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Effect of gamma radiation on antioxidant marker and microbial safety of fresh bitter gourd (*Momordica charantia* L.)

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Abstract

Bitter gourd *(Momordica charantia L.)* is one of the most popular vegetables in some Asian countries for their antioxidant properties and bioactive compounds to lower of diabetic diseases. The Objective of this study was to investigate the effect of gamma radiation on the antioxidant marker compounds (total phenol content, flavonoid content and ascorbic acid content) of fresh bitter gaurd. Microbial safety (total bacterial count, total coliform count, and total Salmonella count) was also examined. Phenolic content was significantly ($p < 0.05$) higher in bitter gaurd irradiated with 0.25 kGy compared to the control. Increased flavonoid content was observed in irradiated sample (0.25 and 0.5 kGy) compared to control sample. Ascorbic acid content decreased significantly with the increase of irradiation doses. Gamma irradiation (0.25 and 0.50 kGy) significantly reduced the microbial count in bitter gourd to keep the samples microbiologically safe.

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Introduction

Bitter gourd (*Momordica charantia* L.) is one of the popular cucurbitaceous vegetables grown in Bangladesh and other tropical regions. Emerald green young fruits are eaten as vegetables that turn to orange-yellow when ripe (Grover and Yadaf, 2004). Alkaloid momordicine produced in fruit and leaves is responsible for bitter flavour (Din *et al.*, 2011). The fruits are used as antidiabetic, antitumorous, anticancer, anti-inflamatory, antiviral, and cholesterol lowering effects etc. (Ahmed *et al.*, 2001). Bitter gaurd is a good source of Vitamin C, Vitamin A, phosphorus and iron (Sultana and Bari, 2003; Paul *et al.*, 2009). It is also a good source of phenolic compounds including gallic acid, gentisic acid (2, 5-dihydroxyl benzoic acid), catechins, chlorogenic acid and epicatechin (Horax *et al.*, 2005). These phenolic compound posses potent antioxidant activity (Budrat and Shotipruk, 2008; Myojin *et al.*, 2008) that play an important role in human nutrition as preventative agents against several diseases, protecting the body tissues against oxidative stress with their antioxidant, anti-mutagen, anti-tumor, anti-inflammatory, and anti-carcinogenic properties (Chiou *et al.*, 2007; Lee *et al.*, 2008).

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 leaves no toxic residues 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 food and food products 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.

Irradiation has influence on phenolic content of food and their by-product. Gamma irradiation increased the amount of phenolic acid content in clove and nutmeg while phenolic content remained unchanged in cinnamon (Variyar *et al.*, 1998). While the total phenol content and its antioxidant activity of bitter gaurd has been reported (Horax *et al.*, 2005; Kubola and Siriamornpun, 2008; Aminah and Anna, 2011). To the best of our knowledge no studies incorporating bitter gaurd to determine the effect of gamma irradiation on total phenol content and ascorbic acid content have been published. The present study was thus designed to determine the effect of gamma irradiation on total phenol and flavonoid content and ascorbic acid of bitter gourd.

Materials and methods

Bitter gourds were purchased from a local supermarket when required and transported to the laboratory. Good quality fresh and tender raw bitter gourd of uniform medium size and dark green colour free form infestation, mechanical injury, blemishes, dirt or mud were selected for all experiment. Samples were placed in perforated low-density polyethylene (LDP) zipper bags (0.05 mm) measuring 165 mm X 150 mm. All the samples were divided into two groups, viz. control (without radiation) and irradiated.

Gamma irradiation was carried out in a 50 kCi Co^{60} gamma irradiator (dose rate 6.4kGy/hr) located at Institute of Food and Radiation Biology, Bangladesh Atomic Energy Commission. Samples were irradiated at a dose of 0.25 and 0.5 kGy.

Antioxident marker

Total phenol content

Total phenol content was determined according to the Folin-Ciocalteu (FC) method (Singleton and Rossi, 1965). Sample tissue (1 g) was homogenized with a pestle in a mortar with 75% methanol (10 ml) and the homogenate centrifuged at 5000 rpm for 10 min at room temperature. The supernatant was filtered through filter paper (Whatman 1, 7.0 cm) and diluted with distilled water (1:10). A diluted sample (0.5 ml) was added to diluted 2N FC reagent (1:10) (2.5 ml). After 3-4 min, 7.5% sodium carbonate solution (2 ml) was added to the mixture and covered for 2 hr at room temperature. The absorbance of the solution was measured spectrophotometrically at wavelength 765 nm. Gallic acid was used as a calibration standard (R^2=0.998). The data was expressed as ppm (mg/l) gallic acid equivalents.

