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The effects of Gamma irradiation on the Microbiological quality, Sensory evaluation and Antioxidant activity of Spinach

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Abstract : This research was conducted to study the effect of transactions radiation on the assessment of microbial quality, sensory evaluation and antioxidant activity of Spinach. Spinach was treated by using three doses of gamma radiation, 0.5, 1.0, 1.5 and 2.0KGy. The results of microbial quality revealed that radioactive transactions have led to a significant reduction (indicate level of significant p< o.o5) in E.coli, total number of Bacteria, yeasts and fungi. However, a significant decrease (p< 0.) in the appearance, color and texture with the dose of 1.5, 2.0 KGy was observed compared with control. The antioxidant activity, total phenols and total flavonoids of aqueous extract of Spinach have been evaluated. The results showed a high antioxidant activity when radioactive transactions gave a significant increase (p< 0.0) in the content of total phenols and total flavonoids while the value decreased of the samples treated with 1.5, 2.0 KGy. The evaluation of antioxidant activity of aqueous extracts of Spinach using DPPH gave a significant decrease (p< 0.0) in free radical scavenging activity of the samples treated with radiation compared to untreated samples.
Keywords: Radiation, Spinach, Microbial quality, Sensory evaluation, Antioxidants activity.

Introduction

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[1,2]. 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[3,4]

Pathogenic bacteria internalized in leaf tissues are not effectively removed by surface treatments but it has been shown that irradiation can inactivate such bacteria. [5]investigated the relative efficacy of a sodium hypochlorite wash versus irradiation to inactivate E. coli 0157:1-17 internalized in leaves of romaine lettuce and baby spinach. A cocktail mixture of three isolates of E. coli 0157:147 were drawn into the leaves after which the leaves were then washed with a sodium hypochlorite sanitizing solution or treated with ionizing radiation (0.25-1.5 kGy). Results showed that treatment of the leaves with irradiation (but not the chemical sanitizers) effectively reduced viable E. colt 0157:117 cells internalized in the leafy green vegetables in a dose-dependent manner. A more complex response to irradiation was observed in the spinach leaves than in romaine lettuce leaves, with a marked tailing effect in spinach at higher doses as compared with a linear response in the lettuce. The specific doses to be used should be determined for each product based on the patterns of antimicrobial efficacy and specific product sensory responses[6]. Overall, irradiation at doses of 1 and 2 kGy may be employed to enhance microbial safety of fresh-cut Iceberg lettuce and spinach while maintaining quality[7]

Generally, Spinach and other raw fresh herbs are widely used for flavoring as well as garnish in a variety of dishes without further cooking. However, Spinach is considered as one of the high-risk herbs when it comes to microbial contamination[8]. Gamma irradiation is effective in eliminating E.Coli bacteria from infected Spinach leaves, and extending the shelf-life of non-contaminated spinach leaves. However, it can adversely affect Spinach leaf smell, texture, and appearance, if the dose level is high[9].

The Food and Drug Administration is amending the food-additive regulations to provide what it calls the safe use of ionizing radiation for just the two leafy greens which are lettuces and Spinaches. Therefore, FDA (2008) indicated that consumption of irradiated spinach and iceberg lettuce is not harmful to human health. Some consumers have raised concerns about the effects of irradiation on food in terms of taste and nutritive value. But the studies conducted found that irradiation does impact vitamin A and folate levels in spinach, but it does not affect nutrients overall[10,8].

Thus, the aim of this research was to study the effect of gamma rays at dose rates of 0.5 , 0.1 , 1.5 and 2.0KGy on the microbiological quality , sensory evaluation and antioxidants activity , total phenols , total flavonoids, free radical scavenging activity of Spinach.

Materials and Methods

Spinach samples:

The samples of fresh Spinach were purchased from a local market in Riyadh, Saudi Arabia .Then, the Spinach 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 be subjected to chemical analysis, microbial qualities and sensory tests (five replicates for each group).

