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Gamma radiation processing under modified atmosphere packaging effects on microbial quality and antioxidant activity of fresh leafy vegetables during storage

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ABSTRACT

Fresh leafy vegetables are great source of vital nutrients, to promote health and prevent diseases but they can transmit pathogenic microorganisms to human beings. Shelf life of these products is very limited post harvest and after three days at refrigeration temperature, they will spoil. Radiation processing combined with modified atmosphere packaging and refrigeration temperature is a practical treatment to ensure safety and enhance vegetables shelf life even to be used in international trades. The effects of irradiation doses at 0, 0.25, 0.5 and 1 kGy on fresh leafy vegetables packed under air, N2 and vacuum atmospheres up to10 days of storage at 4 °C were studied. According to the results of microbial tests, and antioxidant activity of DPPH°, gamma radiation at dose of 0.5 kGy under N2 packing atmosphere are recommended as optimal storage conditions up to 10 days at 4 °C for fresh garlic chives, basil, mint and parsley.

***Keywords*:** Gamma, Modified atmosphere packaging (MAP), Fresh leafy vegetables, Microbial safety, Antioxidant.

I. Introductions

The waste of vegetables is more than 27% of production in Iran. Therefore, by using appropriate processing, in addition to supply fruits and vegetables in the domestic market and reduce its waste, it is possible to have exports into international markets [1]. Pathogens such as Escherichia coli are the main causes of foodborne diseases. Modified atmosphere packaging is a technology to control the microflora of products with minimal processing. However, it is not effective enough [2]. No single treatment can significantly reduce the number of microorganisms in raw vegetable. The microbial population after washing with disinfectants increased faster than the population of water-washed products during long-term storage. Concerns about the toxicity of chlorinated products formed in the presence of organic matter lead to a continuous search for more effective and safer disinfectant methods [3]. Gamma ionizing radiation processing as an alternative treatment is a well- known physical and non-thermal method of food preservation. Food irradiation causes minor changes in the flavor, color, nutrients, taste and other quality characteristics of food. It has been reported that under certain conditions, the concentration of phytochemicals in plants may increase after irradiation [4]. Moderate radiation treatments at 1-10 kGy accompanied by refrigeration storage can

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extend the shelf life of fresh vegetables with no adverse effect on their taste and texture [5]. Modified atmosphere packaging affects the pathogen's behaviour towards the beam and reduces the beam sensitivity of fresh produce regardless of tissue damage. Therefore, combination treatments can reduce the required dose to control the microorganisms and preserve production nutritional quality [6]. In this study, due to the importance of hurdle technology to reduce the required dose, the combination of radiation processing, modified atmosphere packaging and storage at 4 °C has been used.

II. Experimental

A. Preparation of the materials

Vegetables (garlic chives, basil, mint and parsley) were cleaned and then washed three times with tap water and excess water was removed. Different types of vegetables were packed separately in bags made of food grade low density polyethylene polymer under modified atmosphere (air, vacuum and N_2). Packages were irradiated by 60Co, in Gamacell 220 (Nordion, Canada) at doses of 0 (control), 0.25, 0.5 and 1 kGy at room temperature and a dose rate of 1.98 Gy/s [7]. Gamma cell was calibrated by using ferric dosimeter. Samples were stored at 4 °C up to 10 days.

B. Microbial analysis

Aerobic Mesophilic total count [8], spore forming bacteria count [9], total yeast/mold count [10], coliforms count [11], Staphylococcus aureus [12,13] and Escherichia coli presence/absence [14] were evaluated in triplicate. Next, the appropriate plates containing 30–300 bacterial colonies and 15–150 fungal colonies, respectively were selected and counted [15]. The sample microbial contamination was calculated and reported in terms of cfu/g.

