

Antioxidant, Microbial and Sensory Evaluation of Fresh Mint Leaves Irradiated with Various Doses of γ -Irradiation

Amnah M. Al-Suhaibani and Amal N. Al-Kuraieef

Nutrition and Food Sciences Department, Princess Norah bint Abdulrahman University, Riyadh, KSA.

ABSTRACT

This research was conducted to study the effect of gamma radiation on the antioxidant activity, quality assessment of microbial and sensory for fresh mint leaves. Fresh mint leaves were treated by using three doses of gamma radiation, 0.5, 1.0, 1.5 KGy. The antioxidant activity, total phenols and total flavonoids of aqueous extract for mint leaves have been evaluated. The results showed a high antioxidant activity when radioactive gamma radiation gave a significant increase in the content of total phenols and total flavonoids compared to non-irradiated samples. The evaluation of antioxidant activity of aqueous extracts for mint leaves using DPPH gave a significant decrease in free radical scavenging activity of the samples treated with radiation compared to untreated samples. The results also revealed that radioactive gamma radiation have led to a significant reduction in total number of bacteria; yeasts and fungi. However, a significant decrease in the appearance, color and texture with the dose of 1.5 KGy was observed compared with control.

Key words: Radiation, Mint, Antioxidants, Microbial quality, Sensory evaluation.

Introduction

Since fresh fruits and vegetables are grown, processed or packaged in areas that may be exposed to microbial pathogen contamination. In fact, a number of diseases outbreaks were linked to the consumption of contaminated fresh pre-cut fruits and vegetables. Some of the microbial pathogens associated with fresh vegetables include *Listeria monocytogenes*, *Salmonella spp.*, *Shigella spp.*, enteropathogenic strains of *Escherichia coli*, etc (Shurong *et al.*, 2005 and Horak *et al.*, 2006). The possible sources of contamination in these products involve the incoming raw vegetables, the plant workers, and the processing environment. When vegetables are chopped or shredded, the release of plant cellular fluids provides a nutritive medium in which microorganisms are able to grow (Bandeekar *et al.*, 2003). The high moisture content of fresh vegetables, the lack of lethal process to eliminate microbial pathogens and the potential for temperature abuse during preparation, distribution, storage and handling further intensify the risk of food-borne illnesses. Eliminating the risks is difficult, and managing them is based on identifying and controlling those factors that are important in preventing contamination or limiting growth of pathogenic microorganisms between the farm and processing plant (Ramamurthy *et al.*, 2004).

Preserving food through radiation is considered a promising method to enhance the safety of the microbial quality of the food as it prolongs the period of validity. Based on that, the radiation is considered a safe method in preserving food from microbial deterioration and consequently prolonging its marketing period (WHO, 2000 and Haruvy and Deschenes 2003). Irradiation has the potential to enhance food safety for both fresh foods that will be consumed raw and for raw foods that will be further processed. However, exposing food material to radiation 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. In addition, irradiation is a direct, simple and efficient one-time process. Irradiation treatment combined with proper refrigeration for storage can prolong the shelf-life of these food items without affecting the flavor and texture (Manrique *et al.*, 2005 and Shurong *et al.*, 2005).

Researches indicate that medium radiation treatments 1-10 KGy helps in disposal of deterioration and eliminate microbial yeasts and fungi (Clivir 2002). Low-dose irradiation can reduce or totally eliminate the majority of spoilage organisms in foods such as vegetables and fruits and other easily spoiling foods like meat and poultry (Monk *et al.*, 1995).

Mints and other raw fresh herbs are widely used for flavoring as well as garnish in a variety of dishes without further cooking. However, mint is considered as one of the high-risk herbs when it comes to microbial contamination (Hsu *et al.*, 2010).

So that, the aim of this research is to study the effect of gamma rays at dose rates of 0.5, 0.1, 1.5 KGy on the antioxidants activity, total phenols, total flavonoids, free radical scavenging activity, microbial quality and sensory evaluation of fresh mint leaves.

Materials And Methods

Mint samples:

The samples of fresh mint were purchased from a local market in Riyadh, Saudi Arabia. Then, the mint leaves were washed and dried well by exposing them to air and then placed in polyethylene bags (250 grams in each bag). The bags were divided into groups to conduct chemical analysis, microbial qualities and sensory tests (five replicates for each group).