Irradiation process:

Irradiation process was conducted using Cobalt -60 at gamma call-220 at King Abudlaziz City for Science and Technology (KACST) in Riyadh. The dose rate was 14.2514 KGy/h at the time of experiment. The spinach samples were exposed to different doses of gamma radiation 0.5, 1.0, 1.5 and 2,0 kGy in addition to control (non-irradiated Spinach)

Microbial content assay

According to the[11] method, microbial content of the Spinach samples was evaluated through total plat count (TPC) of the microbial content of E.Coli , bacteria, yeasts, and fungi. The estimation was done by taking 10 gm of Spinach 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), Trypone (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 35ºC 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-irradiated Spinach**.**

Sensory evaluation

Organoleptic Test: The Spinach samples were subjected to sensory evaluation by 10 panelists. 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[12,13].

Chemical analysis

Preparing of spinach extract samples:

Thirty grams of fresh Spinach leaves which exposed to radiation in various doses and 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, the samples 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[14].

Total antioxidants assay:

The antioxidant content has been estimated as an equivalent to Quercetin according to [15]. The 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 at 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. The 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 as demonstrated by[16] where 0.2 ml of the previously prepared aqueous extract of Spinach leaves have been added to 1 ml of Folin-Ciocalteu's reagent (0.2), then (0.8ml) of Na_2CO_3 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 reported by[15]. 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[15] with some modification through using DPPH substance. The water extraction was added to spinach 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 following equation: % 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.

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 ± SD (standard deviation(. The P value of <0.05 was considered significant[17].

Results and Discussion

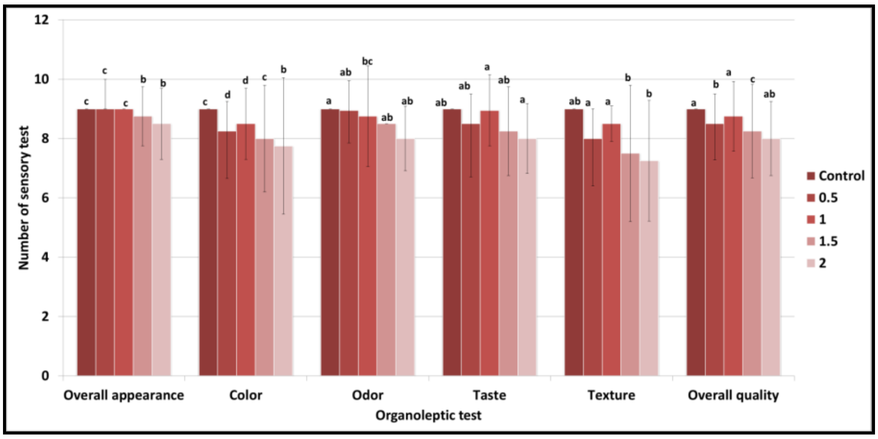
The effects of γ-irradiation with various doses on microbiological aspects of Spinach are shown in table (1). The difference between irradiated units and non-irradiated (control) one indicated that most microbial counts were higher for fresh samples (control) and lowest for irradiated ones. The use of 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 E. coli O15:H7 and total aerobic bacterial counts of fresh Spinach by 2.5×10 and 6.1×10^3, whereas, it decreased the E. coli O15:H7 and total aerobic bacterial counts at the dose of 1.0, 1.5 and 2.0kGy to < 10. Fresh Spinach leaves irradiated with various doses of γ-irradiation showed a great reduction in total yeasts and molds count that reached to < 10.

Table – 1. : Microbiological quality of Spinach irradiated with various doses of γ-irradiation.

Radiation Dose (kGy)	E.coli	Total Aerobic Count	Total yeasts & molds count
Control	**3.1×10^5**	**4.2×10^6**	**3.2×10^3**
0.5	**2.5×10**	**6.1×10^3**	**< 10**
1.0	**< 10**	**< 10**	**< 10**
1.5	**< 10**	**< 10**	**< 10**
2.0	**< 10**	**< 10**	**< 10**

The higher reduction in total aerobic bacterial counts of Spinach 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 [18,19,20] *Salmonella* on radish and bean sprouts and minimally processed pineapple [21]*Listeria* and *Yersinia* on minimally processed capsicum, fresh coriander leaves and sliced carrots[22,23] and viable *E coli* 0157:H7 internalized in fresh lettuce leaves and baby Spinach [24] 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[25,20]. 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*[26].[29, 30] showed that Spinach requires a dose of >1.06 kGy to achieve undetectable levels of E. coli O15:H7 [27]but a dose of 1.0 kGy immediately decreases ascorbic acid levels (vitamin C) by as much as 25% [28] 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[29,30].

