.417 and 1644.143 mg/100g) in the first and the second seasons respectively at the irradiation treatments of 0.75 KG and it brought down at the highest irradiation treatments of 1.25 KG. Generally our results indicated a very large increasing in total antioxidant capacity with increasing irradiation doses and as storage progressed. The results revealed enhancement in the phenolic content and antioxidant capacity of *Mentha viridis* L in the two seasons of all samples until 9 days after irradiation. Thus, it seems that 0.75 KG of irradiation might induce some chemical reactions in components of *Mentha*, which possibly degrade or decompose large molecules into small phenolic molecules, which are readily soluble in methanol and may also be beneficial for the antioxidant properties of the plants. [34] studied the effects of ionizing radiation (0, 0.5, 1, and

2 KG; followed by storing at 8 °C for 8 days) on antioxidant capacity, phenolics content, and tissue browning of three vegetables (Romaine and Iceberg lettuce, and endive). Their results revealed enhancement in the phenolic content and antioxidant capacity of both tissue types (midrib and non-midrib leaf tissues) of all vegetables at days 4 and 8 after irradiation. This increase in phenolic content and antioxidant capacity was attributed to increased phenolic synthesis contributing to the total antioxidant capacity.

Ascorbic acid Content

Ascorbic acid content of *Mentha viridis* L leaves treated with γ -irradiation is shown in (Fig.1and Fig.2). Control samples were rich in ascorbic acid (211.810 and 212.567mg /100g) in the first and second seasons respectively, similar to other reports [35,36]. Ascorbic acid content significantly decreased with increasing radiation dose at

0.25 to 1.25kGy upon storing at (4°C, 98% RH).A does of 1.25 KG significantly decreased (by 90.19 % and 89.77%)in the first and second seasons respectively, similar to other studies [22,37]. This indicated that ascorbic acid oxidized primarily against ionizing radiation. However, no linear correlation was confirmed between the content of ascorbic acid and absorbed dose. Furthermore, cold samples contained (20.76% and 20.61%), (30.46% and 29.64%) and (45.60% and 45.51%) less ascorbic acid after 3, 6 and 9 days of storage than controls in the first and second seasons respectively. Ascorbic acid acts as an antioxidant,

protecting cells against oxidative stress [38].Ascorbic acid was the major water soluble antioxidant in plant leaves. Our results are in disagreement with [39], who found that, marked increase in ascorbic acid under stress adaptation in the mulberry.

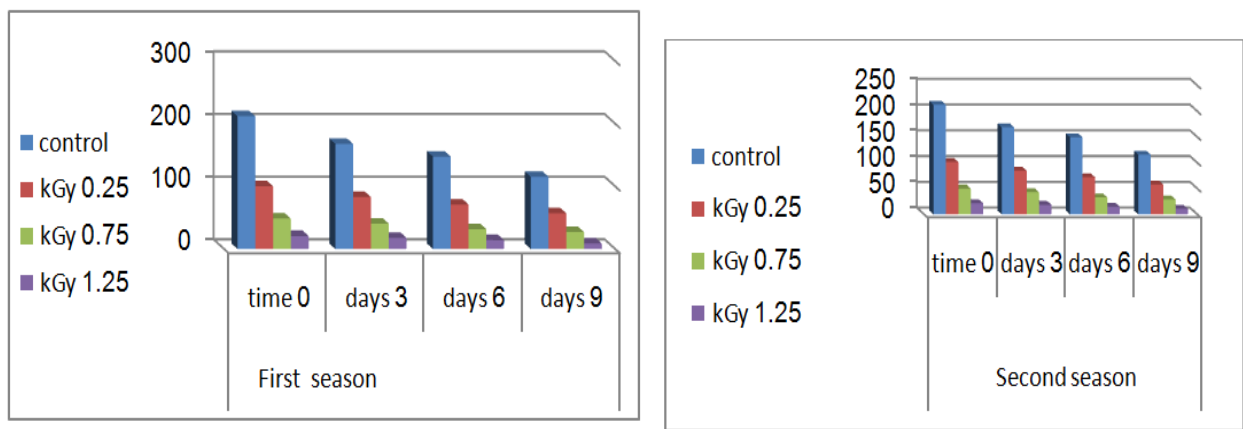


Figure 1. Effect of γ - irradiation on ascorbic acid content(mg/100g) in leaves of “*Mentha viridis* L.”during different cold storage period at (4°C, 98% RH) during2016 and 2107 seasons