Irradiation process:

Irradiation process was achieved using cobalt -60 at gamma call-220 at King Abudl Aziz City for Science and Technology (KACST) in Riyadh. The dose rate was 14.2514 KGy/h at the time of experiment. The mint samples were exposed to different doses of gamma radiation 0.5, 1.0, 1.5k Gy in addition to control which was not treated.

Preparing of mint extract samples:

30 grams of fresh mint leaves which exposed to radiation in various doses were extracted by mixing them with distilled water and stirring and turning for 15 minutes, then separated in Centrifugal Concentrators for 10 minutes (1000×g). Afterward, they were re-extracted several times, and kept as aqueous extract to make other tests. After filtering and extraction, a 110 ml were obtained and five replicates have been made after each test analysis (Pellegrini *et al.*, 2003).

Total antioxidants assay:

The antioxidant content has been estimated as an equivalent to Quercetin according to Meda *et al.*, 2005. 0.75 ml of aqueous extract was added to 1.5 ml of 1,1,-diphenyl-2-picryl-hydrazil (DPPH) solution in alcohol methanol by 0.02 mg/ml concentration. Then, leaving the mixture at the room temperature for 15 minutes, and reading the absorbent degree level through using the spectrophotometer device by the wavelength of 517 nm and using 0.75 ml of water with 1.5 ml of alcohol methanol as a blank. Results have been compared with similar cases when using the Quercetin 6 mg/ml concentration. IC50 value was determined in which the effective concentration of the antioxidant activity was 50% as the (DPPH) radical was scavenged by 50%.

Total phenolic assay:

Folin-Ciocalteu's phenol reagent has been used in the same way demonstrated by Negi *et al.*, 2003 where 0.2 ml of the previously prepared aqueous extract of mint leaves have been added to 1 ml of Folin-Ciocalteu's reagent (0.2), then (0.8ml) of Na₂CO₃ solution (7.5%) was added and left at room temperature for 30 minutes, and later reading the absorbent degree by the spectrophotometer device at wavelength of 765 nm and using the water as a blank. The phenols have been estimated as Catechin equivalents by using the Catechine in concentration of 0.5 mg/ml.

Total flavonoids assay:

This is accomplished by using the method used by Meda *et al.*, 2005. Five ml of aluminum chloride solution has been added in methanol by 2%, leaving the mixture for 10 minutes, and then reading the absorbent degree at wave length of 415 nm. 5 ml of the extraction with 5ml of methanol is used as a blank. Results have been compared with similar cases using Quercetin by 6.25 mg/ml concentration.

Free radical scavenging activity:

This was accomplished by applying the method used by Meda *et al.* (2005) with some modification through using DPPH substance. The water extraction was added to mint by using different concentrations to a known amount of the DPPH 0.75 ml of the aqueous extract and 1.5 of the DPPH substance is added in the methanol (by 0.12 mg/ml concentration) in a way that the overall concentration DPPH is 0.2 mmol. After adding the aqueous extract to the DPPH, it was left for 15 minutes at room temperature. Measuring the degree of absorbent by wavelength of 517 nm with the spectrophotometer device and using 0.75 ml of distilled water with 1.5 ml of the DPPH solution as a blank. The activity was calculated according to the formula: Inhibition % = [(AC- AS) / AC] x 100

Where AC is the absorbance value of the control and AS is the absorbance value of the test solution.

Microbial content assay:

According to the A.P.H.A, 1985 method, microbial content of the mint samples was evaluated through total plat count (TPC) of the microbial content of bacteria, yeasts, and fungi. The estimation was done by taking 10 gm of mint and applying 90 ml of sterilized physiological substance (saline) to obtain a dilution of 1/10. The required dilution was prepared and the AJAR Media culture was prepared as following: agar (15g), Trypnone (5g), dextrose as glucose (1g) and yeast extract (2.5g). The pH value was 7 ± 0.2 . The AJAR Media are placed in Petri dishes which have been prepared in advance, then sterilized and incubated at degrees of 35°C for 48 hours. Five replicates after each test analysis was made and the total count was calculated for each (1 g) of the samples of radiated and non-radiated mint.

Sensory evaluation:

Organoleptic Test: The mint was submitted to 10 panelists for evaluation. The ranking method was used in combination with scoring based on the hedonic scale with 9 scores (1 = dislike extremely but 9 = like extremely). The results were analyzed using analysis of variance (WHO 1999 and Resurreccion *et al.*, 1995).

