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Gamma Irradiation of Export Oriented Moringa Leaf (*Moringa oleifera*) Powder and Flakes for Microbial Safety

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Abstract

Gamma irradiation is a well-accepted innovative technology. This study was carried out to evaluate the effect of different gamma irradiation doses on physical parameters, and microbial quality of export oriented Moringa powder and flakes in Sri Lanka. Homogenized Moringa samples were collected from a particular exporter. Samples were irradiated at doses of 0 kGy, 2 kGy, 4 kGy, 6 kGy, 8 kGy and 10 kGy by industrial Co-60 gamma irradiator. Under physical parameters, water activity, moisture content, colour and infusion colour (in Hunter colour scale) were measured for each of the treatment. Under microbial safety total plate count (TPC), yeast and mold count and coliform counts (total coliform, fecal coliform and E. coli) were measured. All treatments were triplicated. Average moisture content of irradiated Moringa powder and flakes were 6.32±0.04% and 6.09±0.03% respectively. Average water activity of irradiated Moringa powder was 0.41±0.00 and irradiated Moringa flakes was 0.44±0.03. Mean values of colour and infusion colour of irradiated Moringa powder and flakes were not significantly different ($P<0.05$) with the control sample. The average TPC in control sample of Moringa powder was $1.29\times10^7\pm2.46\times10^5$ CFU/g and Moringa flake was $4.20\times10^5\pm3.71\times10^5$ CFU/g, where irradiated samples show drastic reduction with the dose. The average Yeast and Mold counts in the control sample of Moringa powder was $3.11\times10^2\pm6.80\times10^1$ CFU/g and Moringa flake was $2.30\times10^2\pm2.06\times10^2$ CFU/g, where irradiated samples show sterilized conditions. E. coli were identified in 0 kGy and 2 kGy samples only. All irradiated samples showed significant reduction ($p<0.05$) of TPC, yeast and mold and coliform counts in both Moringa powder and flakes. It is concluded that the 6 KGy is better for Moringa flake and 8 kGy is better for Moringa powder for microbial safety while preserving the physical and chemical properties.

Keywords: Moringa, Gamma irradiation, Dose, Microbial safety, Quality changes

1. INTRODUCTION

Moringa oleifera is the most widely cultivated species in the genus *Moringa*, the only genus in the plant family Moringaceae. Products from the tree have multiple uses. The plant's dried leaves are marketed for a wide range of health benefits and are used as herbal tea. *Moringa oleifera* is marketed as a 'superfood' in international markets and has become very popular all around the world (Vergara-Jimenez *et al*., 2017). Based on the composition, *M.oleifera* leaf powder is commonly marketed as a supplement for supporting immune health, improving general health, increasing energy levels and supporting weight management. These claimed benefits are based on their high level of antioxidant activity and chemical composition (Rajput *et al*., 2017). It contains many bioactive compounds such as vitamin A, vitamin B1, B2, B3, B6, folate, vitamin C, calcium, potassium, iron, magnesium, phosphorus, zinc, amino acids, beta-carotene, antioxidants, anti-inflammatory nutrients and omega 3 and 6 fatty acids (Razis & Razis, 2014).

Gamma irradiation as a means for sterilization is a proven technology used in over 50 countries for various applications (Fernstrom, 2010). It is a process that exposes food to ionizing energy for a specific length of time, depending on the purpose of treatment (Prejean, 2001). The term "food irradiation" may be applied to any process that exposes food either to electromagnetic radiation or to high-energy particles. Electromagnetic energy can be generated by radioactive isotopes, as in the case of gamma ray irradiation (Farkas, 2000). Radiation is absorbed by the food, and more particularly, by the microbial organisms in the food. Since food irradiation is a physical process, it does not leave any residue in the treated food products (Farkas, 2000). Irradiation is a completely safe and clean method of hygienization of products, with no harmful by-products or increase in temperature (Molins, 2001; Farkas, 2000).

Since gamma irradiation is an innovative technology, it has been used for sustainable development in the food industry. The aim of this study is to determine the impact of gamma irradiation on the physical, chemical and microbial

parameters of export oriented Moringa powder and flakes before and after irradiation. Also, it is expected find out the effective irradiation dose for microbial decontamination of commercially available export oriented Moringa powder and flakes.