The sensory tests (appearance, color, odor, taste, texture and quality) were scored by the trained panelists on Spinach irradiation. The score of hedonic scale test were analyzed by analysis of variance as shown in figure1. The data showed that doses of 0.5 and 1.0 didn't affect the Spinach attributes. However, a significant decrease ($p< 0.05$) in the appearance, color and texture with the dose of 1.5and 2.0 KGy was observed compared with control. Therefore, beyond this dose of irradiation, treatment may not be suitable for Spinach extract.

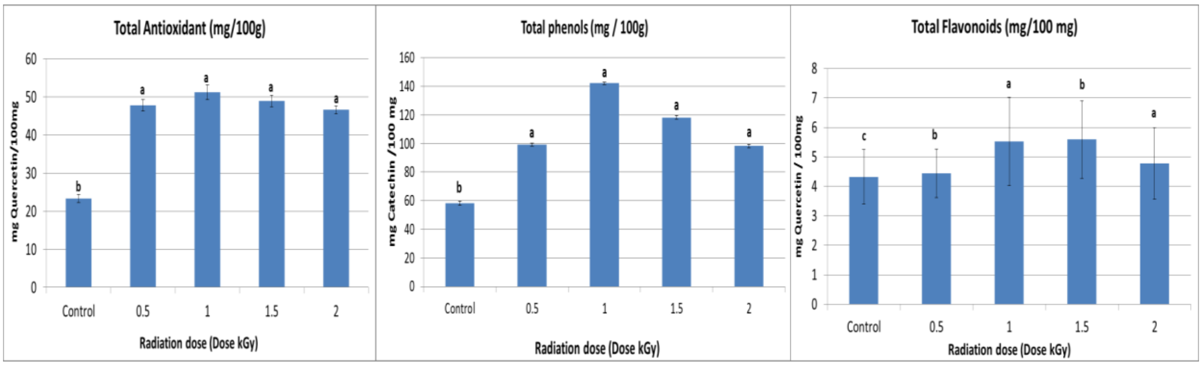


All determinations are mean of 5 samples; results were expressed as mean ± SD (standard deviation . (Values having different letters in the same column are significantly different (P < .05).

Figure – 1 : Sensory evaluation of Spinach irradiated with various doses of γ-irradiation.

These results were agreed with [9]who indicated that treatments up to 0.5 KGy did not change the texture of different types of Spinach. Also, studies by [31,32] 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[33]. Gamma radiation could cause injury to succulent vegetables which are sensitive to irradiation[29,34].

The effects of γ-irradiation and the total antioxidant, total phenols and total flavonoids are shown in figure 2. It showed a significant (p< o.o5) increase in total antioxidant contents recording47.73±1.55, 51.20±1.92, 48.92±1.49, 46.54± 1.4 mg/100g for irradiated Spinach at 0.5, 1.0 ,1.5 and 2.0 KGy, respectively, compared with 23.32 ± 1.06mg/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 Spinach at 0. 5, 1.0, 1.5 and 2.0 KGy had higher phenolic compounds than non-irradiated control in the Spinach extract. The significant increases (p<0.05) in the phenolic contents were 99.17 ± 0.99, 142.19 ± 0.83 ,118.09 ± 1.24 and 98.20 ± 1.30 mg/100g for samples irradiated at 0.5, 1.0, 1.5 and 2.0 KGy, respectively, compared to the content in case of non-irradiated control (58.17 ± 1.47 mg/100g). The total flavonoids contents were increased significantly (p<0.05) by 4.44 ± 0.82, 5.25 ± 1.50, 5.59 ± 1.31 and 4.78 ± 1.21 mg/100g for Spinach irradiated at 0.5, 1.0, 1.5 and 2.0 KGy, respectively, when compared to their level in non-irradiated sample (4. 32 ± 0.92mg/100g). The maximum increase was obtained at 1.5 and 2.0 KGy dose.