Statistical analysis:

The experimental data were subjected to analysis of variance for the completely randomized block design that was used. Averages and least significant differences were calculated using the SAS system version 9.1.3. (cary, NC). Results were expressed as mean \pm SD (standard deviation). The P value of <0.05 was considered significant (Ott, 1984).

Results And Discussion

The effects of γ -irradiation and the total antioxidant, total phenols and total flavonoids are shown in table 1. It showed a significant increase in total antioxidant contents recording 80.06 ± 1.66 , 83.53 ± 2.03 , 81.25 ± 1.60 mg/100g for irradiated mint at 0.5, 1.0 and 1.5 kGy, respectively, compared with 55.65 ± 1.17 mg/100g of the non-irradiated control. It was obvious that 1.0 kGy irradiation dose led to the highest increase in the total antioxidant. The data demonstrated that the irradiated mint at 0.5, 1.0, 1.5 KGy had higher phenolic compounds than non-irradiated control in the mint extract. The significant increases in the phenolic contents were 165.06 ± 1.90 , 208.20 ± 1.74 and 184.10 ± 2.15 mg/100g for samples irradiated at 0.5, 1.0 and 1.5 kGy, respectively, compared to the content in case of non-irradiated control (124.18 ± 2.58 mg/100g). The total flavonoids contents were increased significantly by 1.95 ± 0.14 , 2.81 ± 0.82 and 3.15 ± 0.63 mg/100g for mint irradiated at 0.5, 1.0 and 1.5 KGy, respectively when compared to their level in non-irradiated sample (1.83 ± 0.24 mg/100g). The maximum increase was obtained at 1.5 kGy dose.

It is known that antioxidants are any substance that can prevent or delay the oxidation. Phenols are found in food either freely or as glycosides. There are more than 8000 kinds of natural phenols that are known to be in plants. Phenols exist in food are divided into two main divisions, aromatic phenols and

Table 1: Mean \pm SD of contents of total antioxidant, total phenols and total flavonoids of fresh mint leaves irradiated with various doses of γ -irradiation.

| Radiation dose (kGy) | Total antioxidant (mg / 100g) | Total phenols (mg / 100g) | Total flavonoids (mg / 100g) |
|----------------------|-------------------------------|---------------------------|------------------------------|
| Control | 55.65 ± 1.17^b | 124.18 ± 2.58^b | 1.83 ± 0.24^c |
| 0.5 | 80.06 ± 1.66^a | 165.06 ± 1.90^a | 1.95 ± 0.14^b |
| 1.0 | 83.53 ± 2.03^a | 208.20 ± 1.74^a | 2.81 ± 0.82^a |
| 1.5 | 81.25 ± 1.60^a | 184.10 ± 2.15^a | 3.15 ± 0.63^b |
| LSD | 1.61 | 2.11 | 0.45 |

Values having different letters in the same column are significantly different ($P < .05$).

flavonoids (Halliwell and Gutteridge 1999 and Kim and Lee 2004). The multi-phenols exist in foods have a major role in protection from several diseases. This could be attributed to their antioxidant features. Flavonoids are vegetables dyes spread in different parts of the plants. They are divided into several various divisions that include Flavones. More precisely, Flavones are considered to be compounds that their color range varies from colorless to light yellow, and can be found in citrus, cumin, and mint (Mazur *et al.*, 1996 and Alessandro *et al.*, 2003).

The present findings are in good agreement with that of Taipina *et al.*, (2009) who reported that there was no loss of the antioxidants content of pecan nuts as a result of gamma irradiation while Huang and Mau (2006)

found that antioxidant content of the methanolic extract of *Agaricus blazei* was 45 mg/100g and this amount was increased to 106 mg/100g after 20 kGy of γ -irradiation. On the contrary, Ahn *et al.*, 2005 found that gamma irradiation significantly reduced the phenolic contents in Chinese cabbage. A study done by De Oliveira *et al.*, 2009 on soybeans showed no loss of flavonoids as a consequence of radiation treatment up to 10 KGy irradiation dose, while at a dose of 10 KGy there was an increase in the flavonoids content. Recently, Zhu *et al.*, 2010 reported that the 2 kGy irradiation dose, applied to rice grains, reduced the flavonoid content and the maximum loss was noticed at the 10 kGy irradiation dose. This could be attributed to the effect of the γ -irradiation which might induce chemical reaction in the components of mint by degrading and decomposing the large molecules into small molecules readily soluble in extract. This data is in agreement with the studies done by Huang and Mau (2006) and Hayat *et al.* (2012).

