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Effect of gamma irradiation on nutritional properties and antinutrient contents of *Citrus jambhiri* Lush. Fruits

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Abstract
Kachai lemon (*Citrus jambhiri* Lush.) is one of the most potential fruit crops in northeast India. The present study was conducted to improve the nutritional quality of *Citrus jambhiri* fruits under storage using permissible doses of gamma irradiation at 0.25, 0.5, 0.75 and 1.0 kGy. Proximate content such as moisture, ash, crude fat, crude fibre, crude protein and energy was increased at low doses of gamma rays (0.25-0.5 kGy) and decreased at higher doses (0.75-1.0 kGy). However, available carbohydrates and energy increased with increase in doses of gamma rays. The ascorbate content of fruit increased at 0.25 kGy and decreased at higher doses. Higher reduction in anti-nutrient content such as lipid peroxidation and tannin content was obtained at higher doses of 0.75-1.0 kGy. The results of the present study shed light on improving nutritional properties of *C. jambhiri* fruits under storage using gamma irradiation.

Keywords: anti-nutrients, *Citrus jambhiri*, gamma irradiation, proximate analyses

1. Introduction
Kachai lemon (*Citrus jambhiri* Lush.) is indigenous to Kachai village of Ukhrul, Manipur India. This is one of the largest horticultural commodities produced in this state which contains a unique quality of ascorbic acid to the tune of 50%, the highest so far available in the realm of citrus fruits. Storage, transportation and marketing of citrus fruits is a great concern in this marginal, fragile and inaccessible agro-ecosystem. On the other hand, storage of this fruit over one month reduces the quality parameters which fetch less market value to the growers. Improper storage invites pathogen or physical damage which cause direct loss of product quality through stimulate senescence (Aked, 2002) [1]. Chances of food-borne pathogen contamination is more in stored citrus fruits as it is often consumed as raw and/or after minimal processing (Warriner *et al*., 2009) [15].
Irradiation technology has been used extensively in fruits and vegetables for enhancing shelf life and retaining quality parameters during storage. Although, irradiation technology is not so popular in food industries in northeast India, the advantage of radioactivity in food preservation and processing would be an emerging scope in this region. The joint expert committee of FAO, IAEA and WHO has approved the permissible dose upto 10 kGy in food processing as it has no hazardous effect on nutrition and microbes (Khalil *et al*., 2009) [10]. Food irradiation is a more efficient physical method for preservation compared to other common means of preservation, which best retains standard of food and agricultural outputs. Irradiation of food products leads to minimum changes in the flavor, color, taste, nutrients and other related properties of food (Alothman *et al*., 2009) [2]. Physical mutagens such as gamma rays found more effective in food processing over the chemical mutagens for long term storage of fruits and vegetables. Low to medium dose of gamma irradiation (0.5, 1, and 2 kGy) resulted in significant changes in proximate contents, phenolics, flavonoids and antioxidant capacity on storage (Oufedjikh *et al*., 2000; Fan, 2005) [13, 8].
The study on effect of gamma irradiation on *Citrus jambhiri* fruits is still untapped. This study has been carried out to investigate the influence of gamma irradiation on nutritional aspects of the important Kachai lemon fruit of this region during storage. The findings of this study would shed light on ensuring food and nutritional security in this region in a sustainable way.

2. Material and Methods
2.1 Crop material and treatment conditions
Fruits of *Citrus jambhiri* were collected from the Kachai village of Manipur, India. Healthy uniform fruits were cleaned properly with running tap water.

All fruit samples in a lot of 10 fruits were packed in perforated transparent polyethylene bags and sealed prior to gamma irradiation. The fruits were irradiated with gamma rays from ^{60}Co source in a Gamma Chamber-5000 (Hyderabad, India) at ambient temperature of 25ºC at Quality Control Laboratory, Prof. Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad, India. The fruit samples were irradiated with 0.25, 0.5, 0.75, 1.0 kGy having dose rate of 0.509 kGy/h and stored at room temperature (25±2 ºC) for 30 days. Untreated fresh fruits stored in the similar condition was considered as control. Observations on nutritional properties and anti-nutrient content such as moisture content, ash content, crude fat, crude fibre, crude protein, available carbohydrate, ascorbate content, energy, MDA content and tannin.

2.2 Estimation of proximate contents

Proximate content such as moisture, ash, crude fat, crude fibre, crude protein, available carbohydrates and energy of the treated and un-treated fruit samples were determined according to the AOAC (2012) [3].

2.3 Estimation of ascorbate content

Ascorbate content (AsA) was determined following the methods of Mukherjee and Choudhuri (1983) [12]. AsA was estimated from 0.2 g of fruit sample with 5 ml of 6% trichloroacetic acid. Two ml of extract was mixed with 1 ml of 2 % dinitrophenyl hydrazine (in acidic medium) followed by one drop 10 % thiourea (in 70 % ethanol). The mixture was boiled for 20 min in a water bath. After cooling to room temperature, 5 ml of 80 % (v/v) H_2SO_4 was added to the mixture at 0°C (in an ice bath). The absorbance was recorded at 530 nm. The concentration of AsA was calculated from a standard curve plotted with a known concentration of ascorbic acid.