2. MATERIALS AND METHODS

2.1. Moringa samples

Representative amount of homogenized export oriented Moringa leaf powder (mesh size 40 sever) 2kg and Moringa flake (oven dried moringa leaf) 2kg samples were collected from exporters. Collected samples were weighed separately and packed in sterilized sample bags.

2.2. Gamma irradiation

Irradiation was conducted at Sri Lanka Gamma Centre at Biyagama, Sri Lanka by using industrial Co-60 gamma irradiator (Panoramic with wet source storage, confirming to ANSIN 43.10.1984 and IAEA safety, Series number 107-AERB/RF/IRRAD/SS-6 (Rec-1) 2007) at dose rate 5.3 Gy/min. The given radiation doses were 0, 2, 4, 6, 8 and 10 kGy. The absorbed doses were measured by Harwell Amber Perspex dosimeters. The actual received doses were 2.03, 4.07, 5.44, 7.47 and 9.99 kGy.

2.2.1. Analysis of physical parameters

1. Water activity

Water activity was determined according to the AOAC 978.18 modified method. The water activity of Moringa was determined by using a water activity meter *(NovasinaLabMASTER Water activity meter, Swizterland)* at 25 °C temperature.

2. Moisture content

The moisture content was determined according to the AOAC 925.10 modified method. Moisture content of Moringa was determined by using a Moisture analyzer *(MRS 120-3 moisture Balance analyzer) at 105±1°*C.

3. Colour and infusion colour

Colour was determined according to the (Ali, Yusof, Chin, Ibrahim, & Basra, 2014) method. Colour of Moringa was determined by using a Chroma meter *(Konica Minolta CR-400/410)* using Hunter Lab colour scale. The Hunter colour L*, a*, and b* values were reported through the machine. ΔE was calculated by using the following equations.

ΔL = L observed - L standard (1)

Δa = a observed - a standard (2)

Δb = b observed - b standard (3)

*L, *a, *b standard = Average of non-irradiated samples

$\Delta E = \sqrt{\Delta L^2+\Delta a^2+\Delta b^2}$ (4)

2.2.2. Analysis of Microbial Parameters

Total Plate count (ISO 4833-1: 2013), Yeast and Mold count (ISO 21527-2: 2008) and Coliform count (ISO 4831: 2006) of Moringa were determined. All measurements were performed in triplicate of three independent experiments. Analyses were carried out in duplicates for each dilution series and three replicates were used for each dose.

2.3. Statistical Analysis

Data analysis was done for descriptive and inferential statistics by using Minitab 15 software. The individual observations of Moringa samples were analysed and expressed in terms of mean and standard deviation (SD). One-way ANOVA and Andersen-Darling tests were used to analyse physical and chemical parameters and Kruskal-Wallis test was used to analyse microbial parameters of control and irradiated samples.

3. RESULTS AND DISCUSSION

3.1 Water activity of *Moringa oleifera*

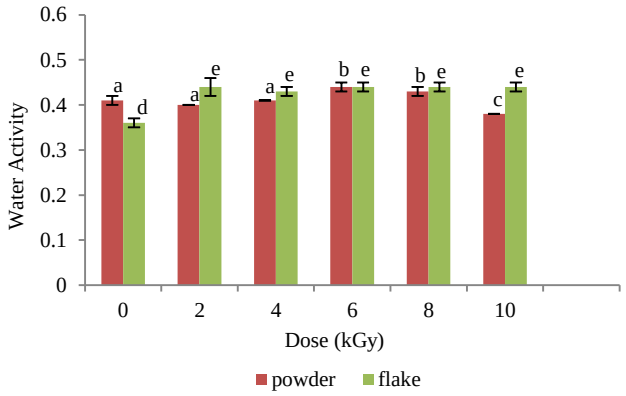

Fig. 1. Mean water activity of Moringa irradiated at different doses (Different letters indicate significant differences at level *P = 0.05* and the error bars indicate the standard deviation of mean).

In Moringa powder, the highest mean water activity was observed in 6 kGy sample (0.44±0.01) and the lowest was observed in 10 kGy sample (0.38±0.00). The highest mean water activity of Moringa flake was observed in 2 kGy (0.44±0.02), 6, 8 and 10 kGy (0.44±0.01) samples and the lowest was observed in the control sample - sample without application gamma radiation (0.36±0.01). Both flake and powder, the differences were significantly higher ($P< 0.05$) compared to control (ANOVA, df=5, 12, P=0.00). Beauchat *et al.*, (2013) said that the drying process can reduce the amount of water available for microbial growth and 0.6 is the minimum water activity that microorganisms can grow. However, treatments may be necessary in order to eliminate the pathogens in contaminated low-moisture products. Several non-thermal technologies have been reported to improve low-moisture food safety (Syamaladevi *et al.*, 2016). In this study water activity of irradiated Moringa was very low for microbial growth.

