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Microbial decontamination of gamma irradiated black tea and determination of major minerals in black tea, fresh tea leaves and tea garden soil

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ABSTRACT

The purpose of this research was to establish an effective dose of gamma irradiation to eliminate any possible bacterial contamination as well as assess the levels of essential major elements [sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe)] present in black tea. The samples were compared to fresh tea leaves and soil from the tea gardens of two distinct tea cultivating regions (Sylhet and Moulvibazar) in Bangladesh. Maximum coliform bacterial contaminants (4.0×10^2 cfu/g) were completely eliminated following an irradiation dose of 2.5 kGy, whereas a viable bacterial load of 9.0×10^3 cfu/g was completely eliminated at 5.0 kGy. Major elements were present at higher concentrations in black tea samples originating from Sylhet compared to those from Moulvibazar. Among the analyzed elements, only Na and Fe were present at higher concentrations in the fresh tea leaves from Sylhet, whereas K, Ca and Mg were observed at higher concentrations in the tea leaves from Moulvibazar. The amount of each mineral in the black tea is acceptable for daily intake and is safe for consumption. It is concluded that black tea from Bangladesh is a rich source of minerals and could be free of microbial contamination after receiving 5.0 kGy of gamma radiation.

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1. Introduction

Tea is one of the most popular non-alcoholic beverages in the world due to its pleasant taste, aroma and incentive physiological functions, produced from the leaves of the *Camellia sinensis* shrub (Dufresne & Farnworth, 2001; Zhu, Hackman, Ensunsa, Holt, & Keen, 2002). Processed teas are primarily classified into four types (black, green, oolong and brick tea) based on their degree of fermentation (Karak & Bhagat, 2010). In Asia, consumers primarily prefer fully fermented black and semi-fermented green teas (Zaveri, 2006). The trend of tea drinking has long been promoted due to its association in reducing blood cholesterol and risk of cardiovascular disease, cancer, immune disorders and Parkinson's disease (Fujita & Yamagami, 2008; Hamer, 2007; Siddiqui, Raisuddin, & Shukla, 2005). Polyphenols, amino acids and vitamins are the primary biologically active components in tea, which have been confirmed to exhibit antioxidant, antibacterial, antitoxin, antiviral, anti-inflammatory and anticancer activities (Friedman, 2007; Korte et al., 2010).

Tea is a good source of minerals, and the presence of both essential and non-essential minerals in tea from different countries has been reported in some of the previous studies (Karak & Bhagat, 2010; Srividhya, Subramanian, & Raj, 2011). The human body requires these elements within a certain limit for proper growth and development because of their vital roles in metabolic processes and general welfare for many individuals. However, excess intake of these elements can lead to chronic toxicity (Koche, 2011). Therefore, determining the levels of macro-elements present in

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beverages, water, plant materials and soil has become important. Moreover, to date, green leafy plants are considered to be the major source of these minerals (Ansari, Norbaksh, & Daneshmandirani, 2007). The metallic constituents of tea leaves vary largely depending on its type (black or green) and growing climate (moisture, pH, organic and mineral content of soil). Usually, plants absorb minerals from the growth medium and distribute them to all other parts of the plant as required (Ansari et al., 2007; Khan, Biswas, Saha, & Motalib, 2012). It has been reported that anthropogenic contamination of soil, water and atmosphere may occur via atmospheric dusts, motor vehicle exhausts and other gaseous and liquid pollutants (Gorgulu, Kipcak, Ozdemir, Derun, & Piskin, 2014; Kaličanin & Velimirović, 2013; Pereira & Dantas, 2016; Pohl et al., 2016; Szymczycha-Madeja, Welna, & Zyrnicki, 2013). This process may arise when associated with the agricultural practices or other processes associated with the growth and cultivation of the medicinal plants including the use of plant protective agrochemicals, fertilizers and/or herbicides and irrigation utilising contaminated water (Kaličanin & Velimirović, 2013; Pereira & Dantas, 2016; Pohl et al., 2016; Szymczycha-Madeja et al., 2013). As a result, contamination of medicinal plants or teas with toxic elements such as arsenic (As), aluminium (Al), cadmium (Cd), mercury (Hg) and lead (Pb) (Pohl et al., 2016) may occur.

Bangladesh has developed a promising and sustainable tea industry due to its suitable geographical location for tea plantation where tea plants are fairly cultivated on the hilly area of greater Sylhet, Moulvibazar, Comilla and Chittagong (Mamun & Ahmed, 2011). Bangladesh produces approximately 63 million kg of tea annually for both local and international consumers, a number that continues to grow. Today, tea has become an important cash crop in Bangladesh and is considered a major agricultural commodity for sustaining the country's economy. With the rapid urbanization and changes in the socio-economic status of the population, tea consumption is increasing daily (Khan et al., 2012). Nevertheless, the importance of a nutritional assessment of tea from Bangladesh has been nothing more than an afterthought.

