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Biochemical composition and effects of radiation on sensory, biochemical and physiological quality of fresh spinach (*Spinacia oleracea L.*)

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Abstract

Leafy vegetables have become an important part of the diet because of their nutritive value and low calorie content. But leafy vegetables are very perishable and susceptible to postharvest damage and spoilage which limit the storage period and quality of them. Irradiation is being used in the preservation of leafy vegetables. The effects of irradiation (0.5 and 1.0 kGy) on sensory, biochemical, physiological attributes of spinach were investigated during 12 days of post-irradiation storage at 12°C ± 60% RH. Spinach treated with 1.0 kGy showed acceptability in color, flavor, texture and overall acceptability till 12 days during storage period whereas at the same period, control and 0.5 kGy treated samples were spoiled. Reduced rate of weight loss was observed in irradiated samples compare to control over the whole storage period. A minor decrease in the ascorbic acid content was the only adverse effect observed in irradiated spinach compare to control sample and no other major changes occurred in TSS, pH value of treated and untreated sample of spinach. Radiation processing of spinach at 1.0 kGy extends the shelf life without affecting sensory and nutritional qualities. Thus, radiation can be used for the shelf -life extension of leafy vegetables and is not harmful to health.

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Introduction

Tropical and subtropical climates make Bangladesh, one of the notable growers of a variety of fruits and vegetables. In Bangladesh, the production of vegetables is about 2.7 million ton (Bangladesh Bureau of Statistic, 2008) which can hardly fulfill 50% requirements of the country (Hossain, 2010). Though fresh fruits and vegetables are perishable, a significant portion of fruits and vegetables (25-30 %) is loss due to improper postharvest handling and processing (Hasan *et al.*, 2010) reported that the monetary loss as calculated from the postharvest quantitative loss of selected fruits and vegetables in Bangladesh is about 420 million US dollar (3442 crore taka) based on retail price. The loss would be much more if the losses of all available fruits and vegetables were added. As a result the country is overburdened by the enormous annual monetary losses as well as the consumers are deprived from the consumption of the highly nutritious fruits and vegetables. As developing country, Bangladesh facing poverty, food insecurity and malnutrition problems that can be remedied in great measure by increasing the production and consumption of traditional leafy vegetables. Hasan *et al.*, reported that the present consumption rate of fruits and vegetables in Bangladesh is 126 g/day/capita (23 g leafy vegetables, 89 g non-leafy vegetables and 14 g fruit), which is far below the minimum average requirement of 400 g/day/capita (FAO, 2003). The fresh vegetables industry is constantly growing mainly due to the consumer's tendency of health consciousness and their increasing interest in the role of food for maintaining and improving human well-being (Ragaert *et al.*, 2004; Huxley *et al.*, 2004; Allende *et al.*, 2006).

Leafy vegetables are highly perishable due to high moisture content and susceptible to rapid depreciation of nutritive value soon after harvest, so require special processing treatments to prevent post-harvest losses (Makobo *et al.*, 2010). Many techniques have been studied in order to overcome these problem and extend the shelf life of fresh produce, for example, low temperature and high humidity, controlled and modified atmosphere packaging (Wang, 2010). Leafy vegetables to be preserved by canning, freezing or dehydration are normally blanched in order to obtain good quality products (Mepba *et al.*, 2007). The maintenance of the quality of fresh produce is still a major challenge for the food industry. Irradiation is an economically viable technology for reducing post-harvest losses and maintaining hygienic quality of fresh produce (Andrews *et al.*, 1998).

Irradiation of foods is now legally recognized in many countries as a safe and effective method for improving food safety (Kume *et al.*, 2009) and it has toxic effects on treated products (Fapohunda *et al.*, 2012). It is also an effective technique for the elimination of food spoilage organisms, preservation of nutritional components and extension of shelf life of fresh produce and reduction of losses (Hajare *et al.*, 2006; Dionisio *et al.*, 2009; Nagar and Bandekar, 2011). It provides an effective alternative to chemical fumigants and preservatives currently used by the food industries which are being phased out owing to their adverse effects on the environment and human health.