3.2 Moisture content of Moringa

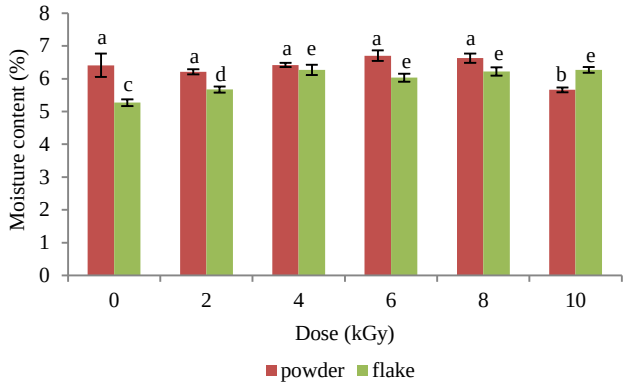

Fig. 2. Mean moisture content of Moringa irradiated at different doses (Different letters indicate significant differences at level *P = 0.05* and the error bars indicate the standard deviation of mean).

According to the figure 3.2, the highest mean moisture content of Moringa powder was observed in the 6 kGy sample (6.70±0.16) and the lowest was observed in 10 kGy sample (5.66±0.07). In flake samples the highest mean moisture contents were observed in the 4 kGy (6.27±0.16) and 10 kGy samples (6.27±0.09) and the lowest was observed in the control sample (5.27±0.10). When compared with moringa powder and moringa flake samples highest moisture content was observed in the moringa powder sample. The differences were significantly higher (P<0.05) compared to control. According to the proximate analysis from Mahima *et al*, (2014), fresh *Moringa oleifera* leaves contain 72.39% of moisture. The average moisture after drying was 7.87%. This value is less than the accepted maximum and this low moisture content can inhibit bacterial, fungal and yeast growth assuring the shelf life (Denis & Harm, 2012). Moisture content has been accelerated by the metabolic activity of the microbial population in non-irradiated samples (Gurtler *et al*., 2014). The highest moisture content was found in irradiated sample and it is well below the accepted maximum. The varaiations might be due to the inherent variations in the samples.

3.3. Colour of Moringa

Table 1
Hunter colour mean values of L*, a*, b* and E of Moringa samples with different irradiation doses.

Dose	L*	a*	b*	ΔE
0 kGy				
Powder	27.87±1.90	-3.36±0.50	9.44±1.20	-
Flake	27.98±0.73	-3.79±0.27	9.74±0.34	-
2 kGy				
Powder	29.62±0.12	-3.72±0.03	10.38±0.05	9.60±0.07
Flake	27.86±0.73	-3.88±0.41	9.52±0.39	0.75±0.32
4 kGy				
Powder	29.76±0.92	-3.80±0.22	10.43±0.48	9.71±0.64
Flake	27.96±0.10	-3.69±0.24	9.97±0.66	1.04±0.34
6 kGy				
Powder	30.00±0.20	-3.70±0.05	10.55±0.11	9.84±0.16
Flake	27.73±2.26	-3.91±0.18	9.54±1.07	1.93±0.97
8 kGy				
Powder	30.02±0.69	-3.82±0.14	10.82±0.38	10.04±0.38
Flake	27.61±1.80	-3.51±0.25	9.58±0.84	1.43±0.97
10 kGy				
Powder	28.29±0.08	-3.62±0.04	10.01±0.13	9.08±0.13
Flake	27.00±1.79	-3.35±0.73	8.98±1.08	2.05±1.21

L* indicates whiteness ranges from (black at 0 to white at 100), a* indicates red when positive and green when negative (a*+ = redder, a*- = greener), b* indicates yellow when positive and blue when negative (b* + = yellower, b* - = bluer) and ΔE value indicates the overall colour combination on powder and flakes of Moringa in Hunter Lab colour scale. Higher ΔE value indicates better color development in samples. The colour measurements of food materials are used in an alternate way to determine the quality variations as they are faster than a complete physicochemical examination. Higher L* values and lower a*, b* values are preferred in dried food products (Ali et al., 2014).