Radiation is a physical treatment for the preservation of many different types of food commodities and has been applied in an increasing number of uses due to its various advantages (Diehl, 1999). Being a cold process, regulated irradiation can eliminate microbial contamination while retaining the color, flavor, taste and aroma of the material, which may undergo severe deterioration following heat treatment (Mishra, Gautam, & Sharma, 2006). Although the final drying step of tea processing utilizes steam treatment at 80 °C to reduce the high bacterial and fungal load, post-processing steps such as storage, handling and packing may result in further microbial contamination of processed teas (Bouakline, Lacroix, Roux, Gangneux, & Derouin, 2000). Moreover, utilization of hot water for the preparation of tea infusion may reduce microbial risks, although the use of water at a sub-boiling temperature may not eliminate all pathogens, including bacteria spores (Mishra et al., 2006).

To date, there are limited data available on the effects of radiation in various tea proprieties, including in *Camellia sinensis* and in others plants extracted by infusion. Some of them also analyzed de fungal contamination, which is normally more radio-resistant than bacteria. Thus, the present study aimed to investigate the optimal gamma radiation dose for bacterial decontamination of black tea as well as to determine the major minerals [primarily sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe)] in the most popular tea produced in Bangladesh (black tea). The levels will be compared with those of fresh tea leaves and soil of the corresponding tea gardens from two main tea growing regions in the Sylhet and Moulvibazar districts in Bangladesh.

2. Materials and methods

2.1. Chemicals and reagents

The standard reference elements Na, K, Ca, Mg and Fe were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). The chemicals used for the digestion technique included 65% nitric acid (HNO_3) and 37% hydrochloric acid (HCl), which were of analytical grade, and were purchased from Merck (Darmstadt, Germany).

2.2. Sample collection and preservation

A total of 30 samples, including fresh tea leaves (n = 10), black tea (n = 10) and soil samples (n = 10) from the tea gardens, were randomly collected from two main tea-growing regions in Bangladesh (the Sylhet and Moulvibazar districts). Among these, five fresh tea samples (500 g) were randomly collected from five different locations in the Sylhet district and the Moulvibazar district and were authenticated by a botanist from the same laboratory. The soil samples (n = 10) were also taken from the corresponding tea gardens where the samples were collected. In addition, five black tea samples were collected from the local markets of Moulvibazar and Sylhet, which sold tea from their respective local tea gardens. All of the collected samples were kept in clean polyethylene bags and immediately transferred to the laboratory of Agrochemical and Environmental Research Division (Institute of Food and Radiation Biology, Bangladesh Atomic Energy Commission, Savar, Dhaka).

2.3. Irradiation treatment

The samples were packed into sterilized (15 kGy radiation dose) low-density polyethylene (LDPE) plastic bags before being sealed. Three samples were prepared for each sample type. The packets were individually labeled, and a series of doses (0.0, 2.5 and 5.0 kGy) were applied to each packet. In this study, the radiation source was an 1850 terabecquerel (50 kCi) 60 Co gamma-irradiator (Model: 400094, Board of Radiation and Isotope Technology, V. N. Purav Marg. Mumbai, India) located at the Institute of Food and Radiation Biology (IFRB) [Atomic Energy Research Establishment (AERE), Bangladesh Atomic Energy Commission, Bangladesh]. Non-irradiated samples were kept as a control. Following the radiation treatment, both the irradiated (at dose rate of 6.4 kGy/h) and the non-irradiated samples were analyzed for the presence of bacteria based on the method described below.

2.3.1. Total viable bacterial count and total coliform count

The total viable bacterial count (TVBC) and total coliform count (TCC) were determined based on the method described by Zaman, Alam, Ahmed, Uddin, and Bari (2014). Briefly, stock sample suspension was prepared by homogenizing 1 g of tea sample in 225 mL sterile saline water (0.85 g/L sodium chloride). From the homogenized sample, 1 mL aliquot was transferred into the test tube containing 9 mL of sterile saline water and was uniformly mixed with a vortex mixture to prepare a $1{:}10^{-1}$ dilution. Again the step was repeated to prepare a mixture with a dilution of 10^{-2}. By conducting four times further dilutions, the final dilution was 10^{-6} which was subsequently used in the experiment.

The appropriate dilutions (0.1 mL of aliquot from the final dilution) were plated on tryptic soy agar (Oxoid Ltd., Hampshire, England) and incubated at 35 °C for 24 h for TVBC. The bacterial count was reported as colony forming unit/g of sample (cfu/g) (Zaman et al., 2014).

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