Total flavonoid content

Total flavonoid content was measured by the aluminium chloride ($AlCl_3$) colorimetric method (Shibata, 1975) using quercetin as a standard. An aliquot (125µl) of plant 80% methanolic extract or standard solution was added to 625µl Distilled water (DW) followed by 37.5µl of 5% $NaNO_2$. After 5 min, 10% $AlCl_3$ (75µl,) was added. At room temperature after 6 min, 1M NaOH was added followed by 137.5µl of DW. Finally after well mixing, the absorbance was measured at 490nm. The flavonoid content was calculated from a quercetin standard curve and expressed as quercetin equivalent (µg/ml).

Ascorbic acid

Ascorbic acid was determined by 2,6-dichloroindophenol titrimetric method (Rangana, 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 aliquout (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.

Microbiological analysis

The microbiological counts of the bitter gourd samples were done using decimal dilution technique followed by standard pour plate technique as describe by (Collins, 1976). Blended control and irradiated samples (10gm) were taken in sterile saline water (90ml) and vortexed for 10 minutes. Subsequent serial dilutions were prepared and the appropriate dilutions were plated in triplicate. The media used for microbiological study were Nutrient agar (Difco, USA) for the total bacterial counts, Mackonky agar for the Coliform count and Salmonella Shigella agar for the total Salmonella and Shigella counts. The plates were incubated at 37 °C for 24h and the developing colonies were reported as colony forming units (cfu/g).

Statistical analysis

All determinations were obtained from triplicate measurements and results were expressed as mean ± standard deviation. Data were analyzed by the SPSS (Statistical Package for Social Sciences) software. Statistical significance was declared at $p <$ 0.05.

Results and discussion

Total phenolic (TP) and flavonoid (TF) contents

Plant phenolics present in fruit and vegetables have received considerable attention because of their potential antioxidant activity (Dziedzic *et al.*, 1983). The effect of radiation on total phenolic and flavonoid contents of irradiated and control (unirradiated) bitter gourd was shown in Figure 1and 2. The total phenol contents increased significantly ($p < 0.05$) in 0.25kGy sample compared to control (Figure 1). The flavonoids also increased significantly with increasing dose of radiation compared to control sample (Figure 2). Irradiation induces the accumulation of phenolic compounds and flavonoids in plants as a defense mechanism against irradiation, also the increase in TP and TF can be attributed to the phenylalanine ammonialyase activity, which is one of the key enzymes in the synthesis of phenolic compounds in plant tissues (Frohnmeyer *et.al.*, 2003; Gitz *et al.*, 2004; Stevens *et al.*, 1998 and Brown *et al.*, 2001) found an increase in the activity of phenylalanine ammonialyase in peaches and cabbage seeds after UV exposure. Increase in phenolic compounds of irradiated plant produce has also been attributed to depolymerization and dissolution of cell wall polysaccharides, which facilitated higher extractability (Bhat *et al.*, 2007).

However, sample with 0.50 kGy exhibited lower phenolic contents compared to 0.25 kGy and control samples (Figure:1). Higher dose of radiation reduced the phenolic contents probably due to decomposition of phenolic compounds. Similar result was found by Ahm *et al.* (2005) in cut Chinese cabbage.

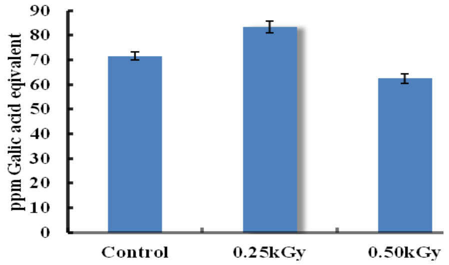

Fig. 1. Effect of radiation on total phenol content.

