

Effect of Electron Beam Irradiation on Microbial Decontamination and Phytochemical Contents of Spices

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ABSTRACT: The effects of electron beam irradiation on microbial load, total phenolic content, total flavonoid content has been investigated on commercially available spices. The initial total bacterial count were found to be in the range of 10^6 cfu/g, whereas the fungal counts were in the range of 10^4 cfu/g in both spices. Exposure to electron beam irradiation in the dose range of 6-12 kGy was sufficient to reduce the total microbial load at acceptable quality limits and did not produce any significant ($p < 0.05$) effect on total phenolic and total flavonoid content in both red chilli and coriander powder. The dose response plot has been studied at various dose and found to be exponentially decreasing. The D_{10} value for the total bacterial and fungal count in these spices was found to be in the range of 2 to 2.5 kGy.

This paper describes the feasibility and effectiveness of electron beam irradiation for microbial decontamination of dry spices, and effect on biologically active compounds present in both spices.

Keywords: Coriander, Electron accelerator, Flavonoids, Phenolics, Red chilli

INTRODUCTION

An electron beam radiation processing facility based on 10 MeV electron LINAC is operational at RRCAT. The facility is well equipped with measurement systems to study & demonstrate the effectiveness of electron beam technology for shelf life extension of food products, microbial decontamination of spices-herbs and dry fruits as well as sterilization of medical products. A microbiology laboratory has been setup in order to perform the microbiological analysis of the electron beam (EB) irradiated food, agro and medical products. In present study red chilli and coriander powder, which are being grown at large scale in Malwa region and are being exported due to their special taste, aroma, flavour & pungency, were electron beam irradiated and analyzed.

Red chilli (*Capsicum annuum*) and Coriander (*Coriandrum sativum* L.) are popular spices which are consumed around the world. India is world leader in chilli production (36% share in global production) followed by China and Pakistan [1]. India is also the biggest producer, consumer and exporter of coriander in the world with an annual production averaging around 3 lakh tonnes. Rajasthan (54%) and Madhya

Pradesh (17%) are the two largest producing states in the country contributing over two-thirds to the country's total coriander production [2]. We have selected these spices as their consumption is increasing due to their flavor and also the presence of low amount of sodium & fat [3]. In addition to being a flavoring agent, coriander and red chilli also possess pharmaceutical and antioxidant properties.

The environmental conditions (high temperature, humidity & rainfall) in tropical regions as well as the harvesting, transport, threshing, drying, storage conditions are potential sources of contamination with bacteria, fungi & insects [4, 5]. Therefore, high level of microbial contamination is often present in spices. As per ICMSF (International Commission on Microbiological Specifications for Foods) spices with $< 10^4$ cfu/g counts are considered to be acceptable quality and $10^4 - 10^6$ cfu/g are considered marginal quality [4].

The conventional methods of microbial decontamination in spices have been fumigation with gaseous ethylene oxide & methyl bromide which are prohibited in many countries (Japan, UK) due to their carcinogenic effect and MtBr is an ozone depleting

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substance comes under the Montreal Protocol & will be phased out globally [6].

Use of ionizing radiation, as a physical method of microbiological decontamination of food including spices & herbs, is a better alternative approach as it doesn't possess any toxicological hazard and is not associated with toxicity in food [7, 8]. As per international agencies overall average dose 10 kGy can kill most microorganisms without any deterioration effect on quality of food. In our country most of the irradiation studies on agricultural commodities have been carried-out with Co-60 radioisotope and exploration using electron beam is limited due to non-availability of suitable accelerators. The present work has been carried out using an electron beam irradiation facility at RRCAT, to study the effect of electron beam radiation on microbial load and phytochemical contents of dried red chilli powder and coriander powder.

MATERIAL AND METHODS

Sampling & materials

The samples of chillies and coriander powder in packets were obtained from local market. These samples were repacked in LDPE packages of thickness, 300 gauge approximately 200 g each under aseptic condition. Microbiological culture media, all chemicals and organic solvents were used of reputed firm & analytical grade.

Irradiation set-up & Irradiation of samples

The electron beam irradiation was carried out in 10 MeV electron Linac. The Linac is oriented in horizontal plane and the accelerated electron beam is scanned in vertical plane using a time varying magnetic field. The spices to be irradiated, were exposed to the radiation field with the help of a belt conveyor at a constant speed. Desired doses were given by controlling the number of passes. The accelerator was operated at beam energy of 7.8 MeV with beam power of 1.5 kW and beam parameters were optimized to deliver uniform surface dose within 5%.