L* value of Moringa powder was increased up to 8 kGy and decreased in 10 kGy. Highest L* value was observed in 8 kGy sample (30.02±0.69) and lowest was observed in the control sample (27.87±2.16). Thus L* value was increased up to 8 kGy there is no significant difference was found (P>0.05). According to the results irradiated Moringa powder show better luminescence than control sample (ANOVA, df=5, 12, P=0.082). L* value of Moringa flake was slightly decreased with irradiation doses, except in 4 kGy sample (28.00±1.2). Highest L* value was observed in control sample (27.98±0.73) and lowest was observed in the 10 kGy sample (27.00±1.79). Thus L* value was decreased with dose, there is no significant difference (P>0.05) (ANOVA, df=5, 12, P=0.952).

The colour measurements of food materials are used in an alternate way to determine the quality variations as they are faster than a complete physicochemical examination. Higher L* values are preferred in dried food products (Ali et al., 2014). When compared with the Moringa flake, Moringa powder have higher L* value.

The highest a* value of Moringa powder was observed in control sample (-3.36±0.45) and lowest was observed in 8 kGy sample (-3.82±0.14). However, there is no significant difference (P>0.05) was found in the samples for a* values (ANOVA, df=5, 12, P=0.195). In Moringa flake the highest a* value was observed in 10kGy sample (-3.35±0.73) and lowest was observed in 6 kGy sample (-3.91±0.18). 2 and 6 kGy samples have low a* value, when compared with the control sample. However, there is no significant difference (P>0.05) was found in the samples for a* values (ANOVA, df=5, 12, P=0.494).

As temperature and drying time increased the colour of leaves became dark green. The natural green colour of leaves is due to mixture of chlorophyll which is directly related to magnesium. During drying, the magnesium molecules are changed to pyro pheophytin and pheophytin. Therefore, at higher temperatures greenness is reduced. Visually, dark green colour of the leaves seemed as dull green-yellow due to degradation of chlorophyll (Ali et al., 2014). As irradiation is a cold process it does not affect to the colour of

the product (WHO, 1988). Lower a* values are preferred in dried food products (Ali et al., 2014).

With high irradiation doses up to 8 kGy b* value of Moringa powder was slightly increased. The highest b* value was observed in 8 kGy sample (10.82±0.38) and lowest was observed in control sample (9.44±1.20). There is no significant difference (P>0.05) found in b* value in Hunter scale (ANOVA, df=5, 12, P=0.112). In Moringa flake, when compared with the control, b* value was slightly decreased in irradiated samples. The highest b* value was observed in control sample (9.94±0.34) and lowest was observed in 10 kGy sample (8.98±1.08). There is no significant difference (P>0.05) found in b* value in Hunter scale (ANOVA, df=5, 12, P=0.871).

The increase in b* value with increase in temperature showed that leaves became more yellow. The lower b* values are preferred in dried food products (Ali et al., 2014). According to this study when compared with the irradiated Moringa powder, irradiated Moringa flake have low b* values.

ΔE value of Moringa powder was increased up to 8 kGy. In this study highest ΔE value was observed in 8 kGy sample (10.04±0.38) and lowest was observed in 10 kGy sample (9.08±0.13). The ΔE values of irradiated Moringa powder were in the range of 9.08-10.04. However, the differences were not statistically significant (P>0.05) (ANOVA, df=4, 10, P=0.061). In Moringa flake, ΔE value was increased with the irradiation doses. In this study the highest ΔE value was observed in 10 kGy sample (2.05±1.21) and lowest was observed in 2 kGy sample (0.75±0.32). The ΔE values of irradiated Moringa powder were in the range of 0.75-2.05. However, the differences were not statistically significant (P>0.05) (ANOVA, df=4, 10, P=0.331). When compared with Moringa powder and flake, Moringa powder has higher ΔE value.

Colour is an important sensory attribute, which determines the products acceptability rate (Muthukumar *et al*., 2012). The color of dried food is considered, as the first quality parameter even before it tasted evaluated by end user. It is critical in the product acceptance (Ali *et al*., 2017). According to the results of Alves *et al*. (2017) average L, a, and b values of Moringa powder were 56.70±0.63, -10.55±0.35 and 26.90±1.11 respectively.

3.4 Infusion colour of Moringa

Table 2
Infusion colour mean value of L*, a*, b* and E of Moringa samples with different irradiation doses.