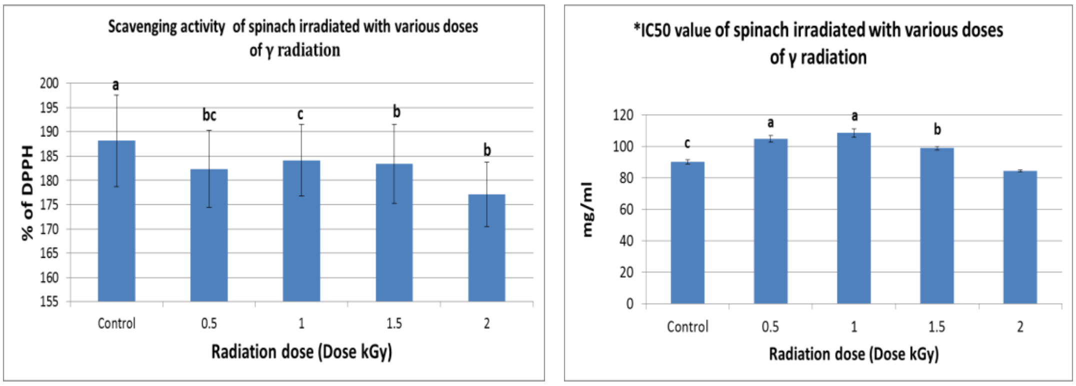


All determinations are mean of 5 samples; results were expressed as mean ± SD (standard deviation . (Values having different letters in the same column are significantly different (P < .05).

Figure – 2 : Contents of Total antioxidant, Total phenols and Total flavonoids of Spinach irradiated with various doses of γ-irradiation.

The present findings are in good agreement with [35] that of the antioxidants content in spinach there was no change in concentration with increasing doses (0.0, 0.5, 1.0, 1.5, 2.0 kGy) of irradiation while, [36] who reported that there was no loss of the antioxidants content of pecan nuts as a result of gamma irradiation. On the contrary [37] found that gamme irradiation significantly (p<0. 0.05) reduced the phenolic contents in Chinese cabbage. A study done by[38] on soybeans showed no loss of flavonoids as a consequence of radiation treatment up to 10 KGy irradiation dose. Recently [39]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 Spinach by degrading and decomposing the large molecules into small molecules readily soluble in extract. This data is in agreement with the studies done by[10,40].

The DPPH radical scavenging activity and IC_{50} for the aqueous extract of irradiated Spinach are shown in figure3. The results indicated that the DPPH radical scavenging activity of Spinach aqueous extracts for irradiated sample at 0.5, 1.0, ,1.5 and 2.0 kGy were found to be 182.39 ± 7.95, 184.14 ± 7.38 ,183.43 ± 8.11 and 177.08 ± 6.61 %, respectively, which were found to be less than of non-irradiated control (188.15 ± 9.45). In contrast, IC_{50} values of aqueous extracts for Spinach irradiated at the above mentioned applied doses were increased 104.76 ± 2.03, 108.53 ± 2.70 ,98.82 ± 1.19 and 84.32 ± 0.52 mg/ml compared to control (90.26 ± 1.36 mg/ml). [41]found that the DPPH radical scavenging activity of soybean receiving doses ranging from of 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, showed a significant increase (P< 0.05) in the scavenging ability against DPPH radicals [42,43].



***IC50 value : the effective concentration at which the antioxidant activity was 50%; the (DPPH) radical was scavenged by 50%. All determinations are mean of 5 samples, results were expressed as mean ± SD (standard deviation(.Values having different letters in the same column are significantly different (P < 0.05).**

Figure – 3 : Scavenging activity and IC50 values of Spinach irradiated with various doses of γ-irradiation.

Conclusion

To sum up, that radioactive transactions had led to a significant reduction (p<0.05) in E.coli, total number of bacteria, yeasts and fungi. However, a significant decrease in the appearance, color and texture was detected with the dose of 1.5, 2.0 KGy compared with control. In addition, irradiation processing increased the total antioxidants, total phenols and total flavonoids of fresh Spinach. Also, the evaluation of the antioxidant activity using the DPPH radical-scavenging-activity indicated some losses of the antioxidant activities in irradiated samples. Generally, the results of this study indicated that the use of irradiation is a suitable method for controlling microbial contamination , however suitable dose should be used to prevent the undesirable changes in sensory quality of the end product.

Recommendation

Accordingly, the present study recommends utilizing γ-irradiation (at dose ranged from 1.5 to 2.0 KGy) for Spinach 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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