

## Impact of Gamma Irradiation on Physical Parameters, Microbial Safety and the Total Polyphenolic Content of Commercially Available Ceylon Black Tea (*Camellia sinensis* L.)

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### ABSTRACT

The study was carried out to evaluate the impact of gamma irradiation on the physical parameters, microbial safety and the total polyphenolic content (TPPC) of Ceylon black tea (*Camellia sinensis* L.). Commercially available Broken Orange Pekoe Fannings (BOPF) black tea samples of 5 distinct brands (n=120) were irradiated at 0, 1, 2, 5, 10 and 30kGy doses using Co-60 gamma irradiator. Samples were analyzed for water activity ( $a_w$ ), color of tea and tea infusion, microbial safety and TPPC. Water activities of samples were within the range of 0.32 to 0.58 and no effect had been observed due to the irradiation. The mean value of L, b and E hunter parameters of tea infusion was increased where the “a” value was decreased with the increment of irradiation dose when compared to the control sample. High irradiation doses resulted in darker color of the tea infusion. Irradiated samples showed a significant reduction of the total plate count. After 5kGy dose, irradiated samples were in sterilized condition. Yeast and mould counts were gradually decreased with the irradiation dose. TPPC of tea leaves of both irradiated and non-irradiated samples ranged from 9.17- 37.98 GAE/240 ml and TPPC values were increased with the irradiation dose.

Results conclude that 5kGy is the optimum dose for the effective microbial safety, preserving the physical parameters and TPPC of commercially available Ceylon black tea.

**Keywords:** Ceylon black tea, color of tea, irradiation, microbial safety, physical parameters, total polyphenolic content

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## INTRODUCTION

Tea is one of the major agricultural produces in Sri Lanka, approximately contributing 2% of the national GDP, 70% to the agricultural export earnings and nearly 15% of total export earnings (Herath & Weersink, 2009). The health benefits of tea include prevention of cancers and cardiovascular diseases, strengthening of immune systems and tea is a source of natural, -antioxidant (Fujita & Yamagami, 2008; Hamer, 2007).

Black tea is the most important one among all other teas consumed all over the world and made sometimes difficult to comprehend by the tea consumers as tea testers developed their own languages to describe various quality attributes of black tea infusion (Liang et al., 2005). There are two main categories of tea, which are orthodox black tea known as broken leaf grade and whole leaf grade. Broken leaf grade has three distinct types namely, Dust No.1, Broken Orange Pekoe (BOP) and Broken Orange Pekoe Fannings (BOPF). BOPF consists of particle size of 500 – 850  $\mu\text{m}$  (Ratnasooriya & Muthunayake, 2014).

Black tea preparation mainly consists of harvesting, withering, rolling, fermentation and drying (Vargas & Vecchietti, 2016). Withering is the crucial step which facilitates the rolling process by disrupting cell structure and the beginning of the tea fermentation (Luczaj & Skrzydlewska, 2005). Contamination of microbes can easily occur during the withering stage due to high increment in the humidity level. Tea leaves and tea buds of plants are collected and taken for processing without cleaning and washing (Mishra et al., 2006). Kausar et al. (2013) indicated that microbes easily contaminated tea during harvest, drying, fermentation, and storage. When the tea is carrying microorganisms, it gives a potential health risk to consumers (Mishra et al., 2006).

Food Irradiation is a treatment, which uses ionizing radiations to improve the microbial safety and storability of food (Mali et al., 2011). Food irradiation causes some issues such as low microbial efficiency, color deviations, flavor changes and functional property losses compared to other preservation technologies that are applied for decontamination of dried foods. Mishra et al. (2006) showed that gamma irradiation could be used as a processing technique which would improve the microbial quality and safety of tea. However, tea manufacturers were concerned about the irradiation process which could result in chemical changes, vitamin depletion, transformation of xenobiotic and survival of bacterial toxins on tea. According to Kausar et al. (2013), instead of irradiation, chemical fumigation with Methyl Bromide (MeBr) and Phosphine ( $\text{PH}_3$ ) is the conventional decontamination method of tea. However, it has been prohibited or been restricted gradually due to their safety or environmental concerns, necessitating the development of alternative methods. Only a limited number of researches conducted on the effect of irradiation on black tea (Mishra et al., 2006; Rashid et al., 2016; Fanaro et al., 2014). Further studies show that different doses of gamma irradiation influence on the chemical and physical structure differences in microorganisms (Farkas, 2006) and thereby reduction of microbial growth can happen.