2.4 Estimation of antinutrient contents

2.4.1 MDA content

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content according to the method of Buege and Aust (1978) [6]. One gram sample was homogenized in 5 ml of 0.6 % (v/v) TBA solution in 10 % (v/v) trichloroacetic acid. The homogenate was centrifuged at 12,000 g for 15 min and the supernatant was heated in a boiling water bath for 15 min and then cooled quickly in an ice bath. The resulting mixture was centrifuged at 12,000 g for 15 min, and the absorbance of the supernatant was measured at 532 nm. Measurements are corrected for unspecific turbidity by subtracting the absorbance at 600 nm. MDA concentrations were calculated by means of an extinction coefficient of 155 $(mmol/L)^{-1}cm^{-1}$ (Zhangyuan and Bramlage, 1992) [16].

2.4.2 Tannin content

Tannin was determined by using Vanillin hydrochloride method (Robert, 1971) [14], with slight modifications. In this method, 0.5 g of sample was ground in 5 ml methanol and was centrifuged after 20-28 hr. To 1 ml of the supernatant, 5ml of vanillin hydrochloride reagent was quickly added, mixed and read in a spectrophotometer at 500 nm after 20 min. A blank was prepared with vanillin hydrochloride reagent alone. Catechin was used as standard and the results were expressed as catechin equivalents.

2.5 Statistical analysis

All parameters were observed in three replications with duplicate determinations. Statistical analyses were carried out in complete randomized design (CRD) following analysis of variance (ANOVA) and significance was tested at probability level $P \leq 0.01$.

3. Results and Discussion

The proximate compositions of irradiated and non-irradiated fruit at 30 days of storage showed significant variations for moisture content, ash, fat, fibre, protein, carbohydrate, ascorbate and energy (Fig. 1 A-H).

Moisture content in whole fruit and peel was significantly increased under 0.25 and 0.5 kGy and decreased at higher dose of 0.75 and 1.0 kGy at 30 days of storage (Fig. 1A). However, moisture content in juice (pulp) was decreased significantly with increasing doses of gamma rays. Moisture loss occurs due to respiration during storage which was lower in low dose of gamma irradiation (Arthur and Wiendl, 2000) [4].

Similarly, ash (Fig. 1B), crude fat (Fig. 1C) and crude fibre (Fig. 1D) contents were decreased significantly with increasing doses of gamma rays irrespective of whole fruits, peel and juice. Our study is in accordance to the findings of Bamidele and Akanbi (2013) [5] who found that the crude fat and fibre slightly decreased under gamma irradiation. However, crude protein content (Fig. 1E) and ascorbate content (Fig. 1G) were increased at lower doses of gamma rays (0.25- 0.5 kGy) and decreased at higher doses (0.75- 1.0 kGy). De Figueiredo *et al* (2014) [7] observed an alteration in ascorbate content in gamma ray induced papaya fruit. Ascorbic acid involved in several metabolic processes and scavenging reactive oxygen species (ROS) in fruits (De Figueiredo *et al*., 2014) [7]. Mehta and Nair (2011) [11] opined that the protein content of fruits decreased at higher doses of gamma rays. On the contrary, available carbohydrate (Fig. 1F) and energy (Fig. 1H) were increased with increasing doses of gamma rays. Irradiated samples exhibited increased carbohydrates over non-irradiated samples (Imadad *et al.*, 2010; Bamidele and Akanbi, 2013) [5] which probably resulted in higher energy (kcal/g). In our study, it has been observed that the lower doses of gamma rays (0.25- 0.5 kGy) induced less variations in proximate content.

