



## Comparative evaluation of ethylene oxide, electron beam and gamma irradiation treatments on commonly cultivated red chilli cultivars (Kunri and Hybrid) of Sindh, Pakistan

Muzzammal Ahmed Muzzafar<sup>a</sup>, Shinawar Waseem Ali<sup>a,\*</sup>, Munawar Iqbal<sup>b</sup>, Maryam Saeed<sup>a</sup>, Mateen Ahmad<sup>a,c</sup>, Muhammad Rizwan Tariq<sup>a</sup>, Abdikhaliq Mursal Yusuf<sup>a,d</sup>, Ayesha Murtaza<sup>e</sup>, Aftab Ahmed<sup>f</sup>, Shazia Yaqub<sup>g</sup>, Muhammad Riaz<sup>h</sup>

<sup>a</sup> Department of Food Sciences, University of the Punjab, Lahore, Pakistan

<sup>b</sup> College of Statistical Sciences, University of the Punjab, Lahore, Pakistan

<sup>c</sup> Institute of Human Nutrition and Dietetics, Gulab Devi Educational Complex, Lahore, Pakistan

<sup>d</sup> Kaalo Aid and Development Organisation, Puntland state, Somalia

<sup>e</sup> Department of Food Science & Technology, University of Central Punjab, Lahore, Pakistan

<sup>f</sup> Department of Nutritional Sciences, Government College University, Faisalabad, Pakistan

<sup>g</sup> Punjab Food Authority, Lahore, Punjab, Pakistan

<sup>h</sup> Department of Food Safety & Quality Management, Bahauddin Zakaria University, Multan, Pakistan

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### ABSTRACT

Chillies are considered a universal ingredient for imparting flavor and pungency to foods. Pakistan stood in the top twenty countries worldwide by producing 82 thousand Tons of chillies during 2022-23. Chilli fungal contamination and aflatoxin production during drying is a common problem during post-harvest process. Gasses treatment and Ionizing radiations are efficient methods for reducing toxigenic and pathogenic microbial growth in food items. The current study was designed to compare the effects of ethylene oxide (ETO), gamma (GB) & electron beam (EB) treatments on two red chilli local cultivars (Kunri and Hybrid) of Pakistan. After treatment, the chilli samples were analyzed for aflatoxins, physicochemical, quality & safety attributes. All results were subjected to Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), dendrogram and ANOVA to check the correlations, grouping and level of significance within the varieties and treatments. The results showed that moisture and water activity mainly designated PC-2 directions and are slightly positively correlated. Conversely, both fat and proteins have a negative correlation with moisture, ash and water activity. Besides, carotenoids and ABTS assay mainly designated PC-2 directions and are slightly positively correlated. Color, flavonoids and TPC also possess positive correlations among them. ETO depicts effectiveness in the reduction of *E. coli* but is not effective in saving antioxidant potential such as total flavonoids. Similarly, gamma irradiations showed strong reduction trends in fungal and pathogenic count, however same trend was observed in ascorbic acid too. Besides, the electron beam with dosage levels of 12 and 15 kGy has shown effectiveness against *Aspergillus* spp., aflatoxins and pathogenic microbial load in addition to saving antioxidant potential (phenolics and flavonoids), physicochemical parameters and color values compared to other applied methods especially in Kunri variety. It

\* Corresponding author.

E-mail address: [shinawar.foodsciences@pu.edu.pk](mailto:shinawar.foodsciences@pu.edu.pk) (S.W. Ali).

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was evident from the research that varietal combination in addition to applied treatment must be specially considered while designing a treatment for chillies.

## 1. Introduction

Chilli (*Capsicum annum* L.) exhibited various significant health benefits such as anticancer, anti-inflammatory, anti-neoplastic, anti-arthritis, antifungal and antioxidant properties [1]. Besides, chillies are denoted as a good source of carotenoids, flavonoids, polyphenols, saponins, nitrogenous compounds, minerals and various vitamins including ascorbic acid [2]. According to the statistical survey of Pakistan, 82 thousand Tons of chillies were produced over an area of 31 thousand Hectares during 2022-23 [3]. Chillies are considered a universal ingredient for imparting flavor and pungency to food stuff.

Chilli is classified as a highly perishable commodity and thus great chances prevail for postharvest complications such as color changes, weight loss and other quality defects. Moreover, chilli comprises almost 80 % moisture content during harvesting, therefore, it is very susceptible to fungal and insect attacks during pre and post-harvest phases [4,5]. Contamination is a big problem possessed during value addition chains like handling, drying, transportation and storage. Fungal contamination like *Aspergillus* spp. such as *Aspergillus flavus* and *Aspergillus parasiticus* (known for substantial production of aflatoxins) are common microbial contamination in chilli [6,7]. The aflatoxins possess mutagenic, carcinogenic, teratogenic and other toxic characteristics [8]. Climatic conditions like temperature and relative humidity are the most important environmental factors for mold growth in tropical and subtropical areas of the world. Likewise, aflatoxin contamination of chilli and other spices is a serious and common problem in Pakistan. Consequently, realistic and efficient techniques are required to mitigate this serious health concern. Different chemical and physical treatments have been employed to reduce microbial contamination and also the production of toxins in chilli including fumigation, direct application of chemicals, etc. Nonetheless, these techniques have their health risks and thus several countries discourage the use of chemicals or fumigation methods [9]. Due to these problems, special consideration has been given to non-thermal preservation techniques that are capable of producing quality products [10].

Ethylene oxide is considered an influential agent in reducing the microbial load from the product [11]. However, although ETO is considered effective some scientists consider this a carcinogenic and mutagenic agent especially when inhaled for a long time [12]. Moreover, ETO disperses from applied commodities and thus reacts with bromide and chloride under ambient conditions to formulate mutagenic and carcinogenic compounds such as 2-bromoethanol and 2-chloroethanol which are stubborn than their parent compounds [13,14]. Therefore, it is important to calculate the exact amount of ETO to be applied to the specific commodity. In this context, irradiation depicted a proficient way to improve food safety while maintaining the nutritional and physicochemical quality of the food products [15].

Ionizing radiations like gamma irradiation [16] and electron beam [17] are efficient methods for reducing toxigenic and pathogenic microbial growth in food items. Different researchers assessed the impact of irradiation treatment on the reduction of microbial contamination on different spices like red chilli, rosemary, turmeric powder, black pepper, and rosemary [18,19]. Other researchers check the effect of X-ray, gamma-ray, and electron beam radiation on black pepper [20] and red chilli powder [6]. Gamma radiation was applied to minimize the toxins levels in black and white pepper and red chilli [21]. However, the radioactive sources of gamma rays and the comparatively longer running time of the equipment pose some problems for the food industry. Moreover, the cost of cobalt-60 and the reluctant behavior of the consumers toward radioactive substances trigger the need for comparing this irradiation source with other irradiation techniques like electron beam [22].

The electron beam (EB) technique is robust, instant, and able to deliver a uniform dose in a shorter period, and requires no radioactive substance like cobalt-60 [23]. However, the dose of irradiation is very important to avoid any health-related issues. According to the World Health Organization (WHO), irradiation dose up to 10 kGy on foods is considered safe to avoid any nutrient losses and production of toxic substances [5]. However, in advanced countries like the USA, an irradiation dose of up to 30 kGy is considered safe for dried food items like chilli powder [24]. EB can reduce microbial contamination through direct or indirect means. Directly, radiations hit the DNA or RNA of microbes [25], while indirectly, they affect the radiolysis of water in molecules. Even though the EB technique is routinely used for the decontamination of packaging materials and medical devices its application to food items is still limited [15].