Chlorophyll (A, B) and Carotenoids S

Effects of irradiation and storage on chlorophyll content (chlorophyll a, chlorophyll b) and total carotenoids are listed in (Table 4).The results indicated that irradiation decreased allchlorophyll content andtotal carotenoids of fresh mint in the two seasons. Significantly decreases in chlorophyll a, chlorophyll b, and total carotenoids were observed at 0.25, 0.75, and 1.25KG compared to control. The results also suggested that storage had a major impact on chlorophyll content and total carotenoids. Chlorophyll a, chlorophyll b and total carotenoids increased by storage significantly at 3 days and sharply decreased by 6 days and 9 days in the two seasons (Table 4). Some

studies reported the benefit of low-dose irradiation without affecting the chlorophyll contents of fresh produce [40]. indicated that irradiation at 1 to 3 KG did not significantly affect the total chlorophyll content of fresh coriander leaves stored at 8 to 10°C for 1 week. The chlorophyll content of alfalfa sprouts over 2 weeks storage period was not significantly different from controls when 0.85 to 2.57 kGy were used [11].Our results suggested that fresh unirradiated mint at 3 days had higher chlorophyll a, chlorophyll b and total carotenoids contents than controls. Similarly [41], indicated less storage-induced progressive loss in chlorophyll content for refrigerated capsicum when irradiation doses up to 3 KG were used.

Volatile oil Study

Most studies that have evaluated the effect of γ - irradiation on medicinal plants and species were conducted on aqueous or organic extracts where volatile oil is one of the most important fractions in plants. Data in Table (5) show the effect of irradiation 0.25, 0.5, 1.25 KG doses and the different cold storage periods at 4°C, 98% RH on volatile oil percentage of *Mentha viridis* L. As shown in (Table 5), the extracted oil percentage was higher for control samples (0.20 and 0.22%) in comparison with the irradiated samples in the first and second seasons respectively. Also the volatile oil percentage slightly decreased by increasing the storage period from 3 to 9 days. the maximum volatile oil percentages were 0.20 and 0.22 %, respectively for control samples

stored at 4°C, 98% RH, while the minimum volatile oil Percentages were 0.05 and 0.07 for samples which irradiated by 1.25 KG stored at 4°C, 98% RH for 9 days in the first and second seasons respectively . The higher yield of volatile oil extraction verified for the irradiated samples has been usually attributed to radiation-induced disruption of the cell wall structure, providing a higher extract-ability of oil from the plant tissues. Moreover, changes on volatile oil extraction yield can be due to a recombination of the radio lytic products with time. Specific effects can be observed on a secondary metabolite in different volatile oil even though submitted to the same radiation conditions. The content of a constituent upon radiation is presumably due to its radiation sensitivity at different doses [42].

Table 4: Effect of γ -irradiation on Chlorophyll a, Chlorophyll b and Carotenoids (mg/100g) in leaves of "*Mentha viridis* L. "during different cold storage period at (4°C, 98% RH) during 2016 and 2017 seasons:

treatments kGy	Chlorophyll a					Chlorophyll b					Carotenoids				
	Storage period in days														
	First season														
	0 time	3 days	6 days	9 days	Mean	0 time	3 days	6 days	9 days	Mean	0 time	3 days	6 days	9 days	Mean
control	130.677A	180.227A	68.460 C	35.623 D	103.747	31.167 C	76.000 A	54.187 B	29.180 D	47.634	53.687 B	68.068 A	41.093 D	43.660 C	51.627
0.25	94.653 B	90.467 B	60.410 C	58.777 D	76.077	34.613 A	30.470 B	26.377 C	26.557 C	29.504	41.818 A	40.637 B	34.466 C	33.529 D	37.613
0.75	101.337 B	88.520 B	74.267 C	74.123 C	84.562	42.343 A	37.150 B	36.660 C	27.073 D	35.807	39.204 A	31.812 D	35.544 B	34.895 C	35.364
1.25	95.413 B	79.580 B	38.743 C	38.557 C	63.073	41.077 A	34.240 B	17.863 C	16.937 D	27.529	34.116 A	33.167 B	25.056 C	24.445 C	29.196
Mean	105.52	109.699	60.47	51.77		37.3	44.465	33.772	24.937		42.206	43.421	34.04	34.132	
	Second season														
control	131.457 A	179.337 A	67.863 C	36.553 D	103.803	32.523 C	75.803 A	55.133 B	29.767 D	48.307	57.423 B	75.030 A	42.733 D	47.393 C	55.645
0.25	95.487 B	91.413 B	61.520 C	60.380 D	77.2	35.287 A	30.783 B	25.680 D	26.837 C	29.647	45.330 A	41.907 B	37.210 C	37.827 C	40.569
0.75	101.533 B	89.423 B	75.623 C	75.253 C	85.458	43.347 A	37.803 A	37.753 A	26.573 A	36.369	43.043 A	40.107 B	39.587 B	39.407 B	40.536
1.25	96.617 B	80.907 B	39.450 C	39.627 C	64.15	41.220 A	35.087 B	18.317 C	17.493 C	28.029	41.410 A	36.609 B	35.530 B	35.500 B	37.262
Mean	425.094	441.08	244.456	211.813		38.094	44.869	34.221	25.168		46.8015	48.413	38.765	40.032	

Volatile oil extracted from *Mentha viridis* L. were analyzed, as a quality indicator, before and after the irradiation and the storage at 4°C, 98% RH and their compositions were studied. The obtained results showed the presence of eight compounds that were identified by capillary GC-2010 plus Gas Chromatographs as collected in Table 5 and 6. The identified compounds are ranged from 75.573% in samples which irradiated by 0.75 KG stored at 4°C, 98% RH for 9 days to 100% in fresh control samples. The majority of compounds (3 Monoterpen hydrocarbons) ranged from 2.990% in samples which irradiated by 0.75 KG and stored at 4°C, 98% RH for 6 days to 8.812% in samples which irradiated by 0.25 KG and stored at 4°C, 98% RH for 9 days, while the oxygenated monoterpenes are represented (2 compounds) and ranged from 43.067% in samples which irradiated by 0.75 KG and stored at 4°C, 98% RH for 3 days to 82.327% in fresh control samples. On the other hand the mono terpenoid alcohol are represented (2

compounds) and ranged from 1.552% in samples which irradiated by 1.25 KG and stored at 4°C, 98% RH for 6 days to 16.494% in un stored samples which irradiated by 0.25 KG. The β -caryophyllene was the only sesquiterpene hydrocarbon compound found and ranged from 4.953% in control samples which stored for 3 days to 21.807% in samples which irradiated by 0.75 KGy and stored at 4°C, 98% RH for 3 days. Comparison of the volatile oil fractions in the *Mentha viridis* L. before and after γ -irradiation (0.25, 0.75 and 1.25 KGy) showed changes in chemical constitutes of the oil (Table 5). Carvone was the major component with a content(82.327%) accompanied with other components at relatively low levels: β -caryophyllene (8.213%), carveol (5.350%) and Menthen-2-ol (4.110%).Table 5 shows that γ -irradiation process affects quantitatively but not qualitatively the chemical composition of *Mentha viridis* L. volatile oil, the effect was dose dependent. γ - irradiation with 0.25 kGy caused the increase in

concentration of two components (carveol) that raise from 5.350 % to 12.055% and (β -caryophyllene) that raise from 8.213 % to 21.751% and caused the decrease in concentration of one component (Carvone) that reduced from 82.327% to 48.139%, the content of Menthen-2-ol was similar to the control. When we increase the irradiation dose, the changes are even more pronounced. Thus, for 0.75kGy, there were only two compounds (Carvone 80.165%) and (β -caryophyllene 10.650%). Whereas for 1.25 kGy three compounds (Carvone, carveol and Menthen-2-ol) decreased in irradiated samples while, the content of β -caryophyllene was similar to the control. Our result agree with those obtained by [43]. In fact these authors working on *Mentha peperita* have shown quantitative but not qualitative changes with doses ranging from 0.5 to 2.66 kGy. [44] have also shown that γ - irradiation increased significantly the content of linalool and estragole in the irradiated basil. On the other hand, our results disagree with those obtained by [45]. They did not show any effect of gamma irradiation on *Thymus*, *Eucayptus* and *Lavandula* up to 25 KG. They attributed the differences in results between authors to the type of plant, method of extraction and γ -irradiation (dose and time of exposure). The same authors suggested that the same compound can be affected differently according to the type plant.