The objective of this study was to determine the impact of gamma Irradiation on the physical parameters, microbial safety and total polyphenolic content (TPPC) of commercially available Ceylon black tea. Also, we expected to find out the effective irradiation dose for microbial decontamination of commercially available black tea in Sri Lanka.

## **MATERIALS AND METHODS**

### **SAMPLE SELECTION**

Five brands were selected considering the BOPF. Totally 120 packets, 24 from each brand, of 50 g were bought from the market. Tea samples were carefully selected to obtain the physical parameters, microbial safety and the TPPC. Proceeding with the test of each parameter, three replicates from each brand were selected per each irradiation dose level.

### **Irradiation**

Tea samples were irradiated in a Co-60 irradiator (Sri Lanka Gamma Centre) with the targeted doses of 0, 1, 2, 5, 10 and 30kGy. Actual doses were 0, 1.06, 2.02, 4.97, 9.46 and 29.29kGy respectively. The targeted dose values are used in this context hereafter to represent the dose levels. The source strength was approximately 154 kCi with a dose rate of 4.2 Gy/min. Dosimetry was performed using Perspex dosimeters (Harwell-Type:3042, United Kingdom) and ceric-cerous dosimeters (BRIT, India). Samples were rotated during the irradiation process to achieve uniform doses.

### **Determination of Water Activity**

The water activity was measured using a water activity meter (Novasina LabMASTER, Switzerland). A plastic chamber containing the test tea sample (2 g) was kept in the instrument to obtain the corresponding water activity at 25°C.

### **Moisture Content**

The moisture content was determined on a dry weight basis using moisture balance (Mishra et al., 2006). The tea samples (2 g) were kept in the container of moisture balance (MRS 120-3, USA) and the percentage decrease in weight at  $105 \pm 1^\circ\text{C}$  was conveyed as moisture content.

### **Determination of Color in Tea Powder**

Color analysis on black tea powder was carried out using a chromameter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-200 measuring head. Color was expressed in L, a, and b Hunter scale parameters on the digital screen of chromameter.

The Hunter scale color parameters  $\Delta L$ ,  $\Delta a$  and  $\Delta b$  represented the light–dark, red–green, yellow–blue in color.

$$\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)} \quad [1]$$

### **Determination of Color in Tea Infusion**

Tea samples (5 g of each) were extracted into 240 ml of freshly boiled distilled water for 5 minutes. The infusion sample was cooled into room temperature before it was filtered using Double-ring No 102-filter paper (Xinhua Paper Industry Co Ltd, Hangzhou, China). The obtained filtrate was diluted using 10ml of distilled water up to 250 ml of total volume. Distilled water was used as the control to reduce the errors from different determination conditions of equipment and temperature differences. Color analysis on black tea infusion was carried out using a chromameter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-200 measuring head.

### **Estimation of Total Polyphenolic Content (TPPC)**

The TPPC in tea leaves were determined by colorimetric method using Folin-ciocalteu reagent according to the ISO standard 14502-1:2005(E) (International Organization for Standardization [ISO], 2005). The absorbance was read at 765 nm using UV-visible spectrophotometer (Optima, SP-300, Japan). Gallic acid was used as the standard solution and TPPC in 0.2g of black tea powder was expressed as milligram of Gallic acid equivalent (GAE) per 240ml.

### **Determination of Total Plate Count (TPC)**

The TPC was determined according to the International Standard 4833-1:2013(E) (International Organization for Standardization [ISO], 2013). Plates were incubated in an inverted position for 72 hours at  $30 \pm 1^\circ\text{C}$  in a culture incubator (ICP 600, Germany). Colonies were counted using colony-counting equipment (Rocker, Galaxy 330, India). The numbers of colony forming units (CFU) on plates (containing 25 to 250 colonies) were calculated per gram of sample.