Dose	L*	a*	b*	ΔE
0 kGy				
Powder	58.19±1.17	-0.95±0.29	10.00±0.88	-
Flake	59.52±1.90	-1.58±0.29	12.31±0.80	-
2 kGy				
Powder	61.14±1.94	-1.32±0.22	11.56±1.02	3.39±2.13
Flake	57.80±1.42	-1.44±0.12	11.72±0.62	1.85±1.52
4 kGy				
Powder	56.72±1.41	-0.74±0.19	9.26±0.64	1.70±1.50
Flake	61.91±3.30	-1.69±0.23	13.40±1.06	3.45±2.14
6 kGy				
Powder	62.11±0.92	-1.34±0.22	12.19±0.71	4.51±1.15
Flake	58.81±0.84	-1.27±0.21	11.60±0.78	1.35±0.54
8 kGy				
Powder	58.19±3.30	-1.40±0.28	12.40±1.68	3.71±3.56
Flake	60.84±3.30	-1.40±0.28	12.40±1.62	2.59±2.50
10 kGy				
Powder	59.33±1.31	-0.67±0.12	11.88±0.75	2.31±1.30
Flake	58.98±1.26	-1.68±0.23	13.73±1.11	2.02±0.51

The highest L* value of Moringa powder was observed in 6 kGy sample (62.11±0.92) and lowest was observed in 4 kGy sample (56.72±1.41). There is a significant difference in L* value (P<0.05) (ANOVA, df=5, 12, P=0.033). In Moringa flake, highest L* value was observed in 4 kGy sample (61.91±3.30) and lowest was observed in the 2 kGy sample (57.80±1.42). However, there is no significant difference was found (P>0.05) (ANOVA, df=5, 12, P=0.311). When compared with the Moringa flake, Moringa powder have higher L* value. It proves infusion colour of the Moringa powder is luminescence than the infusion colour of Moringa flake.

In Moringa powder all a* values show less deviation with the control sample. Highest a* value was observed in 10 kGy sample (-0.67±0.12) and lowest was observed in 8 kGy sample (-1.40±0.28). However, there is no significant difference (P<0.05) was found in the samples (ANOVA, df=5, 12, P=0.005). a* values of Moringa flakes were in the range of (-1.27) - (-1.69). The Highest a* value was observed in 6 kGy sample (-1.27±0.21) and lowest was observed in 4kGy (-1.69±0.23) and 10 kGy (-1.69±0.26) samples. However, there is no significance difference (P>0.05) was found in the samples (ANOVA, df=5, 12, P=0.262). Both a* value of Moringa powder and flakes have negative values. When compared with the Moringa flake, Moringa powder have higher a* value. It proves infusion colour of the Moringa powder is redder than the infusion colour of Moringa flake. Because during processing cell rupture may release of cellular compounds such as colorant.

With high irradiation doses up to 4 kGy b* value of Moringa powder was slightly increased. The Highest b* value was observed in 8 kGy sample (12.39±1.68) and lowest was observed in 4 kGy sample (9.26±0.63). There is no significant difference (P>0.05) found in Hunter scale b* value parameter in (ANOVA, df=5, 12, P=0.012). Highest b* value was observed in 10 kGy sample (13.73±1.11) and lowest was observed in 6 kGy sample (11.60±0.78). There is no any significant difference (P>0.05) found in b* value parameter (ANOVA, df=5, 12, P=0.152). When compared with the Moringa powder, Moringa flake have higher b* value. It proves infusion colour of the Moringa flake is yellower than the infusion colour of Moringa powder.

The highest ΔE value of Moringa powder was observed in 6 kGy sample (4.51±1.15) and lowest was observed in 4 kGy sample (1.69±1.5). The ΔE values of irradiated Moringa powder were in the range of 1.69-4.51. However, the differences were not statistically significant (P>0.05) (ANOVA, df=4, 10, P=0.529). In Moringa flake, highest ΔE value was observed in 4 kGy samples (3.45±2.14) and lowest was observed in 6 kGy sample (1.35±0.54). The ΔE

values of irradiated Moringa powder were in the range of 1.35-3.45. However, the differences were not statistically significant (P>0.05) (ANOVA, df=4, 10, P=0.607). When compared with the Moringa flake, Moringa powder has higher ΔE value. That indicates better colour development in Moringa flake samples.