Dosimetric measurements were carried out using the radiochromic films (GEX, USA) and EPR alanine pallets (Bruker, Germany). The optimum thickness (depth at which the exit dose is equal to the surface dose) for one sided irradiation of the spices with density ~ 0.50 g/cc was found to be 5 cm. The thickness of the sample packets to be irradiated was < 3.5 cm. The overall dose uniformity ratio (DUR) as measured by placing the alanine pallets within the

dummy samples was found to be 1.2. The samples were irradiated at the doses of 4, 6, 8, 10 & 12 kGy. All the control samples as well as the irradiated samples were stored at room temperature during the month of April-May.

Microbiological analysis

Total microbial count, yeast & mold counts were carried out by following BAM (Bacteriological Analytical Manual) [9]. Irradiated & non-irradiated (control) samples were suspended in sterile peptone-physiological saline (0.1% w/v neutral peptone, 0.85% w/v sodium chloride, pH 7.2) and homogenized for 2 min. The homogenized solution was serially diluted and appropriate dilution were plated on plate count agar (PCA) for total aerobic mesophilic bacteria (TAMB) count using standard pour plate method and incubated at 37°C for 24-48 hours. Enumeration of total yeast & mold count was made on spread plates on dichloran rose bengal chloramphenicol agar (DRBA) and incubated for 3-7 days at 28°C in the dark and under normal atmosphere. The results were expressed as average cfu g⁻¹.

Phytochemical analysis

Sample preparation

2 gm of dried powdered red chilli and coriander samples were shaken in 20 ml of the extraction solvent (90 ml absolute methanol + 10 ml 0.05% v/v aqueous HCl) for 30 min. at 25°C using a laboratory shaker (Thermo Fisher Scientific, U.S.A.) at 200 rpm. The solid phase was separated using Whatman filter paper no. 42 and the final extract were used to estimate total phenolics and flavonoids content.

(a) **Total Phenolics content:** Total phenolics was estimated by Folin-Ciocalteu method using gallic acid as a standard compound [10]. The standard curve was prepared using different concentration (C) of gallic acid (20- 100 µg/ml). The 200µl standard solution/sample extract was mixed with 2.6 ml of deionized water, 2 ml of 7% (w/v) sodium carbonate, and 200µl of the Folin-Ciocalteu reagent. The mixture was vortexed and incubate at room temperature for 90 min. in dark. The absorbance (A) was measured against a reagent blank at 745nm using UV-1601 UV-Visible spectrophotometer (Shimadzu Corporation). The relationship between concentration C (µg g⁻¹) and absorbance A is found to be linear and expressed as:

$$A = 0.00453C + 0.00893, r^2 = 0.99959$$

This equation is used to calculate the total phenolic content by measuring the absorbance at 745 nm.

(b) **Total flavonoid content:** Total flavonoid content was determined by the aluminum chloride colorimetric assay with slight modification [11]. A calibration graph was prepared using different concentration of quercetin (20-100 µg/ml) which was used as a standard. To determine the flavonoid content 1 ml of standard or extract solution was taken in graduated test tube, containing 4 ml of distilled deionized water and 0.30 ml 5% sodium nitrite was added. After 5 minutes, 0.30 ml 10% aluminum chloride was added to the mixture. At the 6th minute 2ml of 1M NaOH was added and the volume made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against a reagent blank at 510nm using UV-1601 UV-Visible spectrophotometer (Shimadzu Corporation). A linear relationship is observed between the concentration C (µg g⁻¹) and the absorbance A and can be expressed as:

$$A = 0.00123C + 0.0081, r^2 = 0.99177$$

This equation is used to calculate the flavonoid content by measuring the absorbance at 510nm.

Statistical analysis

The data obtained were expressed in terms of mean and standard deviations (SD). The mean values were compared using one-way ANOVA (Analysis of variance) test for significance of their difference ($p < 0.05$). The data were analyzed using the Origin software.