Red chilli, Kunri and hybrid both are unique varieties of Pakistan that have worldwide demand. However, due to a lack of good agricultural practices, both chillies are contaminated with *Aspergillus* toxins (aflatoxins) producing species during and after harvesting which creates a barrier to producing the product as per international standards. Contrary to previous studies the current research is designed to compare the effect of Ethylene oxide, gamma and electron beam irradiation techniques on both varieties to reduce this contamination, which has never been studied earlier. Moreover, the physicochemical and antioxidant profile was also analyzed to evaluate the safety and quality attributes of these cultivars.

## 2. Material and methods

### 2.1. Raw material procurement and preparation

Two frequently cultivated red chilli cultivars (Kunri and Hybrid) of Sindh Province, Pakistan were procured from selected farmers randomly. Undamaged ones having comparable diameter and length were selected and stored at 4 °C. All samples were grinded

through lab grinder pass through US 40 mash. All chemicals of the analytical grade were procured from Sigma Aldrich (USA) and Media from Merck (Germany). Samples were run in triplicates for the determination of parameters.

## 2.2. Treatment plan for red chilli powder

In the current study, a direct comparison between the ethylene oxide, electron beam and gamma irradiation on selected local chilli varieties was done to evaluate the effectiveness of treatments against microbiological load, aflatoxins contaminations and as well as antioxidant properties, quality and safety profile studied in parallel. Samples of both varieties (Kunri and Hybrid) contaminated with Aflatoxin were packed separately in HDPE bags, labeled and irradiated by applying 3, 6, 9, 12 and 15 kGy dose levels of Electron Beam and Gamma Beam treatments and 10,20,30,40 and 50 % of Ethylene Oxide, rest of the quantity is Carbon dioxide %age.

All the treatment plans further divided into three sections on the bases of applied treatments first one include the Ethylene oxide application on both variety listed as Kunri control (KC), Hybrid Control (HC), Kunri ethylene oxide treated (KET10), Kunri ethylene oxide treated (KET20), Kunri ethylene oxide treated (KET30), Kunri ethylene oxide treated (KET40), Kunri ethylene oxide treated (KET50), Hybrid ethylene oxide treated (HET10), Hybrid ethylene oxide treated (HET20), Hybrid ethylene oxide treated (HET30), Hybrid ethylene oxide treated (HET40), Hybrid ethylene oxide treated (HET50).

Second treatment of gamma irradiation listed as Kunri gamma treated (KG3), Kunri gamma treated (KG6), Kunri gamma treated (KG9), Kunri gamma treated (KG12), Kunri gamma treated (KG15), Hybrid gamma treated (HG3), Hybrid gamma treated (HG6), Hybrid gamma treated (HG9), Hybrid gamma treated (HG12), Hybrid gamma treated (HG15).

Third treatment of electron beam irradiation listed as Hybrid E-beam treated (HE3), Hybrid E-beam treated (HE6), Hybrid E-beam treated (HE9), Hybrid E-beam treated (HE12), Hybrid E-beam treated (HE15), Kunri E-beam treated (KE3), Kunri E-beam treated (KE6), Kunri E-beam treated (KE9), Kunri E-beam treated (KE12), Kunri E-beam treated (KE15).

## 2.3. Physicochemical profile and water activity

Physicochemical compositional analyses like crude protein (Kjeldahl method), crude fat (Soxhlet method), moisture (hot air oven method), Ash (muffle furnace) and water activity of chilli samples were determined through their respective methods as described by AOAC (2019).

## 2.4. Total phenolics and flavonoids content

The chilli samples (50 g) were extracted at room temperature for 24 h in the dark with methanol (80 %, 1 L). The extracts were filtered via Whatman 41 filter paper and the solvent was evaporated under a vacuum evaporator at 40 °C. Subsequently, the water portion was removed through a sublimation process.

The total phenolic content of chilli samples was estimated through the method described by Ref. [26]. The freeze-dried chilli extract of 1 g was dissolved in deionized water (5 mL). Folin-Ciocaltea reagent was added (0.5 mL), and after 3 min, 2 mL of NaHCO<sub>3</sub> (20 g/100 mL) was added and put this mixture in boiling water for 1 min and then cooled. Afterward, the absorbance was measured through a UV-Vis spectrophotometer at 750 nm for both blank and sample. The total phenolics content was estimated through the standard curve of gallic acid and values were presented as equivalents of gallic acid µM/g.

The total flavonoid contents of samples were evaluated through the AlCl<sub>3</sub> colorimetric method as described by Ref. [27]. Briefly, freeze-dried chilli extract 1 g was dissolved in deionized water at a level of 1.5 mg/mL to a final volume of 5 mL. Subsequently, 0.3 mL sodium nitrate (5 g/100 mL, w/v) was added to the dissolved extract and stayed for 5 min. Afterward, 0.6 mL of AlCl<sub>3</sub> was added (10 g/100 mL, w/v) and stayed for 1 min then 2 mL of NaOH was added (1 mol/L, w/v) followed by 2.10 mL of distilled water. The absorbance was measured through a spectrophotometer at 510 nm. The total flavonoid content was measured and expressed as rutin equivalents µg/g.

## 2.5. ABTS assay

The chilli samples were assessed for their antioxidant potential by using the ability to quench ABTA radical cation as described by Ref. [28]. Briefly, ABTS<sup>+</sup> was prepared through the interaction between ABTS stock solution (7 mM) with potassium persulphate (2.45 mM) and stored at room temperature for 16 h in the dark. Subsequently, the solution was diluted with ethanol (95 %) and deionized water at a 1:1 ratio to attain an absorbance of  $0.71 \pm 0.02$  at 734 nm using a spectrophotometer. For sample reading, chilli extract (20 µl) was added to diluted ABTS + solution (6 mL) and mixed for 1 min the decrease in absorbance was recorded as ABTS values for the sample and expressed as µM/g.

## 2.6. Determination of ascorbic acid value (AsA)

The methanolic extract of the chilli sample was mixed with trichloroacetic acid and subsequently analyzed through a spectrophotometer as the method described by Ref. [29]. Briefly, 0.5 ml of methanolic extract from chilli samples was mixed with 0.5 ml trichloroacetic acid (7.5 % solution). The mixture was kept at 4 °C for 5 min with occasional stirring and filtered with 4.5 µm filter paper. The filtrate of 0.2 ml was mixed with 0.2 ml Folin Ciocalteu reagent (1:10 v/v) and subsequently diluted with 2 ml of double distilled water and stored at room temperature in the dark for 10 min the absorbance was recorded through a spectrophotometer at

760 nm wavelength.

## 2.7. Carotenoid composition

The total carotenoid composition of chilli samples was determined by following the method stated by Iqbal, Amjad [30]. Briefly, 5 g of chilli sample was homogenized in 50 ml acetone by using an ultra-homogenizer till the level of virtually zero color retention by the chilli samples. Subsequently, the extract (50 ml), diethyl ether (100 ml) and sodium chloride (10 g/100 ml) were added in a separating funnel after being shaken vigorously and allowed to be stagnant for separation. The aqueous phase was discarded and the supernatant was washed with Na<sub>2</sub>SO<sub>4</sub> (2 g/100 ml) 5 times. Afterward, 50 ml potassium hydroxide (5 g in 50 ml methanol) was added and stored in the dark for 60 min for saponification. The aqueous phase was discarded and washing of the organic phase was carried out with 100 ml of NaCl (10 g/100 ml). The organic phase evaporated through a nitrogen pump and the dried extract re-dissolved in small proportions of acetone. After the extraction, the absorbance maxima were recorded at 470 nm in triplicates by using a spectrophotometer.