### **Determination of Total Coliform**

The total coliform count was determined according to the International Standard 4831-1:2006(E) (International Organization for Standardization [ISO], 2006). The prepared samples were placed in a water bath (GFL 1008, India) for 48 hours at  $37 \pm 0.5^\circ\text{C}$ . Tubes were noted which showed growth after 24 hours. When both total and fecal Coliforms were confirmed, a Brilliant Green Bile Broth (BGLB) and an *E. coli* medium tube were

inoculated from each presumptive positive. Then the tubes were carefully incubated for 48 hours at  $37 \pm 0.5^\circ\text{C}$  for total *Coliforms* (BGLB broth) or for 24 hours at  $44 \pm 0.5^\circ\text{C}$  for fecal coliforms (*E. coli* medium). After that tubes were noted which showed growth in the production of gas and recorded the number of positives for each sample dilution.

### Determination of Yeast and Mould Count (YMC)

The YMC was determined according to the ISO 21527-2:2008(E) (International Organization for Standardization [ISO], 2008). Plates were incubated in upright position at  $25 \pm 1^\circ\text{C}$  for 5 days to 7 days in a culture incubator (ICP 600, Germany). Then colonies were counted using colony-counting equipment (Rocker, Galaxy 330, India). The numbers of colony forming units (CFU) on plates (containing <150 colonies) were calculated per gram of sample.

### Statistical analysis

Descriptive and inferential statistical analysis was done by using SPSS 16.0 software. The individual observations of tea samples in case of physical, microbial and TPPC activities were analyzed and expressed in terms of mean and standard deviation (SD). The mean values of control and irradiated samples were compared using one-way analysis of variance (ANOVA) to determine the significance of their difference ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### Water Activity

The water activity of the control and irradiated tea were within the range of 0.32 to 0.58 and the differences were statistically insignificant ( $P > 0.05$ ) (Table 1). Low water activity and low moisture content of foods act as significant barriers to the growth of microorganisms (Fanaro et al., 2014) and the minimum  $a_w$  required for the growth of microbes was 0.6

Table 1  
*Mean value of water activity and moisture content of commercially available Ceylon black tea samples with different irradiation doses*

Dose (kGy)	Water activity	Moisture content (%)
0	$0.46 \pm 0.07$	$5.42 \pm 0.94$
1	$0.49 \pm 0.04$	$5.28 \pm 1.02$
2	$0.44 \pm 0.07$	$5.22 \pm 0.93$
5	$0.49 \pm 0.05$	$5.19 \pm 0.89$
10	$0.51 \pm 0.06$	$5.19 \pm 1.02$
30	$0.51 \pm 0.07$	$5.41 \pm 1.18$

The data represent the mean  $\pm$  SD of samples in each of five different tea brands. The mean values in a column are not significantly different ( $P < 0.05$ ) as analyzed by one-way analysis of variance (ANOVA).

(Beuchat et al., 2013). Water is the major source of ions and radicals, as well as the greatest mass component in most foods. The irradiation process leads to the formation of radiolytic products such as  $e^-$  (aq),  $(H_2O^+)$ ,  $(H_3O^+)$ ,  $(\cdot OH)$ ,  $(H\cdot)$  and  $H_2O_2$  (Breen & Murphy, 1995). The present study showed irradiation treatment did not affect the water activity level of tea.

### Moisture Content

Table 1 shows the moisture content of the control and irradiated tea and they were within the range of 4.17 - 6.59%. Results reveal that the mean moisture content was not differed due to the higher doses of irradiation in each brand and the differences were statistically not significant ( $P > 0.05$ ). Tea approximately contains 6% of moisture during its proper storage, which reduces the microbial level in normal condition. Thomas et al. (2008) reported that the metabolic activities of microbes were accelerated by the moisture content of tea. The present study showed that irradiation treatment does not affect the moisture content of the tea samples.

### Color of Tea and Tea Infusion

Compared with the control sample of tea infusion, hunter scale parameters of L and b increased with the irradiation dose level (Table 2). The present study, Hunter scale 'L' value depicted the enhancement of the lighter color in black tea infusion by irradiation. The 'b' value of infusion was increased with the higher dose levels that resulted in yellow color. When giving high irradiation doses, tea infusion remained darker in color and significant difference ( $P < 0.05$ ) was observed in the ' $\Delta E$ ' value after irradiation compared to the non-irradiated samples. Therefore, irradiation enhances the color of black tea infusion. According to Liang et al. (2005), darker color in tea infusion is considered as one of the main qualities in black tea.