Among other leafy vegetables, spinach (*Spinacia Oleracea L.*) is one of the most popular leafy vegetable in Bangladesh. It is low in calories and a good source of vitamin C, vitamin A and minerals especially iron (Toledo *et al.*, 2003). Recently, the Food and Drug Administration (FDA) allowed the use of ionizing radiation for the shelf life extension of fresh iceberg lettuce and fresh spinach up to a maximum absorbed dose of 4.0 kGy (FDA, 2012). There have been few studies of the effect of irradiation on quality of spinach (Fan and Sokorai,2008; Fan and Sokorai,2011; Lester *et al.*, 2010). However, studies on the effect of irradiation on spinach biochemical and physiological quality and nutrient content are limited.

The present study was attempted to evaluate effect of gamma radiation to extend the shelf life and to improve biochemical (ascorbic acid, total soluble solid, pH value) and physiological quality (weight loss) of fresh spinach stored at 12 ^{o} C for 12 days.

Materials and methods

Plant material

Spinach (*Spinachia oleraceae* L.) of good quality were collected from the local market and transported to the laboratory. After discarding the damaged and wilted leaves, spinach leaves were selected for uniformity of size, shape and colour, and without any defects or mechanical injury.

Irradiation

After discarding the damaged and wilted leaves, spinach leaves were selected for uniformity of colour and without any defects or mechanical injury. For each treatment 25-30 g of spinach was placed into polythene bags (25 cm X 16 cm) and sealed tightly. The sealed polythene bags were labeled by indicating the name of the product and were irradiated with two selected doses of gamma radiation which were 0.5 kGy and 1.0 kGy. Irradiation was applied to the samples with a 50kCi Co^{60} gamma source (dose rate 6.4 kGy / hr) located at Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment, Savar, Dhaka. Both treated and untreated samples were stored at 12°C temperature for 12 days. The both samples were analyzed at every three days interval for visual and chemical analysis.

Sensory evaluation

A quantitative affective test was used to determine liking and attitude of irradiated spinach because it would show whether irradiation affected acceptance (liking) by consumers (Meilgaard *et al.*, 1999).

Sensory evaluation of all formulated spinach was done by taste tasting panel using the Hedonic scales suggest by (Krum, 1955). A panel of ten judges with age ranging from 25-40 years was made on their consistency and reliability of judgment. They were asked to evaluate color, flavor, and texture scoring rate on a 9 points Hedonic scale, where, 0-2 represent disliked extremely, 3-5 for dislike, 6-8 for acceptable or good and 9 for excellent color, flavor and texture. The preference differences were evaluated by statistical analysis of the data for variance

Estimation of moisture

At first, the initial weight of the samples was taken. Then samples were dried in an oven at about 105° C for about 5-6 hours until a constant weight was reached and cooled in a desiccator and weight again. The percentage of moisture content was calculated by the following equation.

Percentage (%) of moisture = (Final weight – Initial weight /Original weight of sample) × 100

Estimation of protein

Determination of protein by micro-Kjeldahl's method was developed by Ma and Zuazaga, 1942. It involves conversion of organic nitrogen to ammonium sulphate by digestion with concentrated sulphuric acid in a microkjeldahl flask. The digest was diluted, made alkaline with sodium hydroxide and distilled. The liberated ammonia was collected in a boric acid solution and was determined titrametrically. The percentage of protein in the sample was calculated by the following equation:

Percentage (%) of protein = (c-b) × 14 × d × 6.25/a × 1000 × 100

Where, a = sample weight (g)

b= volume of NaOH required for back titration and neutralize 25ml of 0.1N H_2SO_4 (for sample)

c= volume of NaOH required for back titration and neutralize 25 ml of 0.1N H_2SO_4 (for blank)

d=normality of NaOH used for titration

6.25= conversion factor of N to protien

14= atomic weight of N

Estimation of ash

The ash content of a sample is the residue left after ashing in a muffle furnace at about 550-600°C till the residue become white (Carpenter,1960). The percent of ash was calculated as follows.