## 2.8. Determination of ASTA color value

The color measurement of chilli samples was determined through the ASTA analytical method 20.1. by using a UV-Vis spectrophotometer (V-560, Japan) as described [31].

Briefly, the powdered chilli sample (100 mg) was added to acetone (100 mL) and stored at 0 °C for 4 h in the dark with intermittent stirring. Subsequently, the absorbance of the aliquot was determined through a UV-Vis spectrophotometer (V-560, Japan) at 460 nm. ASTA color values were calculated by using the following formula;

$$\text{ASTA Color Value} = A \times 16.4 \times C / W$$

Where; A = Absorbance of aliquot extract, C = correction factor of the instrument at 460 nm and W = sample weight in grams – dry basis.

## 2.9. Detection of aflatoxins

ELISA method was used to measure the level of aflatoxins by ELIZA reader (Awareness Technology, Inc. Palm City FL 34990 Model: 4700, serial number 4701-2809). Briefly, the aflatoxins were extracted through organic solvent mixtures like acetonitrile, methanol, or acetone with water. About 50 ml of prepared extract was inoculated into the microliter plate wells. Afterward, enzyme conjugate and anti-aflatoxin antibody solutions (50 ml each) were incorporated into each well, mixed and incubated at 20–25 °C for 30 min. The liquid was drained from the well and the well was washed with buffer twice (250 ml). Subsequently, a substrate solution (100 ml) was added to each well, mixed and incubated at 25 °C for 15 min under dark conditions. Lastly, sulfuric acid (1 N, 100 ml) was added to each well and measured the absorbance at 450 nm.

## 2.10. Microbial enumeration

Twenty grams of samples were diluted in 180 ml of sterilized phosphate buffer and homogenized by using a stomacher (Chemunex, Rennes, France). Afterward, 1 ml of these aliquots were serially diluted in 9 ml of sterilized buffer to make tenfold dilutions. 0.1 ml of diluent was inoculated on each Petri dish or in broth containing selective medium for each test organism such as for Yeasts and molds; *Aspergillus flavus* and *Aspergillus parasiticus* (Potato Dextrose Agar); *Coliforms* (Violet Red Bile Lactose Agar); *E. coli* (Brilliant Green Bile Broth 2 %); *Enterobacteriaceae* (Violet Red Bile Dextrose Agar); *Staphylococci* (BAIRD-PARKER agar with egg yolk); and TPC (Plate Count Agar). All prepared petri plates were incubated at 37 °C for 24 h in an incubator (Herather Compact Microbiological Incubators Catalog number: 50125590) and colonies were counted (Countess™ 3 Automated Cell Counter catalog no.AMQAX2000). Un-inoculated media plates were also incubated in the same manner to identify any possible background contamination.

## 2.11. Statistical analysis

The experimental results were subjected to analysis of variance (ANOVA – one way) using SPSS 22 (IBM SPSS, Chicago, IL, USA), Principle Component Analysis and Cluster Analysis were done by using The Unscrambler X 10.4 (64-bit) and mean comparison was carried out by using Tukey's HSD test ( $\alpha < 0.05$ ). While MS Excel (2016) and SigmaPlot 12.0 were used for making the graphical representation. Whole measurements were taken at least in triplicates and results were described as mean  $\pm$  standard deviation (SD). Chemometrics consists of the application of statistical techniques to comprehend chemical statistics along with correlating a number of physicochemical attributes and quality parameters to the analytical instrument dataset. In the present study chemometric methods i. e. Principal Component Analysis (PCA) and Cluster Analysis were applied to foresee variations in multivariate data.

### 3. Results and discussion

#### 3.1. Physicochemical profile of treated red chilli powder (TRCP)

The analysis of the physicochemical compositional profile is associated with the characteristics of the chilli along with the indication for the effect of treatment on the nutritional profile of the chilli. Moreover, water activity is a unique character that affects the shelf life, microbial growth and other significant changes in the chilli samples. The results of the Physicochemical composition of various chilli treatments are described and discussed in subsequent sections.

##### 3.1.1. Protein content of TRCP

The protein content was varied significantly among all treatments ranging from  $16.01 \pm 0.08$  g/100 g to  $14.73 \pm 0.06$  g/100 g (Table 1). The maximum protein content was found in HE15 i.e.  $16.01 \pm 0.08$  g/100 g while the minimum was observed in KET50 i.e.  $14.73 \pm 0.06$  g/100 g. It was evident from the results that differences were also caused due to the variation of protein content in both varieties such as KC (Kunri Control) contains  $15.20 \pm 0.16$  g/100 g while HC (Hybrid Control) has  $15.93 \pm 0.12$  g/100 g percentage of protein content. The protein content in the treatments was also affected due to the method applied like ethylene oxide, gamma and electron beam irradiation techniques. A close overview of the degradation pattern depicts that ETO has proven more detrimental to the protein content as compared to irradiation techniques, however, surprisingly electron beam irradiation technique has proven effective in preserving the protein content of the chilli samples compared to gamma. That is probably because an electron beam is less likely to disintegrate the larger molecules compared to gamma irradiations.