The DPPH radical scavenging activity and IC₅₀ for the aqueous extract of irradiated mint are shown in table 2. The results indicated that the DPPH radical scavenging activity of mint aqueous extracts for irradiated sample at 0.5, 1.0, and 1.5 kGy were found to be 64.87 ± 7.53 , 66.62 ± 6.96 and 65.91 ± 7.69 %, respectively less than the non-irradiated control (70.63 ± 9.03). In contrast, IC₅₀ values of aqueous extracts for mint irradiated at the above mentioned applied doses were increased 45.03 ± 1.86 , 48.80 ± 2.53 and 41.09 ± 1.02 mg/ml compared to control (30.53 ± 1.19 mg/ml).

Murakami *et al.*, (2002) found that the DPPH radical scavenging activity of soybean receiving doses ranging from 0.5 – 5 KGy of γ -irradiation against DPPH radicals was increased. In another study, done on the green tea leaf extracts with 10 and 20 kGy of irradiation, and rosemary leaves powder extract exposed to 30 kGy of irradiation, it showed a significant increase in the scavenging ability against DPPH radicals (Jo *et al.*, 2003).

Table 2: Mean \pm SD of DPPH scavenging activity and IC₅₀ values of fresh mint leaves irradiated with various doses of γ -irradiation.

| Radiation dose (kGy) | DPPH scavenging activity % | IC ₅₀ (mg/ml) |
|----------------------|----------------------------|--------------------------|
| Control | $70.63 \pm 9.03a$ | $30.53 \pm 1.19c$ |
| 0.5 | $64.87 \pm 7.53 b c$ | $45.03 \pm 1.86a$ |
| 1.0 | $66.62 \pm 6.96c$ | $48.80 \pm 2.53a$ |
| 1.5 | $65.91 \pm 7.69b$ | $41.09 \pm 1.02b$ |
| LSD | 7.80 | 2.50 |

Values having different letters in the same column are significantly different ($P < 0.05$).

The results of microbiological aspects are shown in table (3). The difference between irradiated units and non-irradiated (control) ones indicated is that most microbial counts were higher for fresh samples (control) and lowest for irradiated ones. The use of irradiation treatment might affect the microbial counts. It was noticed that gamma irradiation caused a great reduction in all tested microorganisms and this reduction was proportional to irradiation doses. The lowest irradiation dose of 0.5 kGy decreased the total aerobic bacterial counts of fresh mint by 9.5×10^4 , whereas, it decreased the total aerobic bacterial counts at the dose of 1.0 and 1.5 kGy to < 10 . Fresh mint leaves irradiated with various doses of γ -irradiation showed a great reduction in total yeasts and molds count that reached to < 10 .

Table 3: Mean \pm SD of microbiological quality of fresh mint leaves irradiated with various doses of γ -irradiation.

| Radiation Dose (kGy) | Total Aerobic Count | Total yeasts & molds count |
|----------------------|---------------------|----------------------------|
| Control | 3.2×10^7 | 2.1×10^3 |
| 0.5 | 9.5×10^4 | < 10 |
| 1.0 | < 10 | < 10 |
| 1.5 | < 10 | < 10 |

The higher reduction in total aerobic bacterial counts of mint samples might be due to the direct effect of radiation as well as the indirect effect resulting from radiolysis which is greater in fresh samples than irradiated one. Low-dose irradiation was found to be inactivating as *Listeria monocytogenes* and total aerobic count on broccoli, cabbage, tomatoes, bean sprouts, and celery (Prakash *et al.*, 2000, Fan *et al.*, 2003 and Bari *et al.*, 2005); *Salmonella* on radish and bean sprouts and minimally processed pineapple (Shashidhar *et al.*, 2007); *Listeria* and *Yersinia* on minimally processed capsicum, fresh coriander leaves and sliced carrots (Ramamurthy *et al.*, 2004 and Kamat *et al.*, 2005) and viable *E. coli* 0157:H7 internalized in fresh lettuce leaves and baby spinach (Niemira 2008). Irradiation at doses of 1 to 2 kGy has been found to achieve a 5-log reduction of pathogenic bacteria and prolong the shelf life of fresh product without compromising its sensory attributes (Foley *et al.*, 2004 and Bari *et al.*, 2005). Another study in Northern India revealed the incidence of multidrug resistant of *Salmonella* in coriander and mint; 3 and 24 of tested coriander and mint respectively were found to be positive for *Salmonella* (Singh *et al.*, 2007).