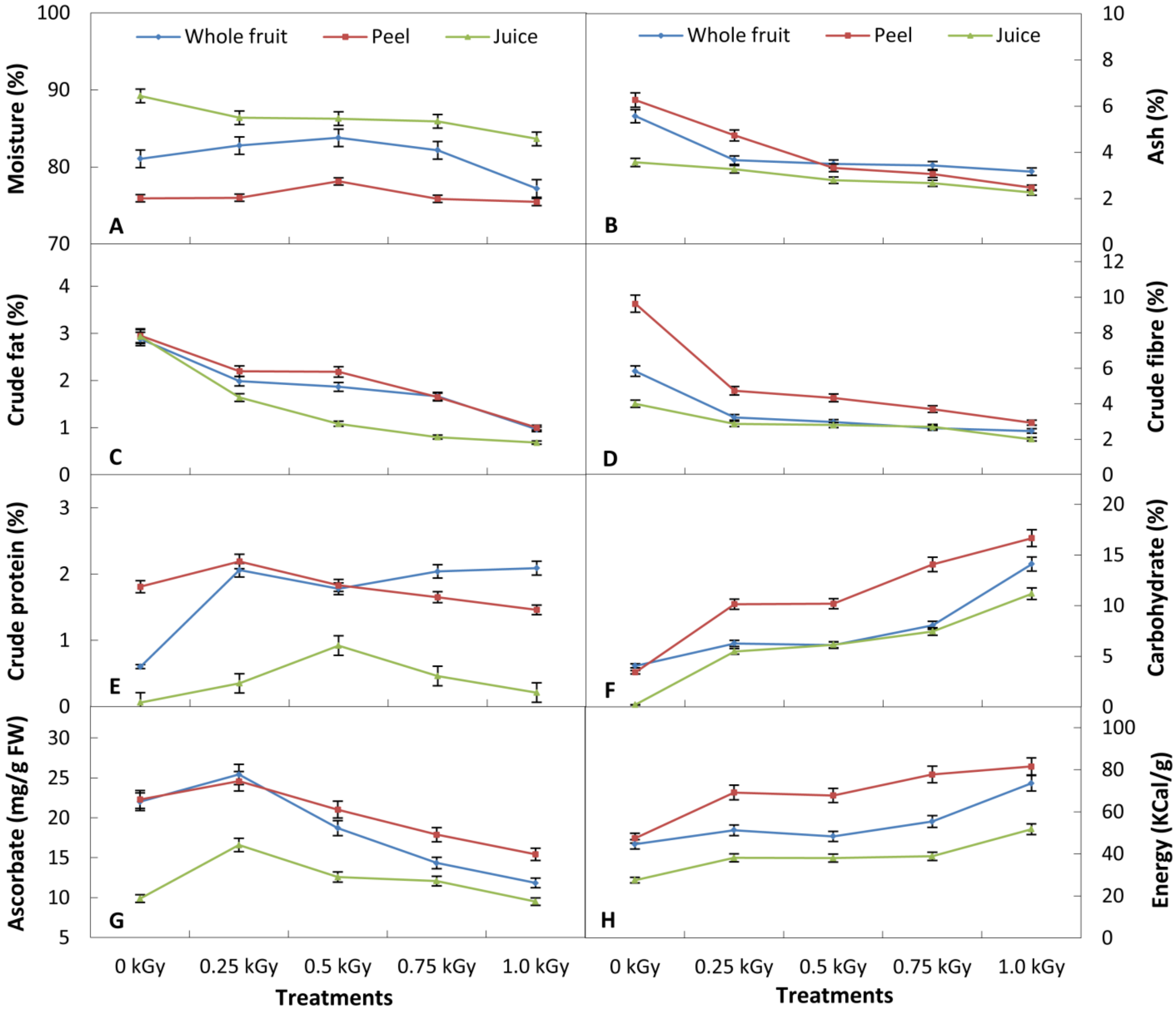

Fig. 1A-H Effect of gamma irradiation on proximate composition of *Citrus jambhiri* fruits

The data on anti-nutrient content *viz.*, MDA content and tannin content are presented in Fig. 2A-B. In our study, both MDA (Fig. 2A) and tannin content (Fig. 2B) was increased at lower doses of gamma rays (0.25- 0.5 kGy) which was decreased highly at higher doses of 1.0 kGy. Hence, higher doses would be imposed for inhibiting the anti-nutrient content in citrus fruits.

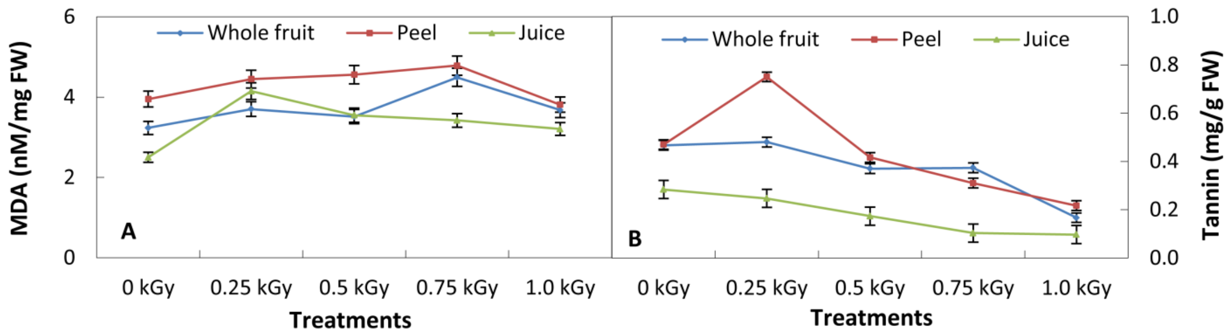

Fig. 2A-B Effect of gamma irradiation on anti-nutrient contents of *Citrus jambhiri* fruits

4. Conclusions

In the present study, it has been concluded that, gamma irradiation at 0.25-0.5 kGy is maintaining the nutritional properties in *Citrus jambhiri*, hence, could be effectively used for minimizing the nutritional loss in citrus fruit during storage. Minimal loss of ascorbic acid during storage, which is a signature component of this fruit, could be achieved at irradiation level 0.25 kGy. However, the higher doses at 0.75-1.0 kGy could be imposed to reduce the total carbohydrate and anti-nutrient content. This study will encourage further investigations of bioactive processes undergone owing to gamma irradiation.

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