**Table 1**  
Physicochemical composition and water activity of Treated Red Chili Powder.

Treatments	Protein g/100g	Fat g/100g	Moisture g/100g	Ash g/100g	Aw
KC	$15.20 \pm 0.16^{ijkl}$	$14.33 \pm 0.05^{gh}$	$5.73 \pm 0.05^{ab}$	$6.02 \pm 0.09^f$	$0.40 \pm 0.06^a$
HC	$15.93 \pm 0.12^{ab}$	$16.27 \pm 0.02^e$	$4.69 \pm 0.05^{fg}$	$13.39 \pm 0.13^c$	$0.34 \pm 0.03^a$
KET10	$15.10 \pm 0.16^{ijklm}$	$13.60 \pm 0.09^j$	$5.21 \pm 0.06^e$	$8.44 \pm 0.06^o$	$0.34 \pm 0.04^a$
KET20	$15.10 \pm 0.14^{ijklm}$	$13.10 \pm 0.12^j$	$5.47 \pm 0.02^{cd}$	$7.53 \pm 0.07^q$	$0.32 \pm 0.03^a$
KET30	$15.13 \pm 0.12^{jkl}$	$12.64 \pm 0.06^k$	$5.93 \pm 0.05^a$	$8.38 \pm 0.04^o$	$0.39 \pm 0.02^a$
KET40	$14.85 \pm 0.04^{lm}$	$13.10 \pm 0.12^j$	$5.35 \pm 0.04^{de}$	$13.36 \pm 0.07^j$	$0.38 \pm 0.03^a$
KET50	$14.73 \pm 0.06^m$	$12.77 \pm 0.07^k$	$5.64 \pm 0.05^{bc}$	$13.02 \pm 0.06^k$	$0.38 \pm 0.05^a$
HET10	$15.62 \pm 0.08^{b-g}$	$18.02 \pm 0.13^a$	$4.39 \pm 0.04^h$	$13.76 \pm 0.07^b$	$0.33 \pm 0.03^a$
HET20	$15.56 \pm 0.04^{b-i}$	$17.22 \pm 0.03^{bc}$	$4.74 \pm 0.10^f$	$13.02 \pm 0.06^{de}$	$0.32 \pm 0.04^a$
HET30	$15.43 \pm 0.06^{d-k}$	$17.11 \pm 0.02^c$	$4.48 \pm 0.02^{gh}$	$12.38 \pm 0.03^g$	$0.32 \pm 0.05^a$
HET40	$15.23 \pm 0.12^{h-k}$	$17.51 \pm 0.11^b$	$4.35 \pm 0.04^{hi}$	$12.77 \pm 0.02^{ef}$	$0.31 \pm 0.02^a$
HET50	$15.57 \pm 0.17^{b-i}$	$17.16 \pm 0.06^c$	$4.31 \pm 0.02^{hi}$	$13.89 \pm 0.05^b$	$0.32 \pm 0.03^a$
KG3	$15.33 \pm 0.07^{f-k}$	$14.10 \pm 0.12^h$	$5.87 \pm 0.04^{ab}$	$9.96 \pm 0.03^m$	$0.41 \pm 0.03^a$
KG6	$15.59 \pm 0.14^{b-h}$	$13.35 \pm 0.05^{ij}$	$5.94 \pm 0.07^a$	$7.51 \pm 0.08^q$	$0.42 \pm 0.06^a$
KG9	$15.46 \pm 0.12^{d-j}$	$13.34 \pm 0.05^{ij}$	$5.92 \pm 0.03^a$	$8.93 \pm 0.05^r$	$0.40 \pm 0.07^a$
KG12	$15.47 \pm 0.12^{d-j}$	$12.69 \pm 0.04^k$	$5.84 \pm 0.04^{ab}$	$7.53 \pm 0.07^q$	$0.42 \pm 0.03^a$
KG15	$15.07 \pm 0.12^{klm}$	$13.22 \pm 0.03^j$	$5.79 \pm 0.08^{ab}$	$6.37 \pm 0.06^s$	$0.41 \pm 0.04^a$
HG3	$15.56 \pm 0.09^{b-i}$	$16.20 \pm 0.08^e$	$4.35 \pm 0.05^{hi}$	$12.37 \pm 0.12^j$	$0.34 \pm 0.04^a$
HG6	$15.36 \pm 0.03^{e-k}$	$14.22 \pm 0.06^h$	$4.14 \pm 0.08^i$	$8.02 \pm 0.08^p$	$0.34 \pm 0.05^a$
HG9	$15.30 \pm 0.06^{f-k}$	$14.98 \pm 0.09^f$	$4.67 \pm 0.09^{fg}$	$8.46 \pm 0.06^o$	$0.32 \pm 0.05^a$
HG12	$15.55 \pm 0.07^{c-i}$	$17.21 \pm 0.07^{bc}$	$4.48 \pm 0.05^{gh}$	$13.96 \pm 0.10^i$	$0.35 \pm 0.05^a$
HG15	$15.32 \pm 0.03^{f-k}$	$15.21 \pm 0.07^f$	$4.35 \pm 0.05^{hi}$	$13.14 \pm 0.06^{cd}$	$0.36 \pm 0.05^a$
HE3	$15.68 \pm 0.04^{a-f}$	$16.73 \pm 0.05^d$	$4.34 \pm 0.05^{hi}$	$14.87 \pm 0.08^a$	$0.34 \pm 0.05^a$
HE6	$15.26 \pm 0.04^{g-k}$	$16.33 \pm 0.04^e$	$4.14 \pm 0.05^i$	$10.79 \pm 0.07^h$	$0.35 \pm 0.03^a$
HE9	$15.47 \pm 0.05^{d-j}$	$16.73 \pm 0.10^d$	$4.67 \pm 0.08^{fg}$	$14.03 \pm 0.06^p$	$0.32 \pm 0.04^a$
HE12	$15.90 \pm 0.07^{abc}$	$16.32 \pm 0.06^e$	$4.49 \pm 0.07^{gh}$	$12.50 \pm 0.07^{fg}$	$0.35 \pm 0.03^a$
HE15	$16.01 \pm 0.08^a$	$16.33 \pm 0.03^e$	$4.36 \pm 0.04^{hi}$	$12.39 \pm 0.07^g$	$0.35 \pm 0.05^a$
KE3	$15.55 \pm 0.05^{b-i}$	$14.12 \pm 0.14^h$	$5.87 \pm 0.04^a$	$6.97 \pm 0.11^t$	$0.41 \pm 0.04^a$
KE6	$15.76 \pm 0.06^{abcd}$	$13.64 \pm 0.06^j$	$5.93 \pm 0.05^a$	$6.51 \pm 0.06^s$	$0.42 \pm 0.05^a$
KE9	$15.45 \pm 0.05^{d-j}$	$13.63 \pm 0.07^j$	$5.92 \pm 0.10^a$	$7.46 \pm 0.06^q$	$0.40 \pm 0.02^a$
KE12	$15.60 \pm 0.08^{b-h}$	$14.63 \pm 0.07^g$	$5.85 \pm 0.06^{ab}$	$6.97 \pm 0.04^t$	$0.41 \pm 0.03^a$
KE15	$15.74 \pm 0.10^{a-e}$	$13.61 \pm 0.07^i$	$5.78 \pm 0.07^{ab}$	$7.47 \pm 0.11^s$	$0.41 \pm 0.02^a$

Means carrying different letters in a column are statistically significant ( $p < 0.01$ ).

Kunri control (KC), Hybrid Control (HC), Kunri ethylene oxide treated (KET10), Kunri ethylene oxide treated (KET20), Kunri ethylene oxide treated (KET30), Kunri ethylene oxide treated (KET40), Kunri ethylene oxide treated (KET50), Hybrid ethylene oxide treated (HET10), Hybrid ethylene oxide treated (HET20), Hybrid ethylene oxide treated (HET30), Hybrid ethylene oxide treated (HET40), Hybrid ethylene oxide treated (HET50), Kunri gamma treated (KG3), Kunri gamma treated (KG6), Kunri gamma treated (KG9), Kunri gamma treated (KG12), Kunri gamma treated (KG15), Hybrid gamma treated (HG3), Hybrid gamma treated (HG6), Hybrid gamma treated (HG9), Hybrid gamma treated (HG12), Hybrid gamma treated (HG15), Hybrid E-beam treated (HE3), Hybrid E-beam treated (HE6), Hybrid E-beam treated (HE9), Hybrid E-beam treated (HE12), Hybrid E-beam treated (HE15), Kunri E-beam treated (KE3), Kunri E-beam treated (KE6), Kunri E-beam treated (KE9), Kunri E-beam treated (KE12), Kunri E-beam treated (KE15)

### 3.1.2. Fat content of TRCP

The fat content was varied among treatments and depicted a range between  $18.02 \pm 0.13$  g/100 g to  $12.64 \pm 0.06$  g/100 g (Table 1). The maximum fat content was observed in HET10 i.e.  $18.02 \pm 0.13$  g/100 g while the minimum amounts were found in KET30 i.e.  $12.64 \pm 0.06$  g/100 g. the variation between the fat values is due to the processing technique i.e. ETO, gamma and electron irradiation application and also due to the difference of fat content in Kunri (KC)  $14.33 \pm 0.05$  g/100 g and Hybrid (HC)  $16.27 \pm 0.02$  g/100 g varieties originally. It was evident from the results that ETO was proven a good technique compared to irradiation in saving fat values of the samples, however, among other two electron beam depicted better results related to fat contents than the gamma irradiation method. The difference in the chilli samples is based on their genetic variation as reported earlier [32]. The significantly higher amount of fat content in the hybrid variety than Kunri is due to the higher percentage of seed content compared to other fruit components as described by Ananthan, Subash [33].