With respect to the effect of different cold storage period at (4°C, 98% RH) on volatile oil constituents of *Mentha viridis* L., data in Table 5 illustrated that leaves of *Mentha viridis* L. when exposed to cold

storage provide new compounds such as monoterpane hydrocarbon (α -pinene, β -pinene and D- limonene) and oxygenated monoterpene (1,8Cineole). On another hand some compounds decreased by increasing the period of storage in the control and all the treatments such as menthen-2-ol, carveol and β -caryophyllene. However carvone recorded different results, it decreased by increasing the period of storage in the control and all the treatments except the 0.25 kGy it increased by increasing the period of storage from 48.139% to 68.525. Moreover slight differences were found in the percentages of the other compounds. Some reported changes in chemical composition are quite ambiguous and might be the result of evaporation or no accurate analyses rather than of more oxidative degradation. For instance, the decline of caryophyllene oxide in stored lemon balm oil [46] or increased amounts of sesquiterpenic hydrocarbons [47] surprisingly differ from results normally expected. Our results are in line with [48] who stated that a volatile compound in different volatile oil is under specific reactional environments and the conditions of each volatile oil submitted to different ways of isomerization, oxidation, and hydroxylation when exposed to γ -radiation provide new compounds. In spite of the exposition to radiation on secondary metabolites studied a long time ago, new studies are necessary to better understand its effect on cell structure and chemical structure of the constituents of the volatile oil from vegetal material. In addition, it seems to be interesting to study whether a modification of the structure of some pure compounds (even in trace) could lead to the formation of toxic, long-lived radicals [45].

Table 5: Effect of γ -irradiation on Volatile oil % and Volatile oil Composition in fresh leaves of "*Mentha viridis* L." during different cold storage period at (4°C, 98% RH) in 2017 season:

Irradiation Treatments kGy	Storage period in days	Volatile oil %		Main constituents percentage								Total Identified	Un Identified
		1 st season	2 nd season	α -pinene	β -pinene	D-limonene	1,8Cineole	Menthen-2-ol	Carveol	Carvone	β -caryophyllene		
control	0 time	0.20A	0.22 A	-----	-----	-----	-----	4.110	5.350	82.327	8.213	100.00	-----
	3 days	0.17B	0.18B	0.517	0.719	5.023	2.260	0.498	1.073	73.148	4.953	88.191	11.809
	6 days	0.15B	0.16 B	0.607	0.750	4.140	2.065	0.383	1.474	71.275	7.334	88.028	11.972
	9 days	0.11C	0.11C	0.618	0.769	4.120	2.015	0.322	1.499	71.122	7.522	87.987	12.013
0.25	0 time	0.19A	0.21A	-----	-----	-----	-----	4.439	12.055	48.139	21.751	86.384	13.616
	3 days	0.17 A	0.19 A	0.941	1.110	6.446	2.188	0.524	1.552	60.048	14.280	87.089	12.911
	6 days	0.15B	0.17 B	0.830	1.048	6.781	2.607	0.416	1.197	68.479	7.545	88.903	11.097
	9 days	0.10 C	0.15C	0.850	1.112	6.850	2.712	0.455	1.200	68.525	7.855	89.559	10.441
0.75	0 time	0.18A	0.19A	-----	-----	-----	-----	-----	-----	80.165	10.650	90.815	9.185
	3 days	0.15B	0.16 B	-----	-----	3.110	1.699	2.965	4.635	74.613	10.310	97.332	2.668
	6 days	0.11C	0.14C	-----	-----	2.990	1.707	2.188	4.637	74.597	10.232	96.351	3.649
	9 days	0.08D	0.13C	-----	-----	3.916	0.663	2.145	4.638	42.404	21.807	75.573	24.427
1.25	0 time	0.15 A	0.17 A	-----	-----	3.149	1.643	1.803	2.837	76.849	8.620	94.901	5.099
	3 days	0.11 B	0.15B	0.977	1.179	6.224	2.291	0.347	1.294	71.641	6.730	90.683	9.317
	6 days	0.08C	0.13C	0.506	0.780	6.151	2.812	0.443	1.109	67.131	7.493	86.425	13.575
	9 days	0.05D	0.07 D	0.511	0.810	6.135	2.911	0.449	1.165	67.125	7.541	86.647	13.353