C. Determination of DPPH° scavenging activity

In this test, the lipophilic DPPH◦ reacts with the hydrogen donator antioxidants and it will be reduced. To perform, 50 µl of different concentrations of the sample was added to 5 ml of 0.004% DPPH solution in methanol. After 30 minutes of storage at room temperature, the absorption of sample was read at 517 nm against the methanol as a blank. The percentage of free radical scavenging was calculated using Equation 1, where, A is absorption of samples [16]:

Eq. 1.

D. Statistical analysis

Duncan's Multiple Range Test (DMRT) was used to compare the means ($p<0.05$) using the SPSS (ver. 19) software and subjected to two-way analysis of variance (ANOVA). All measurements were performed in triplicate. A completely randomized statistical design was used. The interaction of independent test variables was determined by SAS software (ver. 9.1) and Minitab 17. The graphs were drawn using Excel software.

III. Results and Discussion

A. Microbial contamination assessment

The results of analysis of variance showed that the main effects including irradiation dose, storage time and packaging atmosphere and dual interaction of radiation dose × packaging atmosphere had significant impact on aerobic mesophilic, sporulative, coliform, and staphylococcus counts ($p<0.01$). Other dual and triple (radiation dose × packaging atmosphere × storage time) interactions were not significant ($p>0.01$). Neither main effects nor their interactions were significant on mould/yeast counts, as well. The effects of storage time on microbial contamination of fresh vegetables are presented in Table 1.

The effects of storage time on microbial contamination (Table 1) showed that storage time has significant impact on microbial decontamination of the samples ($p<0.05$). In garlic chives, basil, min and parsley at the end of storage (10th day) the microbial counts increased, significantly. However, on the other storage times (0, 3, 7) no significant difference ($p\geq0.05$) were observed on microbial population.

Microbial contamination of different treatments are represented in Figure 1(A-D). Microbial counts

were the least in 4 types of vegetables at the same irradiation dose under N_2 atmosphere ($p<0.05$). Total aerobic mesophilic, Staphylococcus aureus, coliform counts were decreased in garlic chives, basil, parsley and mint under 3 types of packaging atmosphere by increasing irradiation dose, significantly ($p<0.05$). E. coli was only present in non-irradiated samples under all atmospheres of packaging . The radiation energy breaks the bonds in the DNA molecules of the microbes in the food and defects in the genetic instructions. If this damage is not repairable, the organism will die or will not be able to reproduce [17]. In MAP-packed samples, low concentrations of O2 inside the package inhibit the growth of aerobic microbiota [18]. Contrarily, in garlic chives the spore forming bacteria increased by enhancing irradiation doses, but the difference was not significant among irradiated treatments. It can be attributed to this fact that a great part of garlic chive's stem grows in the soil, which increases the probability of contamination with spore-forming bacteria. Spores especially absolute anaerobic spores such as Clostridium, are constructed in adverse growth conditions such as nutrient deficiencies, improper pH and Eh, radiation and other undesirable factors (absence or at low concentration of oxygen) [19]. Unfavorable growth circumstances of spore-forming bacteria after irradiation leads to spore formation and with increasing radiation dose as the intensity of unfavorable factor, spore formation increases. As a result of anaerobic conditions in vacuum packaging their population will increase and spores will find a greater chance to survive. Finally, it seems that at irradiation dose of 0.5 kGy under N_2 atmosphere the microbial counts were acceptable and were in the food hygiene standards up to 10th day of storage.