### 3.1.3. Moisture content of TRCP

Moisture content is a very important characteristic as this considered as an significant factor in depicting the shelf life and microbial infestation in the chilli samples. There are plenty of factors that direct the final moisture levels in the chilli crop. In the current study, genetic, environmental and technique applied to the chilli samples plays a critical role in the final content of moisture. The moisture in the treatments varied significantly and depicted a range between  $5.94 \pm 0.07$  g/100 g to  $4.14 \pm 0.08$  g/100 g. the maximum moisture content was observed in KG6 ( $5.94 \pm 0.07$  g/100 g) while the minimum was observed in HG6 ( $4.14 \pm 0.08$  g/100 g). It was evident from the research that the Kunri variety contains more moisture content i.e.  $5.73 \pm 0.05$  g/100 g compared to the Hybrid variety i.e.  $4.69 \pm 0.05$  g/100 g (Table 1). Interestingly, ETO and irradiation techniques don't impart high change to the moisture content. Several scientists discussed the importance of moisture content in deciding microbial growth and shelf life stability of chilli samples [4,34].

### 3.1.4. Ash content of TRCP

The ash content of chilli comprises the mineral content of the chilli samples. These minerals are not only important due to their nutritional significance but also their functional characteristics in minute quantities. The ash content varied due to the genetic factors as well as the methods of handling, storage and processing of the chilli samples. It was evident from the research that ash content in the chilli samples varied between  $6.02 \pm 0.09$  g/100g to  $14.87 \pm 0.08$  g/100 g (Table 1). Interestingly both varieties depicted variation in the ash content. The maximum ash content was shown in HE3 i.e.  $14.87 \pm 0.08$  g/100 g while the minimum was observed in Kunri

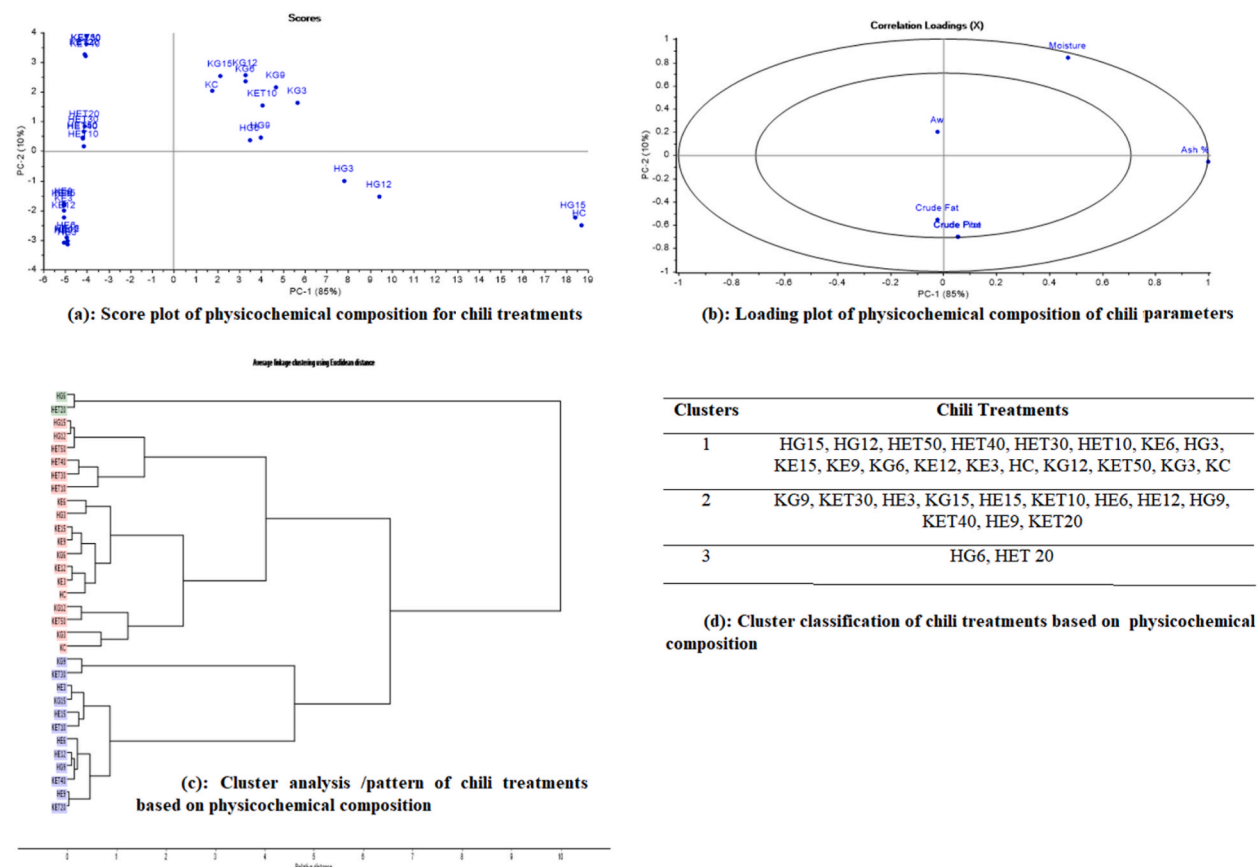


Fig. 1. Chemometric analysis based on Physicochemical profile of TRCP.

control i.e.  $6.02 \pm 0.09$  g/100 g, while the hybrid control variety contains  $13.39 \pm 0.13$  g/100 g ash content. There is no significant effect of all treatments on ash contents variation. The results of the present study are in line with the findings of previous research by Ananthan, Subash [33], who analyzed the different chilli varieties and depicted that the varieties exhibited differences in ash contents. Another study, conducted by Krithika, Radhai [35] exhibited that different chilli varieties contain diverse amounts of different mineral compounds. Recently a group of scientists, Ayob, Hussain [32] studied the chemical composition and antioxidant potential of Himalayan Red chilli varieties and found significant variations in the ash content of all varieties. They described this behavior mainly due to genetic variations and also due to phenotypic and environmental factors. Another group of researchers, Dahiru, Charles [36] worked on mineral quality and proximate values of *capsicum annum* being applied open and sun drying for removal of moisture content. They described very high values of the ash contents i.e.  $\sim 14\%$  and above. In another study, a comparison between nutrients profile of pepper fruit due to the effect of environmental and genetic variations was studied and found vast variations in ash content up to  $12\%$  approximately [37]. Similarly, In another study the ash content was observed elevated i.e.  $12.67\%$  compared to control i.e.  $10.08\%$  by the application of non-thermal techniques such as UV and LED [38].