RESULTS AND DISCUSSION

Effect of electron beam irradiation on microbial profile of red chilli & coriander powder

Total aerobic mesophilic bacterial count (TBC) is known to be the most commonly quality parameter for evaluating the hygienic status of food and food additives. Total aerobic bacterial and yeast & mold contamination in the non-irradiated and irradiated

samples are summarized in Table-1. The initial total bacterial load in red chilli and coriander powder was found to be in the range of 10^6 to 10^7 cfu g⁻¹, and that of yeast & molds in range of 10^3 to 10^4 cfu/g, which is much higher than the acceptable count limit.

The dose response curve of the microbes was obtained by plotting the number of surviving cells against radiation dose, kGy (graph not shown). The D_{10} value of total bacterial count in red chilli and coriander powder was found to be 2.1 kGy and 2.3 kGy, respectively and that of yeast & mold count in both samples were about 2.1 kGy.

The EB irradiation at 6 kGy dose, reduced the total viable bacterial count by 3 log cycles and at 10 kGy dose the total viable bacterial count reduces 4-6 log cycle, to a limit which is too low to count. The yeast and mold count were not detected at 6 and 10 kGy dose. Our results show that irradiation dose up to 6 kGy is sufficient to reduce the microbial contamination to the acceptable quality limit of 10^4 cfu/g. These results are consistent with the results reported with Gamma irradiation, where 4-6 kGy dose is found effective to reduce the microbial load to acceptable label 10^4 cfu/g [12, 13].

The data represents the mean of the triplicate samples \pm standard deviation (SD). ND- not detectable.

Effect of electron beam irradiation on total phenolic & flavonoid content in red chilli and coriander powders

Phytochemicals are the substances which act as free radical terminators, whose presence in relatively low concentrations significantly protect the cells by inhibiting the lipoxygenase activity, scavenging free radicals and chelating metal ions [14]. Phenolic and flavonoid content represent a substantial portion of spice antioxidants. The total phenolics and flavonoid content of the irradiated and non-irradiated red chilli and coriander powder were exhibited in Table-2. The

Table 1
Total bacteriological, yeast & mold count of the dried red chilli and coriander powder

Radiation dose (kGy)	Mean count of microorganisms detected in packed red chilli samples		Mean count of microorganisms detected in packed coriander powder samples	
	Total mesophilic bacterial count (cfu g ⁻¹)	Yeast & mold count (cfu g ⁻¹)	Total mesophilic bacterial count (cfu g ⁻¹)	Yeast & mold count (cfu g ⁻¹)
0	$2.69 \times 10^6 \pm 0.88$	$6.82 \times 10^4 \pm 2.1$	$3.46 \times 10^6 \pm 3.7$	$4.55 \times 10^4 \pm 0.07$
4	$3.76 \times 10^4 \pm 1.96$	$8.5 \times 10^2 \pm 0.70$	$3.13 \times 10^4 \pm 1.1$	$5.75 \times 10^2 \pm 0.35$
6	$5.10 \times 10^3 \pm 3.4$	ND	$8.55 \times 10^3 \pm 0.44$	ND
8	$4.47 \times 10^2 \pm 2.38$	ND	$1.35 \times 10^3 \pm 0.21$	ND
10	ND	ND	$7 \times 10^2 \pm 1.41$	ND
12	ND	ND	ND	ND

methanolic extract shows high phenolic content in red chilli powder (4.58 mg GAE/g dry powder) as compared to coriander powder (2.88 mg GAE/g dry powder). Whereas the total flavonoid content was found to be highest in the coriander powder (3.21 mgQE/g dry powder) when compared to that of red chilli powder (2.48 mgQE/g dry powder). The results revealed that the all used doses for the

microbial decontamination of electron beam radiation did not significantly affect the total phenolics and flavonoid contents of red chili and coriander powder even at 12 kGy samples shown in Fig. 1. These results are consistent with the results reported for gamma irradiation of spices, such as anise, cinnamon, ginger, liquorice, mint, nutmeg, and clove [15, 16, 17].