Table 1. The effect of storage time on the microbial contamination of samples*

Vegetable	Storage time (day)	Microorganism (cfu/g)			
		Total aerobic bacteria	Spore forming bacteria	*Staphylococcus aureus*	Coliforms
Garlic chives	0	$(111.10\pm60*10^3)^b$	$(38.40\pm5.00*10^3)^b$	$(14.30\pm1.00*10^3)^b$	$(12.40\pm1.00*10^3)^b$
	3	$(110.60\pm5.10*10^3)^b$	$(40.50\pm0.40*10^3)^b$	$(15.60\pm1.1*10^3)^b$	$(11.50\pm0.40*10^3)^b$
	7	$(113.20\pm0.20*10^3)^b$	$(40.80\pm0.80*10^3)^b$	$(18.61\pm1.20*10^3)^b$	$(13.80\pm0.10*10^3)^b$
	10	$(136.70\pm1.20*10^3)^a$	$(52.40\pm0.90*10^3)^a$	$(30.61\pm0.10*10^3)^a$	$(20.40\pm0.01*10^3)^a$
Basil	0	$(116.20\pm5.00*10^3)^b$	$(86.20\pm0.50*10^3)^b$	$(20.20\pm1.30*10^3)^b$	$(8.20\pm0.10*10^3)^b$
	3	$(119.10\pm4.30*10^3)^b$	$(84.50\pm0.10*10^3)^b$	$(20.40\pm0.10*10^3)^b$	$(8.50\pm0.05*10^3)^b$
	7	$(120.70\pm1.40*10^3)^b$	$(83.20\pm0.12*10^3)^b$	$(20.60\pm0.30*10^3)^b$	$(8.20\pm0.50*10^3)^b$
	10	$(129.30\pm5.10*10^3)^a$	$(90.70\pm0.17*10^3)^a$	$(32.70\pm0.20*10^3)^a$	$(9.70\pm0.10*10^3)^a$
Mint	0	$(76.20\pm2.40*10^3)^b$	$(71.50\pm4.00*10^3)^b$	$(6.90\pm0.40*10^3)^b$	$(7.50\pm0.20*10^3)^b$
	3	$(77.10\pm3.90*10^3)^b$	$(70.60\pm1.10*10^3)^b$	$(7.05\pm0.20*10^3)^b$	$(7.60\pm0.06*10^3)^b$
	7	$(78.10\pm7.40*10^3)^b$	$(72.60\pm2.40*10^3)^b$	$(7.07\pm0.13*10^3)^b$	$(7.60\pm0.04*10^3)^b$
	10	$(88.20\pm2.80*10^3)^a$	$(88.40\pm4.50*10^3)^a$	$(8.47\pm0.40*10^3)^a$	$(8.50\pm0.09*10^3)^a$
Parsley	0	$(43.20\pm2.30*10^3)^b$	$(16.40\pm0.10*10^3)^b$	$(1.60\pm0.20*10^3)^b$	$(1.40\pm0.10*10^3)^b$
	3	$(42.10\pm1.90*10^3)^b$	$(15.20\pm1.10*10^3)^b$	$(1.69\pm0.01*10^3)^b$	$(1.45\pm0.07*10^3)^b$
	7	$(45.40\pm2.80*10^3)^b$	$(17.80\pm0.20*10^3)^b$	$(1.75\pm0.04*10^3)^b$	$(1.55\pm0.01*10^3)^b$
	10	$(59.50\pm1.70*10^3)^a$	$(25.70\pm0.03*10^3)^a$	$(4.08\pm0.05*10^3)^a$	$(2.78\pm0.00*10^3)^a$

*Values are mean ± standard deviation (n=3); different lowercase letters in the same column show significant difference by Duncan's Multiple Range Test (at $p<0.05$, each parameter compared separately).