Percentage (%) of ash = (Weight of ash / Weight of Sample) × 100

TSS and pH estimation

Total soluble solid (TSS) content was determined by Abbe refractometer, ATAGO 9099, Japan by placing a drop of juice on its prism. Percent TSS obtained as Brix (unit of TSS) from direct reading of the refractometer. The pH of the samples was measured by digital pH meter of type H1 98106 by HANNA at ambient temperature. The sample was taken as juice.

Ascorbic acid estimation

Ascorbic acid was determined by 2,6-dichloroindophenol titrimetric method (Ranganna,1986). Briefly, Sample (2g) was homogenized with 3% metaphosphoric acid (25 ml) and was filtered through filter paper (Whatman 1, 7.0 cm). Then an aliquot (5 ml) of filtrate was titrated with the 2,6-dichloroindophenol dye (standardized by the metaphosphoric acid) to a pink end-point. Results were expressed on a fresh weight basis as mg ascorbic acid equivalent/ 100 g sample.

Weight loss estimation

For physiological analysis weight loss was performed in spinach leaves. Water loss from the fresh leaves was measured by the change in weight of the samples over a storage period. The weight of produce was recorded immediately after treatment then daily during storage in the sealed polythene bags at 12°C for 12 days. Water loss was calculated from the formula.

Weight loss = final weight – initial weight of sample / Initial weight (g) of sample X 100

Statistical analysis

Statistical procedures were performed using SPSS for Microsoft version 18.0 software package (SPSS Chicago, IL). To determine significant difference between treatments least significant difference (LSD) at $P < 0.05$ was used.

Results and discussion

Biochemical composition

A preliminary study was conducted just after harvesting to determine the biochemical composition of spinach (Table 1). 93.64 % moisture content, 2.8 mg/ 100gm total protein content, 3.9 % Total soluble solid (TSS), 1.26% ash content and 6.03 value of pH were found in spinach .

Table 1. Biochemical composition of fresh spinach just after harvesting.

Proximate chemical composition	Amount (Mean ± SD*)
Moisture content	93.74 ± 1.1
(%)Protein (mg/100g)	2.8 ± 0.58
Total soluble acid (%)	3.9 ± 0.11
Ash (%)	1.26±0.11
pH	6.03± 0.15

Values are the mean of 3 replicates

*SD= Standard Deviation

Effect of gamma radiation

Sensory assessment

The average ratings on liking of color, flavor, texture, and overall acceptability are shown in Table 2. The degree of color liking was found to be significantly higher in 1.0 kGy sample compared to the unirradiated sample at 12 days after irradiation. The color liking of other irradiated (0.5 kGy) samples was ranked similarly as the control on the 12 sampling days. Significantly higher ranking of flavor liking was observed only for the 1.0 kGy samples upto 12 days after irradiation. Off-odour was detected in control and 0.5 kGy treated sample at day 12 during storage. In terms of texture, good quality in texture was found in spinach treated in 1.0 kGy up to day 12 during storage period. At the same time sliminess were detected in the samples of irradiated (0.5 kGy) and untreated spinach.

Sensory evaluation of spinach showed that spinach with 1.0 kGy showed more acceptable in color, flavor, and texture up to 12 days of storage compare to control and 0.5 kGy treated sample. After 9 days control and 0.5 kGy treated sample get become spoiled. Fan and Sokorai (2008) also found that 1 kGy radiation dose was acceptable in appearance, smell, and texture of spinach. Fan and Sokorai (2011) also found that the liking of irradiated fresh-cut spinach was not significantly affected by irradiation at doses up to 2 kGy. Neal *et al.* (2010) found that electron beam irradiation did not affect the basic tastes, aromatics, or mouth feels of fresh spinach compare to un-irradiated sample. Prakash *et al.* (2000) found that irradiation with 0.35 kGy has no changes in sensorial attributes such as flavor and appearance of cut romaine lettuce though there was loss of firmness by 10% in irradiated sample.