3.5 Microbial count of Moringa

Table 3
Microbial count of Moringa at different doses.

Dose	Total plate count (CFU g^{-1})	Yeast and mold (CFU g^{-1})	Total coliform (MPN g^{-1})	Fecal coliform (MPN g^{-1})
0 kGy				
Powder	$1.29\times10^{7}\pm 2.46\times10^{5}$	$3.11\times10^{2}\pm 6.80\times10^{1}$	>100 ± 0.00	>100 ± 0.00
Flake	$4.20\times10^{5}\pm 3.71\times10^{5}$	$2.30\times10^{2}\pm 2.06\times10^{2}$	>100 ± 0.00	1.48 ± 0.98
2 kGy				
Powder	$3.75\times10^{6}\pm 1.30\times10^{6}$	0.00 ± 0.00	>100 ± 0.00	>100 ± 0.00
Flake	$3.20\times10^{4}\pm 2.46\times10^{4}$	0.00 ± 0.00	24 ± 0.00	1.44 ± 0.68
4 kGy				
Powder	$1.61\times10^{5}\pm 1.17\times10^{5}$	0.00 ± 0.00	>100 ± 0.00	>100 ± 0.00
Flake	$8.19\times10^{3}\pm 7.33\times10^{3}$	0.00 ± 0.00	14.43 ± 9.86	0.84 ± 0.60
6 kGy				
Powder	$1.30\times10^{3}\pm 6.34\times10^{2}$	0.00 ± 0.00	31.33±12.70	0.44 ± 0.25
Flake	$4.26\times10^{2}\pm 5.77\times10^{0}$	0.00 ± 0.00	2.30 ± 0.00	0.30 ± 0.00
8 kGy				
Powder	$1.02\times10^{3}\pm 2.44\times10^{2}$	0.00 ± 0.00	21.53±21.18	0.30 ± 0.00
Flake	0.00 ± 0.00	0.00 ± 0.00	1.84 ± 0.79	0.30 ± 0.00
10kGy				
Powder	0.00 ± 0.00	0.00 ± 0.00	1.38 ± 0.79	0.30 ± 0.00
Flake	0.00 ± 0.00	0.00 ± 0.00	<0.03± 0.00	0.30 ± 0.00

The sample medians for the six treatments were calculated. With the increment of irradiation dose, both flake and powder show significant reduction ($p<0.05$) in the counts. When compared with the total viable cells and yeast and mold count, Moringa contain less amount of yeast and mold count. With high irradiation doses total coliform and fecal coliform counts were dramatically decreased. Adu-Gyamfi & Mahami, (2014) mentioned that irradiated Moringa leaf powder contains less than 3.72×10^{3} CFU g^{-1} amount of total viable cells, 9.77×10^{2} CFU g^{-1} amounts of yeast and mold cells and 3.33×10^{3} CFU g^{-1} units of coliform count.

Moringa leaves in shady environments of high humidity could promote microbial growth and negatively impact microbiological quality. Many of the practical applications of food irradiation have to do with preservation. Radiation inactivates the food spoilage organisms, including bacteria, molds and yeast. It is effective in increasing the shelf life of product (WHO, 1988). Farkas, (2006) showed that the mechanism behind ionizing radiation is mainly the inactivation of microbes due to its nucleic acid damage. The process of food irradiation ensures the destruction of bacteria, fungi, insects and other parasites that cause food spoilage and diseases (Molins, 2001). Gamma irradiation significantly improved the microbiological quality of both dried Moringa leaf and powder. The process should be integrated into the processing protocol of dried Moringa leaves in order to enhance their quality for the domestic and export markets (Adu-Gyamfi & Mahami, 2014).

4. CONCLUSION

Moringa tree has multiple uses and it increases export market as a food supplement. In this study Gamma irradiation did not cause considerable changes in the physical parameters of Moringa powder and flakes under the doses studied. According to the results of this study, Moringa powder contains high amount of microbial count than the Moringa flake. Drastic reduction of microbial counts could be seen in 6 kGy in Moringa flake and 8 kGy in Moringa powder. The study showed that 6 kGy and 8 kGy are the optimal doses to eliminate the microbial contamination of Moringa flake and powder respectively. This treatment can be used as a safe and efficient method for preventing microbial spoilage during exportation while preserving the other physical and chemical parameters.

Conflict of Interest

No conflicts of interest to declare.

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