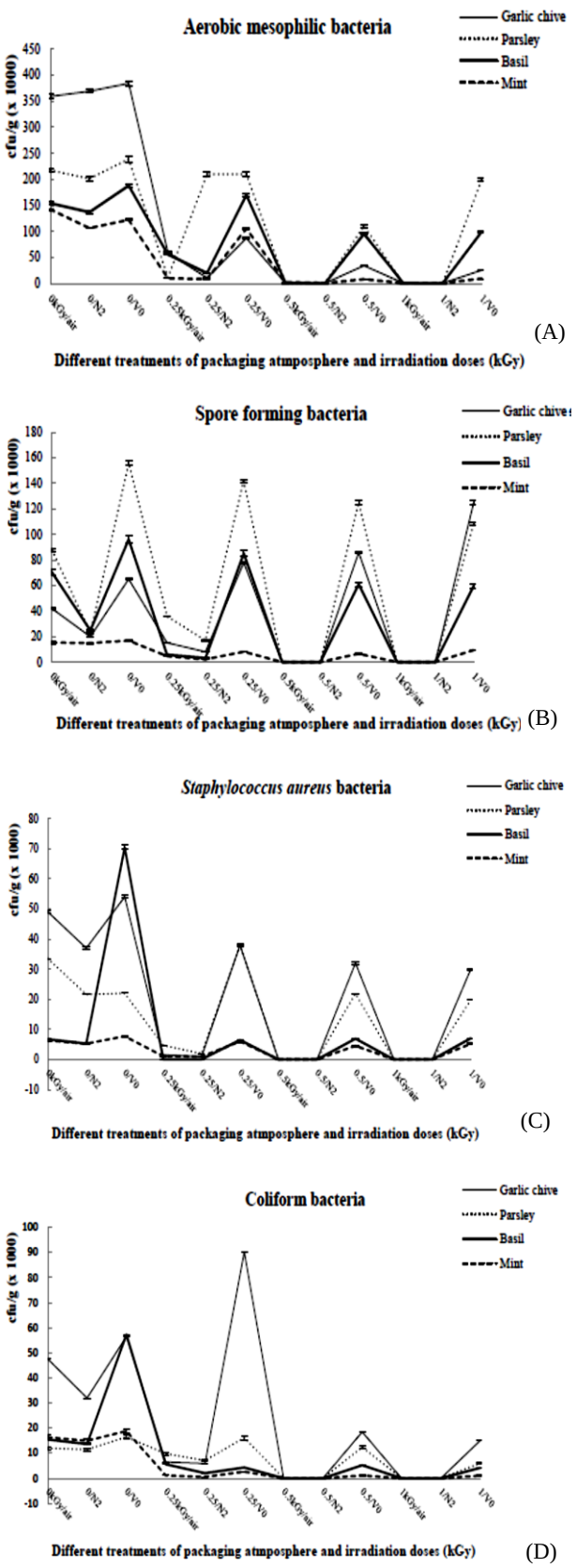

Figure 1. Microbial contamination of non-irradiated and irradiated vegetables (0, 0.25, 0.5 and 1 kGy) under air, vacuum, and N2 atmospheres; **A.** Aerobic mesophilic bacteria, **B.** Spore forming bacteria, **C.** Staphylococcus aureus bacteria, **D.** Coliform bacteria.

B. Determination of DPPH° scavenging activity

According to the results the main effects including irradiation dose, and packaging atmosphere and dual interaction of radiation dose × packaging atmosphere have affected antioxidant activity of garlic chives, parsley and basil, significantly ($p<0.01$). The main effects mentioned above and their double and triple interactions were not significant on DPPH° scavenging activity of mint ($p>0.01$). Considering that only the dual interaction of radiation dose × packaging atmosphere impacts will be illustrated ($p<0.01$). The results are represented in Table 2.

The lowest and the highest antioxidant activity treatments were reported in garlic chives and mint, respectively. The antioxidant activity of garlic chives under all three atmospheres decreased with increasing radiation dose except 0.5 kGy under all atmospheres. In basil the reduction in antioxidant activity increased with increasing radiation dose except in irradiated samples at 0.5 and 0.25 kGy under air and vacuum packaging atmospheres, respectively. In parsley, antioxidant activity decreased with increasing dose in all treatments with the exception of 3 samples were irradiated at 0.5, 1 and 0.25 kGy under air, N_2 and vacuum packaging atmospheres, respectively. Antioxidant activity in mint samples did not show significant difference among all irradiated and non-irradiated treatments ($p>0.05$).