The overall appearance of fresh spinach showed that sample irradiated at 0.5 and 1.0 kGy did not significantly different in overall liking compare to control sample upto 6 days of storage (Table 2). Irradiation treatment with 1.0 kGy showed significant difference at 9 and 12 days during storage compare to control and 0.5 kGy irradiated sample. Gomes *et al.* (2009) agreed with our result in case of lettuce.

Table 2. Sensory evaluation (Colour, flavor, texture and overall acceptability) of spinach treated with 0.5 and 1.0 kGy gamma radiation followed by storage at 12°C in air.

Treatment	day 0	3	6	9	12
			Colour		
Control	8.8a	7.9a	7.8a	6.3a	6.1a
0.5 kGy	8.8a	8.0a	7.8a	6.7b	6.1a
1.0 kGy	8.8a	8.0a	8.0a	7.0c	7.0b
LSD (5%)	***0.3***				
			Flavour		
Control	8.8a	7.5a	7.5a	6.3a	5.3a
0.5 kGy	8.8a	7.7a	7.5a	6.3a	5.0a
1.0 kGy	8.8a	7.8a	7.5a	6.5a	6.4b
LSD (5%)	*0.4*				
			Texture		
Control	9.0a	7.2a	6.7a	5.9a	5.0a
0.5 kGy	9.0a	7.6ab	7.4b	6.0a	5.0a
kGy	9.0a	7.8b	7.7b	6.8b	6.6b
LSD (5%)	*0.6*				
			Overall acceptability		
Control	8.9a	7.6a	7.5a	6.0a	5.5a
0.5 kGy	8.9a	7.6a	7.5a	6.3a	5.4a
kGy	8.9a	7.8a	7.7a	6.7b	6.7b
LSD (5%)	*0.39*				

Values are the mean of 6 replicates

In this and all subsequent Tables, mean values with different superscript letters are significantly different at P = 0.05 and LSD values are at P = 0.05

Biochemical and physiological assessment

Changes in spinach biochemistry and physiology were investigated over the period 0-12 days at 12°C as the sensory study found that the shelf life of irradiated spinach was extended to about 12 days. The effect of 0.5 and 1.0 kGy on ascorbic acid, total soluble solid (TSS) and pH value is given in Table 3.

Ascorbic acid

Ascorbic acid content showed a significant difference ($P<0.001$) between treatments. Spinach treated with 0.5 and 1.0 kGy had a significantly lower ascorbic acid content than control sample. Storage period showed a significant difference ($P<0.001$) with ascorbic acid content of all treatments decreasing throughout the storage period.

Table 3. Ascorbic acid, TSS , pH value of spinach treated with 0.5 and 1.0 kGy gamma radiation followed by storage at 12°C in air.

Treatment	day 0	3	6	9	12	Mean
			Ascorbic Acid (mg/100 gm)			
Control	13.37	11.82	11.72	11.37	7.56	11.17a
0.5 kGy	17.39	11.15	8.59	7.87	5.23	10.0b
1.0 kGy	17.39	9.19	8.59	7.87	3.49	10.0b
LSD (5%)	2.7				1.2	
			TSS (%)			
Control	3.3	3.1	3.3	2.6	3.9	3.2a
0.5 kGy	3.4	3.3	3.4	2.3	3.1	3.1a
1.0 kGy	3.1	3.2	3.1	3.0	2.7	3.0a
LSD (5%)	0.7			0.3		
			pH value			
Control	6.4	6.6	6.0	6.0	6.2	6.2a
0.5 kGy	6.4	6.2	5.9	6.1	6.1	6.1a
kGy	6.3	5.9	5.9	6.0	6.7	6.2a
LSD (5%)	0.4			0.2		