### 3.1.5. Water activity ( $A_w$ ) of TRCP

Water activity ( $A_w$ ) of chilli comprises available moisture content for microbial as well as enzymatic activities. This is considered as chief criterion for the depiction of the shelf life of the products and agricultural commodities. The water in any commodity is the moisture in all three phases i.e. free as well as physically and chemically bound water. However, the water activity depicts the available moisture content for microbial growth, enzymatic activity and Physicochemical reactions. Table 1 shows that water activity in all chilli samples doesn't vary significantly in all treatments. It was also evident from the results that ETO, irradiation techniques such as gamma and electron beam irradiations don't affect the water activity on a large scale. This is perhaps due to the lower exposure time of the

**Table 2**  
Aflatoxin, phytochemical and antioxidant composition of Treated Red Chili Powder.

Treatment	Aflatoxin	Color ASTA	ABTS Assay $\mu\text{M/g}$	Flavonoids $\text{mg/g}$	TPC $\text{mM/g}$	AsA $\mu\text{g/gfw}$	Carotenoids $\text{mg/gfw}$
KC	$8.6 \pm 0.04^i$	$98.89 \pm 5.21$	$3.82 \pm 0.01^d$	$18.51 \pm 21^{k-n}$	$43.9 \pm 0.15^{ij}$	$572 \pm 19^{a-e}$	$19.16 \pm 0.23^a$
HC	$28.2 \pm 0.86^{ab}$	$99.67 \pm 5.19$	$4.13 \pm 0.05^{abc}$	$20.68 \pm 22^{bcd}$	$45.87 \pm 0.29^{gh}$	$598 \pm 11^{a-e}$	$13.19 \pm 0.08^{efg}$
KET10	$8.53 \pm 0.07^j$	$108.9 \pm 3.65$	$4.06 \pm 0.01^{abcd}$	$18.19 \pm 14^{mn}$	$40.3 \pm 0.22^{lm}$	$616 \pm 10^a$	$15.46 \pm 0.24^c$
KET20	$8.12 \pm 0.06^i$	$100.67 \pm 8.18$	$4.13 \pm 0.04^{abc}$	$18.47 \pm 18^{k-n}$	$38.43 \pm 0.29^o$	$598 \pm 12^{a-e}$	$18.41 \pm 0.13^b$
KET30	$7.58 \pm 0.09^{ijk}$	$98.67 \pm 3.86$	$4.02 \pm 0.06^{abcd}$	$17.77 \pm 19^n$	$32.4 \pm 0.41^p$	$613 \pm 12^{ab}$	$13.32 \pm 0.21^{ef}$
KET40	$7.33 \pm 0.08^{ijk}$	$102 \pm 3.56$	$4.22 \pm 0.06^{ab}$	$18.62 \pm 13^{n}$	$38.9 \pm 0.22^{no}$	$560 \pm 8^e$	$13.53 \pm 0.08^{de}$
KET50	$6.98 \pm 0.09^{ijkl}$	$98 \pm 4.9$	$3.94 \pm 0.09^{cd}$	$17.87 \pm 15^n$	$42.42 \pm 0.36^k$	$578 \pm 11^{a-e}$	$13.24 \pm 0.04^{efg}$
HET10	$27.83 \pm 0.24^{abc}$	$99.77 \pm 4.22$	$4.01 \pm 0.04^{abcd}$	$18.93 \pm 7^{h-m}$	$53.4 \pm 0.36^b$	$611 \pm 11^{abc}$	$13.21 \pm 0.08^{efg}$
HET20	$27.17 \pm 0.54^{bc}$	$96.83 \pm 2.1$	$4.18 \pm 0.06^{abc}$	$19.67 \pm 11^{e-i}$	$58.47 \pm 0.33^a$	$599 \pm 15^{a-e}$	$13.14 \pm 0.08^{efg}$
HET30	$28.87 \pm 0.19^a$	$98.73 \pm 2.07$	$4.01 \pm 0.04^{abcd}$	$21.34 \pm 20^{ab}$	$52.35 \pm 0.32^b$	$612 \pm 11^{ab}$	$12.48 \pm 0.14^{jk}$
HET40	$26.93 \pm 0.57^{bc}$	$101.83 \pm 5.1$	$4.04 \pm 0.11^{abcd}$	$19.56 \pm 32^{e-i}$	$52.67 \pm 0.25^b$	$589 \pm 15^{a-e}$	$13.49 \pm 0.18^{ef}$
HET50	$26.4 \pm 0.54^{cde}$	$104.67 \pm 6.6$	$3.99 \pm 0.05^{bcd}$	$21.07 \pm 21^{abc}$	$50.45 \pm 0.33^c$	$613 \pm 10^{ab}$	$12.5 \pm 0.16^{ijk}$
KG3	$8.5 \pm 0.41^i$	$96.75 \pm 3.82$	$4.11 \pm 0.09^{abc}$	$18.27 \pm 31^{lmn}$	$44.76 \pm 0.67^{hi}$	$577 \pm 11^{a-e}$	$12.72 \pm 0.13^{g-k}$
KG6	$7.85 \pm 0.11^{ij}$	$94.43 \pm 2.86$	$4.07 \pm 0.03^{abcd}$	$18.61 \pm 26^{l-n}$	$46.8 \pm 0.45^{efg}$	$583 \pm 7^{a-e}$	$12.71 \pm 0.19^{g-k}$
KG9	$7.25 \pm 0.07^{ijk}$	$97 \pm 4.32$	$4.18 \pm 0.11^{abc}$	$19.45 \pm 31^{e-j}$	$32.8 \pm 0.16^p$	$613 \pm 8^{ab}$	$13.03 \pm 0.05^{e-i}$
KG12	$6.3 \pm 0.08^{jkl}$	$99 \pm 5.72$	$4.01 \pm 0.03^{abcd}$	$19.25 \pm 15^{g-k}$	$42.8 \pm 0.16^{jk}$	$606 \pm 6^{a-d}$	$12.95 \pm 0.04^{f-j}$
KG15	$6.11 \pm 0.07^{kl}$	$98.77 \pm 8.86$	$4.13 \pm 0.07^{abc}$	$19.77 \pm 23^{efgh}$	$39.85 \pm 0.23^{lmn}$	$580 \pm 17^{a-e}$	$13.53 \pm 0.09^{de}$
HG3	$27.87 \pm 0.19^{abc}$	$101.67 \pm 6.8$	$4.28 \pm 0.06^a$	$18.2 \pm 15^{lmn}$	$48.1 \pm 0.36^d$	$569 \pm 13^{a-e}$	$14.04 \pm 0.1^d$
HG6	$27.17 \pm 0.31^{bc}$	$101 \pm 8.52$	$4.13 \pm 0.06^{abc}$	$18.85 \pm 15^{l-m}$	$58.63 \pm 0.29^a$	$549 \pm 11^e$	$13.49 \pm 0.09^{def}$
HG9	$26.27 \pm 0.56^{cde}$	$98.43 \pm 7.73$	$4.21 \pm 0.08^{ab}$	$21.17 \pm 27^{ab}$	$39.17 \pm 0.21^{mno}$	$563 \pm 16^{b-e}$	$13.35 \pm 0.15^{ef}$
HG12	$26.83 \pm 0.62^{bcd}$	$101.53 \pm 8.88$	$4.13 \pm 0.08^{abc}$	$19.05 \pm 10^{g-l}$	$50.63 \pm 0.29^c$	$612 \pm 10^{ab}$	$12.95 \pm 0.11^{f-j}$
HG15	$25 \pm 0.82^{ef}$	$96.77 \pm 5.98$	$4.23 \pm 0.07^{ab}$	$19.37 \pm 19^{f-j}$	$50.57 \pm 0.23^c$	$604 \pm 8^{a-d}$	$13.1 \pm 0.14^{e-h}$
HE3	$26.6 \pm 0.65^{bcde}$	$99.38 \pm 6.14$	$3.96 \pm 0.09^{bcd}$	$21.17 \pm 09^{ab}$	$39.83 \pm 0.46^{lmn}$	$611 \pm 11^{abc}$	$13.22 \pm 0.09^{efg}$
HE6	$25.3 \pm 0.5^{def}$	$100.73 \pm 5.87$	$4.06 \pm 0.09^{abcd}$	$19.67 \pm 18^{e-i}$	$39.27 \pm 0.25^{lmno}$	$549 \pm 11^e$	$12.47 \pm 0.14^{jk}$
HE9	$23.9 \pm 0.67^f$	$102.67 \pm 8.06$	$4.18 \pm 0.11^{abc}$	$18.84 \pm 15^{l-m}$	$38.37 \pm 0.29^o$	$563 \pm 17^{b-e}$	$13.43 \pm 0.17^{ef}$
HE12	$19.5 \pm 0.71^g$	$100.7 \pm 5.23$	$4.28 \pm 0.06^a$	$19.9 \pm 23^{defg}$	$39.07 \pm 0.12^{no}$	$602 \pm 4^{a-d}$	$11.51 \pm 0.13^l$
HE15	$17.43 \pm 0.49^h$	$104.63 \pm 8.41$	$4.23 \pm 0.03^{ab}$	$21.58 \pm 29^a$	$40.4 \pm 0.36^l$	$586 \pm 17^{a-e}$	$13.17 \pm 0.12^{efg}$
KE3	$8.2 \pm 0.15^i$	$97.74 \pm 3.79$	$4.22 \pm 0.1^{ab}$	$20.21 \pm 15^{def}$	$46.37 \pm 0.25^{de}$	$581 \pm 19^{a-e}$	$11.52 \pm 0.17^l$
KE6	$7.8 \pm 0.08^{ij}$	$100.7 \pm 4.92$	$4.14 \pm 0.04^{abc}$	$21.48 \pm 32^{ab}$	$47.63 \pm 0.19^{de}$	$611 \pm 10^{abc}$	$13.2 \pm 0.11^{efg}$
KE9	$5.57 \pm 0.12^{lm}$	$100.33 \pm 8.01$	$4.04 \pm 0.07^{abcd}$	$21.37 \pm 30^{ab}$	$47.23 \pm 0.26^{def}$	$561 \pm 22^{cde}$	$12.31 \pm 0.13^k$
KE12	$4.3 \pm 0.04^{m}$	$101.67 \pm 7.41$	$4.28 \pm 0.08^a$	$20.31 \pm 22^{cde}$	$46.47 \pm 0.34^{efg}$	$549 \pm 15^e$	$15.58 \pm 0.13^c$
KE15	$4.01 \pm 0.08^{m}$	$102 \pm 7.12$	$4.23 \pm 0.06^{ab}$	$21.16 \pm 33^{ab}$	$47.07 \pm 0.25^{d-g}$	$566 \pm 13^{a-e}$	$12.59 \pm 0.17^{h-k}$