Table 6: The classification of volatile oil components (%) offresh leaves of “*Mentha viridis* L. which irradiated with γ -irradiation and stored in different cold storage period at (4°C, 98% RH) in 2017 season:

Irradiation Treatments kGy	Storage period in days	Monoterpene Hydrocarbhone	Oxygenated Monoterpene	Monoterpenoid Alcohol	Ssesquiterpene
control	0 time	-----	82.327	9.46	8.213
	3 days	6.259	75.408	1.571	4.953
	6 days	5.497	73.34	1.857	7.334
	9 days	5.507	73.137	1.821	7.522
0.25	0 time	-----	48.139	16.494	21.751
	3 days	8.497	62.236	2.076	14.280
	6 days	8.659	71.086	1.613	7.545
	9 days	8.812	71.237	1.655	7.855
0.75	0 time	-----	80.165	-----	10.650
	3 days	3.916	43.067	6.783	21.807
	6 days	2.990	76.304	6.825	10.232
	9 days	3.110	76.312	7.6	10.310
1.25	0 time	3.149	78.492	4.64	8.620
	3 days	8.380	73.932	1.641	6.730
	6 days	7.437	69.943	1.552	7.493
	9 days	7.456	70.036	1.614	7.541

Conclusion

In conclusion, the results showed that 0.75 kGy dose was the best one used for *Mentha viridis* L. and it was better than the control, it exhibited the lowest value of discarded % and reduced the rate of weight loss% more than 50% compared to the control, it recorded the highest values in total phenols and total antioxidant capacity. Our results may indicate degradation or in solubilization of flavonoids compounds when they are exposed to γ -irradiation. Flavonoids decreased by increasing the period of storage at (4°C, 98% RH). The data indicates a significant increase in total phenols and total antioxidant capacity in irradiated *Mentha viridis* L compared to control. The results revealed enhancement in the phenolic content and antioxidant capacity of *Mentha viridis* L in the two seasons of all samples until 9 days after irradiation. This increase seems to originate from the cleavage of chemical bonds in polyphenols molecules, which produces more soluble phenols. However, ascorbic acid content significantly decreased with increasing radiation dose at 0.25 to 1.25 kGY upon storing at (4°C, 98% RH). Our results indicated that irradiation decreased all chlorophyll content and total carotenoids of fresh mint in the two seasons. The results also suggested that storage had a major impact on chlorophyll content and total carotenoids. Chlorophyll a, chlorophyll b and total carotenoids increased significantly by 3 days and sharply decreased by 6 days and 9 days in the two seasons. Indeed, volatile oil percentage decreased by irradiation and by storage at (4°C, 98% RH). Furthe rmore, volatile oil was analyzed by capillary

GC-2010 plus Gas Chromatographs and presence of eight compounds that were identified. γ -irradiation process affects quantitatively but not qualitatively the chemical composition of *Mentha viridis* L. while, expose to cold storage provide new compounds such as mono terpene hydrocarbon (α -pinene, β -pinene and D- limonene) and oxygenated monoterpene (1,8Cineole). In summary, low dose γ - irradiation could be used as a decontamination method for *Mentha viridis* L. The increased amount of soluble phenols can be considered positive aspect of the preservation of the product; such compounds are bacteriostatic agents. More detailed work is, however, to be considered for a more in depth study on the chemical compositions, especially the quantitative aspect of phenolic compounds.

Conflict of Interest

The authors declare that: there is no conflict of interest

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