Irradiation is used to improve the microbiological safety of these foods. The irradiation showed to be a feasible process because the doses necessary to ensure good microbiological quality did not change the overall quality of the vegetables and fruits. There was an increase in the shelf life of the irradiated when compared to the non-irradiated food (Bandekar *et al.*, 2003 and Landgraf *et al.*, 2006).

The sensory tests (appearance, color, odor, taste, texture and quality) were scored by the trained panelists on mint irradiation. The score of hedonic scale test were analyzed by analysis of variance as shown in table 4. The data showed that doses of 0.5 and 1.0 didn't affect the mint attributes. However, a significant decrease in the appearance, color and texture with the dose of 1.5 KGy was observed compared with control. Therefore, beyond this dose of irradiation, treatment may not be suitable for mint extract.

These results were agreed with Niemira et al.,(2002) who indicated that treatments up to 0.5 kGy did not change the texture of different types of iceberg lettuce. Also, studies by Hagenmaier and Baker (1997), Gunes *et al.*, (2001) and Prakash *et al.*, 2002 demonstrated that doses of 0.5 kGy did not induce alterations on visual attributes or softening in iceberg lettuce but some changes in the texture of some vegetables and fruits exposed to 0.8kGy. Some studies have shown that Irradiation could accelerate ripening manifested in the loss of chlorophyll and the damage of essential enzyme systems in some vegetables (Gnanasekharan *et al.*, 1992). Gamma radiation could cause injury to succulent vegetables which are sensitive to irradiation (Bandeekar *et al.*, 2003 and Suchada *et al.*, 2005).

Table 4: Mean \pm SD of sensory evaluation of fresh mint leaves irradiated with various doses of γ -irradiation.

| Dose(kGy) | Appearance | Color | Odor | Taste | Texture | Quality |
|-----------|------------------------------|-------------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------|
| Control | 9.0 \pm 0.20 ^a | 9.0 \pm 1.01 ^a | 9.0 \pm 1.31 ^a | 9.0 \pm 1.41 ^a | 9.0 \pm 1.10 ^a | 9.0 \pm 1.20 ^a |
| 0.5 | 9.0 \pm 0.10 ^a | 8.25 \pm 1.60 ^{ab} | 8.95 \pm 1.10 ^{ab} | 8.50 \pm 1.80 ^a | 8.0 \pm 1.60 ^{ab} | 8.50 \pm 1.22 ^{ab} |
| 1.0 | 9.0 \pm 1.20 ^a | 8.50 \pm 1.20 ^{ab} | 8.75 \pm 1.70 ^{ab} | 8.95 \pm 1.20 ^a | 8.50 \pm 1.60 ^{ab} | 8.75 \pm 1.18 ^{ab} |
| 1.5 | 8.75 \pm 1.01 ^b | 8.0 \pm 1.80 ^b | 8.50 \pm 1.30 ^{ab} | 8.25 \pm 1.50 ^a | 7.50 \pm 2.30 ^b | 8.25 \pm 1.58 ^{ab} |
| LSD | 0.55 | 1.15 | 1.07 | 1.25 | 1.25 | 0.99 |

Values having different letters in the same column are significantly different ($P < .05$).

Conclusion:

To sum up, radiation processing increased the total antioxidants, total phenols and total flavonoids of fresh mint leaves. Also, the evaluation of the antioxidant activity using the DPPH radical-scavenging-activity indicated some losses of the antioxidant activities in irradiated samples. In addition, Gamma irradiation caused a great reduction in all tested microorganisms and this reduction was proportional with irradiation dose. Generally, the results of this study indicate that the use of irradiation is a suitable method for food preservation, especially the sensory qualities which are not affected by any radiation dose treatment with the exception of sensory evaluation which were affected at dose level of 1.5 KGy.

Recommendation:

Accordingly, the present study recommends utilizing γ -irradiation for mint preservation and other green vegetables. Further, work is needed to evaluate the in vivo assays after feeding the experimental animals on the irradiated stuff.

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