Means carrying different letters in a column are statistically significant ( $p < 0.01$ ).

Kunri control (KC), Hybrid Control (HC), Kunri ethylene oxide treated (KET10), Kunri ethylene oxide treated (KET20), Kunri ethylene oxide treated (KET30), Kunri ethylene oxide treated (KET40), Kunri ethylene oxide treated (KET50), Hybrid ethylene oxide treated (HET10), Hybrid ethylene oxide treated (HET20), Hybrid ethylene oxide treated (HET30), Hybrid ethylene oxide treated (HET40), Hybrid ethylene oxide treated (HET50), Kunri gamma treated (KG3), Kunri gamma treated (KG6), Kunri gamma treated (KG9), Kunri gamma treated (KG12), Kunri gamma treated (KG15), Hybrid gamma treated (HG3), Hybrid gamma treated (HG6), Hybrid gamma treated (HG9), Hybrid gamma treated (HG12), Hybrid gamma treated (HG15), Hybrid E-beam treated (HE3), Hybrid E-beam treated (HE6), Hybrid E-beam treated (HE9), Hybrid E-beam treated (HE12), Hybrid E-beam treated (HE15), Kunri E-beam treated (KE3), Kunri E-beam treated (KE6), Kunri E-beam treated (KE9), Kunri E-beam treated (KE12), Kunri E-beam treated (KE15)

waves toward the chilli samples. The chief factor that affects the chilli treatments regarding water activity is the varietal differences in all treatments. The main varieties possess differences in water activity *i.e.* Kunri  $0.40 \pm 0.06$  and Hybrid  $0.34 \pm 0.03$ . This is because of the initial drying of the chilli samples that removes the maximum amount of water from the chilli to achieve equilibrium regarding the moisture content/water activity. A group of scientists, assess the effect of water activity on growth patterns of *Aspergillus flavus* in dried chilli samples. They considered water activity as a significant factor that influences the rate of growth of fungus on different food commodities including chilli [31].

### 3.1.6. Chemometric analysis on the basis of physicochemical profile of TRCP

Chemometrics consists of the use of mathematical and statistical techniques to understand the chemical profile along with depicting correlation among physical parameters and quality attributes to analyze data. In the current study chemometric techniques such as Principal Component Analysis (PCA) and Cluster Analysis (CA) were applied to visualize variations in multivariate datasets. The classification capability of PCA has been depicted in Fig. 1. Assessment of the score plot in Fig. 1 (a) depicts that the variety with treatment causes the least variations among the studied chilli varieties. HC and HG15 separated from the rest of the varieties along with PC-1 direction and attains the highest values. The distance between locations of different varieties on the score plot is directly proportional to the degree of similarity or differences among them. The correlation loading plot has been shown in Fig. 1 (b), that illustrates the influence of various variables on PCA. It was presented that the first two principal components elucidated 95 % of the variability in which PC-1 explained 85 % of the total variance while PC-2 explained 10 %. The loading plot depicted the information related to the correlation among investigated parameters. The parameters that exist apart are negative while those lying close to each other are positively correlated. Moisture and water activity mainly designated PC-2 directions and are slightly positively correlated. Conversely, both fat and proteins have a negative correlation with moisture, ash and water activity. Besides, ash content tends to reside in the extreme right of the loading plot identifying that it has an impact on variation in studied chilli varieties.

The chilli varieties that received different irradiation techniques such as electron beam and gamma rays were classified into different groups while applying Hierarchical Cluster Analysis (HCA) based on their physicochemical compositional profile. The chilli treatments were grouped into three main clusters as depicted in Fig. 1 (c). All three clusters could be recognized in the dendrogram obtained by using the centroid clustering technique as shown in Fig. 1 (d). It was evident that chilli treatments such as HG15, HG12, HET50, HET40, HET30, HET10, KE6, HG3, KE15, KE9, KG6, KE12, KE3, HC, KG12, KET50, KG3, KC were grouped in cluster 1. Cluster 3 indicated that only two chilli treatments *i.e.* HG6 and HET 20 were placed in separate groups depicting their distinct characteristics compared to other treatments.

## 3.2. Aflatoxin, phytochemical and antioxidant composition of TRCP

The analysis of variance regarding aflatoxin, phytochemical and antioxidant composition are considered as significant parameters in the assessment of the safety and nutritional quality of chilli. The chilli samples were tested for the presence of aflatoxins, color, ABTS, Flavonoids, TPC, AsA and Carotenoids contents. The results regarding the above-mentioned parameters of various chilli treatments are described and discussed in subsequent sections.