Free radicals and reactive species generated during radiation processing may act as stress signals and cause stress in vegetables, which leads to an increase in antioxidant production (e.g., tocopherols), then confirms the idea of a hormonal effect. The increase in phenolic contents in all irradiated plants has also been attributed to the depolymerisation and dissolution of cell wall polysaccharides, which facilitates their extraction [20,21]. In contrary, oxidation can decrease antioxidants after irradiation [6]. In present study some results which represented an increase in antioxidant activity after irradiation compared to control samples were in the same line with above researches. In addition to, time did not have a significant effect on antioxidant properties of vegetables.

Table 2. DPPH° scavenging activity (%) of irradiated samples (0, 0.25, 0.5 and 1 kGy) under air, vacuum, and N_2 atmospheres*

Vegetable	Dose (kGy)	Packaging atmosphere		
		Air	N_2	Vacuum
Garlic chives	0	(28.98 ± 0.08)^{c}	(20.99 ± 0.01)^{f}	(14.45 ± 0.00)^{h}
	0.25	(26.44 ± 0.05)^{d}	(17.75 ± 0.03)^{g}	(21.06 ± 0.00)^{e}
	0.5	(36.40 ± 0.09)^{a}	(29.63 ± 0.02)^{c}	(33.69 ± 0.01)^{b}
	1	(23.22 ± 0.04)^{e}	(22.06 ± 0.01)^{e}	(19.14 ± 0.02)^{f}
Parsley	0	(57.46 ± 0.31)^{b}	(54.97 ± 0.00)^{c}	(43.35 ± 0.01)^{g}
	0.25	(47.54 ± 0.22)^{f}	(52.42 ± 0.02)^{d}	(66.85 ± 0.01)^{a}
	0.5	(50.68 ± 0.11)^{e}	(37.30 ± 0.01)^{h}	(51.92 ± 0.02)^{e}
	1	(42.92 ± 0.26)^{g}	(51.91 ± 0.01)^{e}	(48.08 ± 0.00)^{f}
Basil	0	(74.88 ± 0.25)^{b}	(81.36 ± 0.00)^{a}	(61.45 ± 0.01)^{e}
	0.25	(55.65 ± 0.09)^{g}	(75.26 ± 0.02)^{b}	(63.02 ± 0.00)^{e}
	0.5	(69.94 ± 0.03)^{d}	(73.86 ± 0.01)^{c}	(58.78 ± 0.02)^{f}
	1	(56.43 ± 0.15)^{f}	(72.70 ± 0.02)^{d}	(46.57 ± 0.01)^{h}
Mint	0	(95.82 ± 0.01)^{a}	(95.99 ± 0.01)^{a}	(95.58 ± 0.01)^{a}
	0.25	(95.48 ± 0.02)^{a}	(95.48 ± 0.02)^{a}	(95.77 ± 0.02)^{a}
	0.5	(95.92 ± 0.01)^{a}	(95.33 ± 0.01)^{a}	(95.48 ± 0.01)^{a}
	1	(95.55 ± 0.01)^{a}	(95.26 ± 0.01)^{a}	(94.38 ± 0.01)^{a}

*Values are mean ± standard deviation (n=3); different lowercase letters in the same vegetable show significant difference by Duncan's Multiple Range Test (at $p<0.05$, each parameter compared separately).

IV. Conclusion

The highest decreases of microbial contamination were found in irradiated samples at 0.5 kGy under N_2 atmosphere. They were 4 logarithmic cycles in basil and parsley for aerobic mesophilic, 6 logarithmic cycles in garlic chives for spore forming, 5 logarithmic cycles in garlic chives and parsley for Staphylococcus aureus and 5 logarithmic cycles in garlic chives, basil and parsley for coliform bacteria. E. coli was only present in non- irradiated samples under different atmospheres. Generally, in most of treatments, the antioxidant activity decreased after irradiation, but irradiated samples at 0.5 kGy under N_2 atmosphere showed acceptable results in 4 types of vegetables. Therefore, irradiation at 0.5 kGy under N_2 packaging atmosphere are recommended as optimal treatments to preserve fresh garlic chives, basil, mint and parsley up to 10 days at 4 °C.

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VI. Conflict of interest

The authors declare that they have no conflict of interest.

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