Table 2  
Mean value of L, a, and b in tea infusion and tea powder of commercially available Ceylon black tea samples with different irradiation doses

Dose (kGy)	Tea Infusion			Tea powder		
	L*	a	b*	L	a	b
0	44.56 ± 2.25	22.12 ± 1.11	37.14 ± 2.24	23.40 ± 0.38	4.03 ± 0.24	5.01 ± 0.59
1	50.41 ± 3.74	20.15 ± 1.78	45.04 ± 3.20	23.54 ± 0.27	4.02 ± 0.27	5.01 ± 0.48
2	48.39 ± 1.93	20.95 ± 1.01	41.83 ± 2.60	23.52 ± 0.27	4.04 ± 0.27	4.99 ± 0.53
5	50.26 ± 1.77	22.03 ± 1.95	45.20 ± 2.78	23.90 ± 0.47	3.85 ± 0.34	4.48 ± 0.69
10	52.14 ± 1.83	21.91 ± 1.49	47.30 ± 2.51	23.90 ± 0.47	3.97 ± 0.31	4.68 ± 0.49
30	53.71 ± 3.05	20.53 ± 2.69	48.03 ± 1.84	23.40 ± 0.39	3.85 ± 0.24	4.30 ± 0.58

The data represent the mean ± SD of samples in each of five different tea brands.

Positive values for L, a and b indicates lighter, redder and yellower accordingly.

\*The values marked are significantly differed ( $P < 0.05$ ) with increasing irradiation doses when compared to the control sample. The analysis was performed by one-way analysis of variance (ANOVA).

Further, there was no significant difference ( $P>0.05$ ) found in hunter scale L, a and b parameters before and after the irradiation of tea powder, hence not in ' $\Delta E$ ' value. Therefore, no effect was found on the color of tea powder due to irradiation.

### Total Polyphenolic Content (TPPC)

The differences in the TPPC of 1, 2 and 5kGy irradiated tea samples were statistically insignificant ( $P>0.05$ ) compared with the control (Figure 1). At higher doses of 10kGy and 30kGy, tea samples had an increment of TPPC when comparing with the control (Figure 1).

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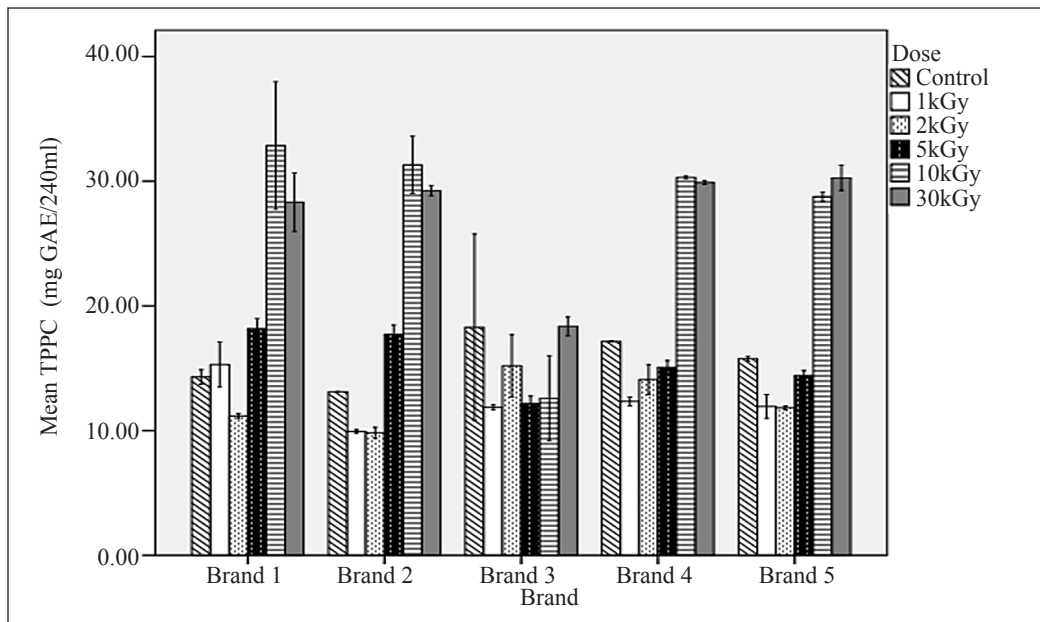