Values are the mean of 2 replicates with 2 treatment units in each replicate

The present study showed that ascorbic acid content of spinach was decreased gradually with the increasing of storage period in both irradiated and control sample. The results also showed that the loss of ascorbic acid is higher in irradiated samples compared to un-irradiated ones. The rapid decrease in ascorbic acid in the irradiated spinach during storage may be a stress response to irradiation (Reyes *et al.,* 2007). Ascorbic acid is a heat-sensitive bioactive compound and gets degraded by oxidative processes, which are stimulated in the presence of light, oxygen, and enzymes like ascorbate oxidase and peroxidase (Davey *et al.,* 2000). Therefore due to irradiation in presence of air, oxidation of ascorbic acid might have occurred contributing significantly for the observed reduction. Also, mild heat might also have been generated during radiation treatment which leading to decreased ascorbic acid contents (Alothman *et al.,* 2009). Fan *et al.,* (2011) also found reduced rate of ascorbic acid in irradiated fresh cut spinach compare to non-irradiated sample during 14 days storage. Kader (2009) reported that high

CO_2 concentration during storage was also found promoting the loss of ascorbic acid in spinach, due to stimulation of the oxidation of ascorbic acid and inhibition of dehydroascorbic acid reduction (Kader, 2009).

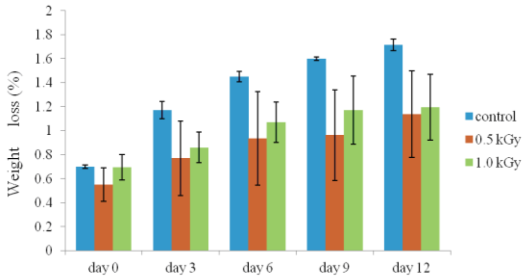

Fig. 1. Weight loss of spinach treated with 0.5 and 1.0 kGy gamma radiation followed by storage at 12°C in air. Values are the mean of 2 replicates with 2 treatment units in each replicate.

Total soluble solid (TSS) and pH value

Statistical analysis showed there was no significant difference in TSS among treatments. Storage time had a significant effect ($P<0.001$) on TSS. TSS values of both control and irradiated sample were stable from 0 to 6 days and decreased at 9 days but again increased at 12 days of storage period.

Statistical analysis of pH value showed that there was no significant difference between treatments. Storage time had a significant effect on pH value ($P<0.001$).

Weight loss

A major problem facing the fresh produces is the loss of water, which can cause reduction in crispness, loss of nutritional quality and undesirable changes in colour. The weight loss is mainly caused by water transpiration and respiration (Nagar *et al.,* 2012). In the present study, the weight loss of spinach stored at 12°C temperature of different storage period presented in Fig. 1. Weight loss was significantly different ($P<0.001$) between treatments with weight loss of 0.5 kGy < 1.0 kGy < control. Storage period had a significant effect ($P<0.001$) on weight loss with the level in all treatments gradually increasing with increasing storage period in both control and irradiated spinach during storage. Weight loss is also minimized by low temperatures and relative humidity (Bell, 1986 ; Cantwell and Reid , 1992).

Conclusions

The present results suggest that irradiation with 1.0 kGy have positive effect on the liking or overall acceptability, biochemistry and physiology of spinach even though irradiation promoted the loss of ascorbic acid during 12 days storage. Results also suggested that irradiation could be a promising technique to improve safety with minimal loss in quality of vegetables like spinach which is very effective in improving the overall quality and economical value of vegetables for developing countries like Bangladesh. For Food manufacturers, radiation technology can be useful as it can result in shelf life extension of different food produce, thereby, reducing post harvest spoilage losses.

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