Table 2
Total phenolics & flavonoids content in dried red chilli and coriander powder*

Radiation dose (kGy)	Red chilli powder		Coriander powder	
	Total phenolics (mg GAE/g dry powder)	Total flavonoids (mg QE/g dry powder)	Total phenolics (mg GAE/g dry powder)	Total flavonoids (mg QE/g dry powder)
0	4.58 ± 0.05	2.48 ± 0.15	2.88 ± 0.08	3.21 ± 0.11
4	4.59 ± 0.09	2.58 ± 0.02	2.82 ± 0.13	3.43 ± 0.09
6	4.49 ± 0.19	2.54 ± 0.09	2.55 ± 0.27	3.11 ± 0.01
8	4.50 ± 0.01	2.39 ± 0.12	2.70 ± 0.01	3.20 ± 0.14
10	4.59 ± 0.06	2.34 ± 0.12	2.61 ± 0.01	3.20 ± 0.15
12	4.56 ± 0.01	2.45 ± 0.05	2.64 ± 0.05	3.25 ± 0.07

*Each value is mean of triplicate samples ± standard deviation (SD). The mean values in a column are not significantly different at ($p < 0.05$), as analyzed by one-way analysis of variance (ANOVA).

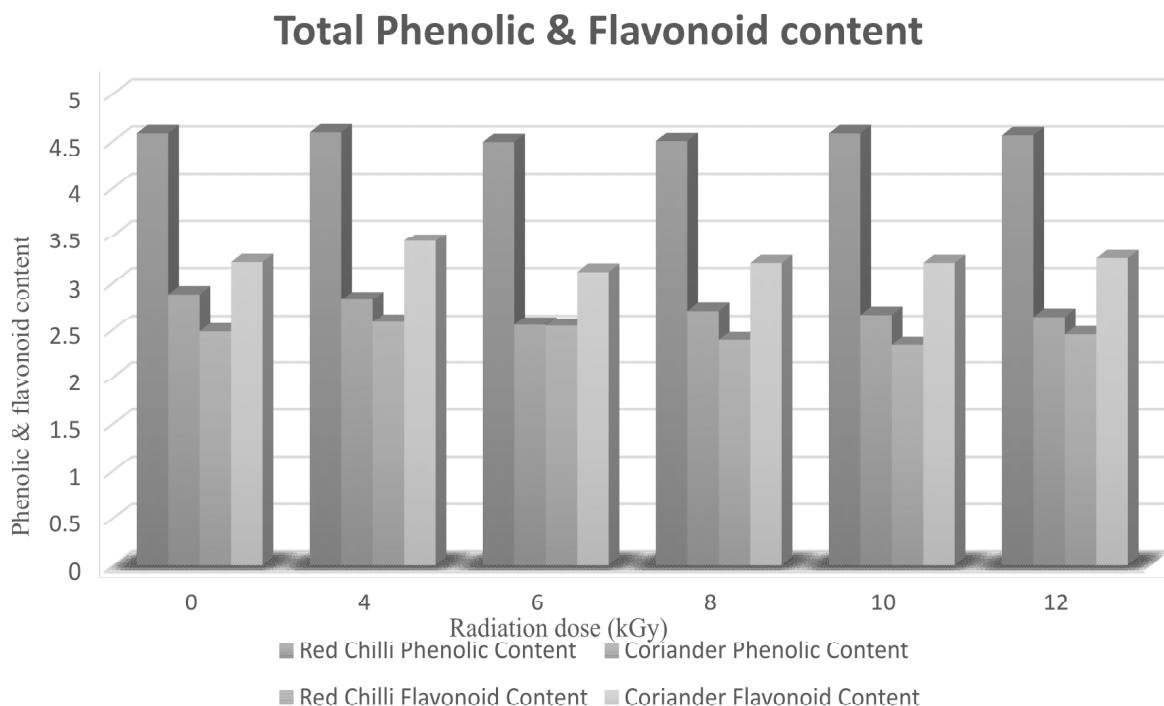


Figure 1: Effect of radiation on total phenolic & flavonoid content of red chilli and coriander powder

CONCLUSION

The red chilli and coriander powder collected from local market were irradiated with electron beam and studied for microbial decontamination and phytochemical contents. The experimental results suggest that electron beam irradiation is effective tool

for microbial decontamination and electron beam dose of 6 kGy is adequate to reduce the microbial load below acceptable quality limit without affecting total phenolic and flavonoid contents. At high dose 10-12 kGy, the microbial load reduces significantly below the non-detectable range, and may be needed only if

initial microbial load present in the samples is high. The total phenolic and flavonoid contents were measured after high dose (12 kGy) irradiation and no significant change is observed. Reliable operation of electron beam irradiation facility at RRCAT Indore, has opened avenues for collaborative studies and research in India.

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