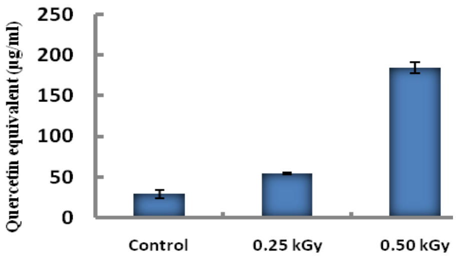

Fig. 2. Effect of radiation on total flavonoid content.

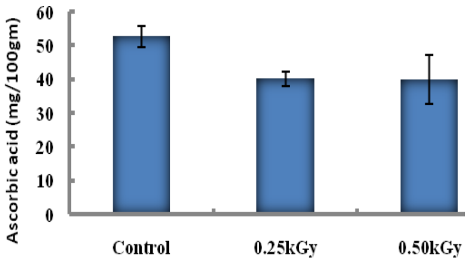

Fig. 3. Effect of radiation on ascorbic acid content.

Ascorbic acid content

In the present study, ascorbic acid content decreased with the increase of radiation dose (Figure 3). 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, during radiation treatment, mild heat might also have been generated leading to decreased ascorbic acid contents (Mohammad *et al.*, 2009). González-Aguilar *et al.* (2007) have reported the same negative effect of UV-C irradiation on ascorbic acid content in mango "Tommy Atkins" fruits when compared with control fruits.

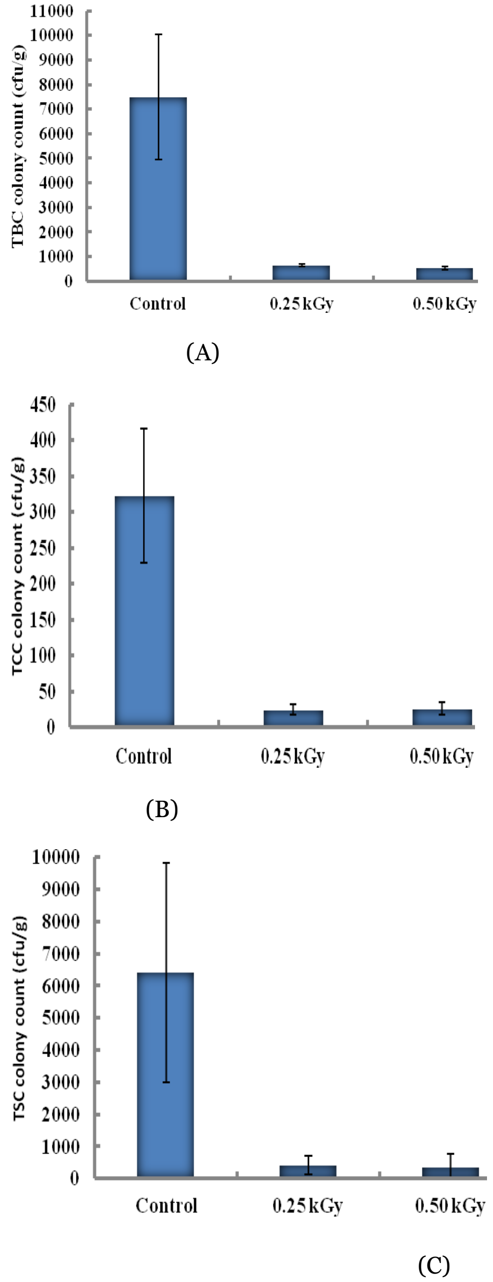

Fig. 4. Radiation effect on microbial load (A,B,C). Each value represents the mean of three samples and results were expressed as colony forming units per gram of the sample (cfu/g).

Microbiological study

Microbiological quality is an important factor to consider the quality of a food product. Ionizing radiation can safely and effectively eliminate the pathogenic bacteria from the food and disinfest the fruits and vegetables (Dionísio, 2009). The effect of gamma radiation on microbial quality of fresh bitter gourd have been shown in Figure 4 (A,B,C). After exposing to gamma irradiation at doses of 0.25 and 0.5 kGy, total bacterial count (TBC), total coliform count (TCC) and total salmonella count was significantly reduced (about one log) than the control samples.

Conclusions

The present study suggests that low dose gamma radiation could be useful for enhancing the antioxidant properties and microbial safety of fresh bitter gourd and thus play a positive role in preventing several physiological disorders and pathological processes in consumers.

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