Figure 1. Variation of mean total polyphenolic content of each brand, according to different irradiation doses

Results of this study revealed that when increasing the irradiation dose level after 5kGy, the TPPC level was increased. Hirashima et al. (2013) indicated that the degradation or in solubilization of phenolic compounds occurred due to irradiation. It may due to the breakdown of the aflavin fractions in tea. Wiseman et al. (1997) explained the breakdown of the aflavins, which resulted in the formation of the arubigins compound, which provided high TPPC levels. According to Mali et al. (2011), differences of the TPPC due to irradiation may be caused by plant type, geographical and environmental conditions, sample state and phenolic content of the sample. Hence the variation of TPPC in distinct brands of different magnitude might be due to the influence of those factors.

## Microbial Safety of Tea

**Total Plate Count (TPC).** This study focused to assess the efficiency in removing bacterial contamination using gamma irradiation of commercially available Ceylon black tea. Hence, commercially available different tea brands were collected and analyzed for distinct types of microbial contaminations.

There was a significant difference ( $P < 0.05$ ) between the values of TPC before and after irradiation in all samples (Figure 2). The TPC in all samples collected was within the range of  $0.45 \times 10^3$  to  $6.64 \times 10^3$  CFU/g. The presence of a high microbial load in the tea samples indicates that tea contamination has been occurred. This study showed that applying 1kGy of irradiation to the tea sample were eliminated approximately half of the viable bacteria count compared to the control sample. Beyond the 5kGy of irradiation dose, complete removal of bacteria can be seen (Figure 2). According to the Farkas, (2006) the mechanism behind ionizing radiation is mainly the inactivation of microbes' due to its nucleic acid damage, direct damage or indirect damage caused by the oxidative radicals originating from the radiolysis of water.

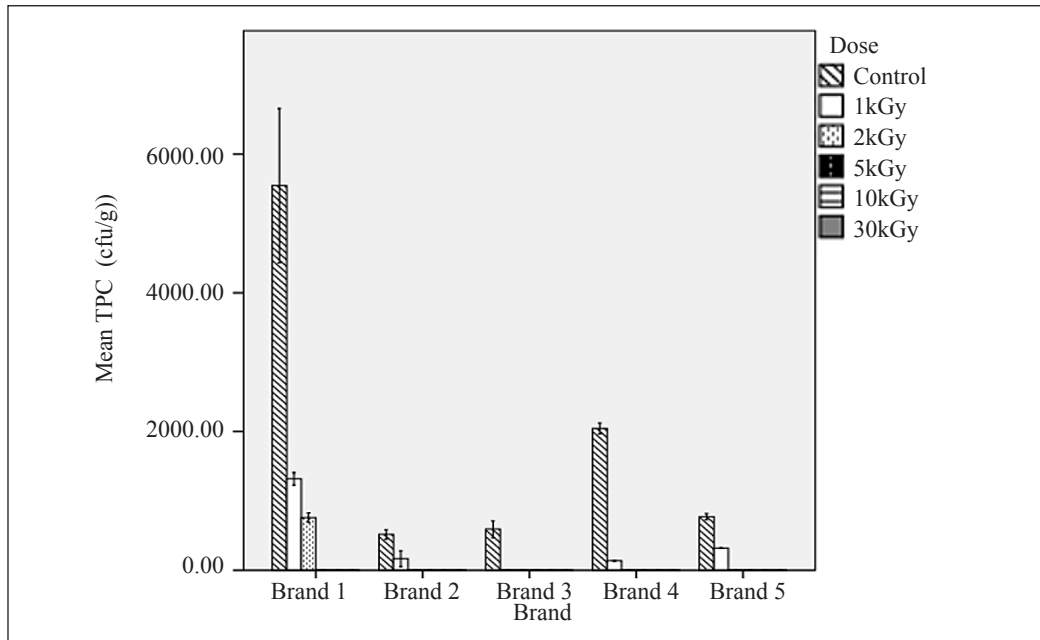


Figure 2. Variation of mean total plate count for each brand, according to the different irradiation doses

**Coliform.** Both Irradiated and non-irradiated Ceylon black tea samples were shown less than 01 coliform counts which confirmed that none of the tea samples were contaminated with coliform bacteria. According to Zhao et al. (1997), the presence of coliform may associate with the fecal contamination of food plant, including tea leaves. This may be the reason behind the low coliform counts, which have been observed in the present study.



**Yeast and Mould Count (YMC).** The yeast and mould contamination of commercially available Ceylon black tea were within the range of  $0.45 \times 10^2$  to  $6.81 \times 10^2$  CFU/g. Results revealed a significant reduction ( $P < 0.05$ ) of the YMC with the increment of irradiation dose in all samples compared to the control (Figure 3). However, the study showed a high resistivity of yeast and mould when compared to the bacteria. Generally, yeast and mould are more resistant than bacteria for radiation (Rashid et al., 2016). According to the Mishra et al. (2006), 5kGy was the most effective dose for elimination of microbial contamination. According to Farkas (2006), the radiation energy amount needed to control microorganisms varies from the number of organisms present in the food and resistivity of the species. Results of the present study shows that, 5kGy irradiation dose ensures the microbial safety of black tea.

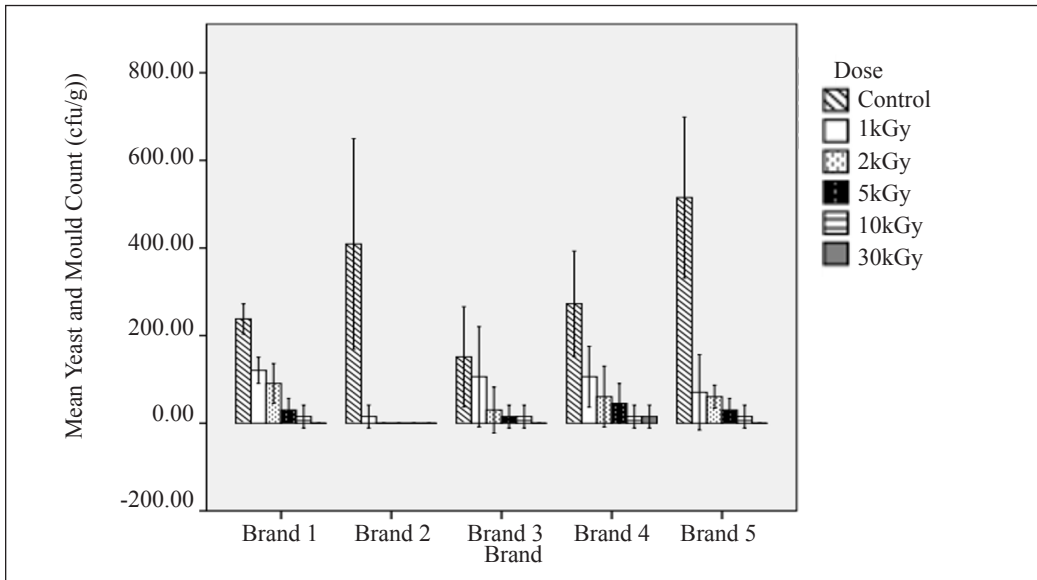


Figure 3. Variation of mean yeast and mould count of each brand, according to the different irradiation doses

Considering the quantity exported per year, the incidents of rejection of Sri Lankan teas from few ports of entry due to high microbial contamination was reported (Lei, 2017). Due to that the growing demand for irradiation as a substitute for chemical fumigation to quarantine the pests has been seen and tea is becoming an ideal large scale local candidate for irradiation (Kausar et al., 2013). Studies carried out on the effects of irradiation on quality of tea were limited in numbers and the studies carried out up to now was limited to green tea. Further, there were no studies conducted in Sri Lanka on irradiation of tea and impact on the chemical components naturally available in tea.

## CONCLUSION

The present study carried out to measure the effectiveness of gamma irradiation on commercially available black tea. The research mainly focused on the behavior of certain attributes of tea. The 5kGy dose was the most effective dose for microbial control of commercially available Ceylon black tea, while preserving physical parameters, microbial safety and TPPC of tea. Therefore, irradiation can be used as a preservation technique in the tea industry with a much-regulated manner.

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