### 3.2.1. Aflatoxins content of TRCP

The aflatoxins developed in agricultural commodities due to fungal contamination. The results depicted that both selected varieties of chilli samples contain different amounts of aflatoxins such as Kunri and Hybrid,  $8.6 \pm 0.04$  and  $28.2 \pm 0.86$  ppb respectively (Table 2). It was evident from the research that the Hybrid contains more aflatoxins compared to the Kunri variety. However, the applied treatments for the reduction of aflatoxins showed a different impact on each variety. Ethylene oxide treatment is less effective against aflatoxins compared to irradiation treatments, and it is worth mentioning that electron beam has shown far superior results compared to other treatments. The electron beam treatment showed a negative correlation with the aflatoxins, which means the more radiation exposure to the chilli the fewer aflatoxin residues in the sample. For instance, the lowest values of aflatoxins were reported in KE15 *i.e.*  $4.01 \pm 0.08$  ppb (Table 2). Also kept the physicochemical profile in good value as depicted in Table 1.

Mozaffari Nejad, Sabouri Ghannad [39] carried out a study to detect aflatoxins in Iranian and Indian spices through the ELISA method. They stated that Iranian spices contain more aflatoxins compared to Indian spices. Besides, they depicted that chilli contains higher concentrations of aflatoxins than black pepper. This study showed the potential threat of aflatoxins in chillies samples.

### 3.2.2. ABTS assay of TRCP

The ABTS assay is used to evaluate the ability of antioxidants to scavenge the ABTS values in aqueous phase compared to the Trolox standard (water-soluble vitamin D analogue). These ABTS molecules are synthesized by the reaction of oxidizing agents such as potassium persulfate and/or potassium permanganate with ABTS salt. The ABTS assay values varied among all treatments ranging from  $3.82 \pm 0.01$   $\mu\text{M/g}$  to  $4.28 \pm 0.06$   $\mu\text{M/g}$  (Table 2). The maximum ABTS assay value was observed in HE12 *i.e.*  $4.28 \pm 0.06$   $\mu\text{M/g}$  while the minimum value was found in KC *i.e.*  $3.82 \pm 0.01$   $\mu\text{M/g}$ . It was evident from the results that both varieties intrinsically showed different results regarding ABTS assay *i.e.* HC  $4.13 \pm 0.05$   $\mu\text{M/g}$ . The ABTS assay values didn't show significant differences among the chilli samples treated with irradiations such as ETO, gamma rays and electron beam as depicted in Table 2.

Similar results were described in earlier studies conducted by Floegel, Kim [40] who compare ABTS and DPPH assays to evaluate the antioxidant potential of chilli and other antioxidant-rich foods. They stated that ABTS assay-based evaluation of antioxidant capacity possesses a strong relationship with oxygen radical absorbance capacity. Earlier, Kim, Lee [41] also estimated the antioxidant capacity of chilli by using ABTS and DPPH assays which were reduced by the drying process. They stated that exposure time incredibly



reduces the antioxidant capacity of chilli samples. The same context, of the present study was observed where the antioxidant capacity was affected by exposure to different treatments.

### 3.2.3. Total phenolic content of TRCP

The total phenolic compounds are considered vital plant constituents having redox characteristics with antioxidant properties [42]. The free radical scavenging activity of these compounds is due to their hydroxyl groups which are measured through the Folin-Ciocalteu reagent. The total phenolic content depicted variation among all chilli treatments from the maximum value observed in KET30 *i.e.*  $32.4 \pm 0.41$  mM/g while the minimum value was seen in HG6 *i.e.*  $58.63 \pm 0.29$  mM/g. It was evident from the results that both varieties also possess different total phenolic contents as Kunri showed fewer values *i.e.*  $43.90 \pm 0.15$   $\mu$ M/g compared to Hybrid which depicts more values *i.e.*  $45.87 \pm 0.29$  mM/g (Table 2).

It was evident from the result that ethylene treatments, especially with the hybrid variety performed well in the context of total phenolic content. This might be due to the extended extraction ability of ethylene form and the stress response of chilli tissues which eventually lead to the formation/excretion of phenolic compounds. Same is the true with gamma irradiation treatment however, it was different in case of electron beam (Table 2). In electron beam treatments the total phenolic compounds showed a lower values compared to other treatment methods. It is perhaps due to the capacity of the irradiation for penetration and destroy characteristics of rays.

In another study, a group of scientists Woldemariam, Kießling [5] studied the influence of the electron beam of red chilli powder. They stated similar results of electron beam on the reduction of polyphenols. In the study, they found that different doses levels of electron beam up to 30 kGy had a significant effect on total phenolic content of red chilli powder. Similar results were reported by another group of researchers Koseki, Villavicencio [43], who stated that total phenolics lost due to irradiation dose level of 10–30 kGy on dehydrated rosemary.

### 3.2.4. Total flavonoid content TRCP

The flavonoids are described as natural substances having variable phenolic structures largely found in fruits, vegetables, tea and other spices such as chilli. These compounds are well known for their health-beneficial effects owing to have antioxidant, anti-carcinogenic, anti-mutagenic and anti-inflammatory characteristics. The total flavonoid content showed variations among all treatments in the range of  $17.77 \pm 19$  to  $21.58 \pm 29$  mg/g as indicated in Table 2. The maximum value was observed in HE15 *i.e.*  $21.58 \pm 29$  mg/g while the minimum value was observed in KET30 *i.e.*  $17.77 \pm 19$  mg/g. It was evident from the results that both chilli varieties possess different flavonoid content such as Kunri showed less total flavonoid content *i.e.*  $18.51 \pm 21$  mg/g compared to the Hybrid chilli varieties such as  $20.68 \pm 22$  mg/g. The treatments such as ethylene oxide and irradiation techniques such as gamma irradiation and electron beam on chilli samples affect the total flavonoid contents. Interestingly, it was observed that electron beam treatment increases the flavonoid profile of chilli samples. It is perhaps due to the fact that an electron beam unfolds the other phenolic compounds and activates them as flavonoids. The unfolding of complex molecules might be another reason for an increase in total flavonoids in chilli samples. However, the ethylene oxide treatments affect the total flavonoid content negatively means reducing the available content as described in Table 2. Total flavonoid contents are important characteristics to evaluate the antioxidant potential of food material. The increase in flavonoid content was also reported by other researchers as a matter of detachment of hydrogen bonds from the cell wall which results in high release [44].

### 3.2.5. Ascorbic acid value (AsA) of TRCP

The ascorbic acid, a significant antioxidant compound found in chilli acts as a crucial substrate for enzyme-based detoxification of hydrogen peroxide [45]. The ANOVA depicts significant variation among all treatments with regard to AsA value. The mean squares regarding AsA values are shown in Table 2. The ascorbic acid value varied significantly among all chilli treatments from the maximum value attained by KET10 *i.e.*  $616 \pm 10$   $\mu$ g/g while the minimum value was observed in HG6 *i.e.*  $549 \pm 11$   $\mu$ g/g. Both chilli varieties showed a little difference in ascorbic acid content such as  $572 \pm 19$   $\mu$ g/g in Kunri while  $598 \pm 11$   $\mu$ g/g in Hybrid chilli samples as presented in Table 2. The results regarding AsA value showed variation among all chilli samples treated with ethylene oxide, gamma irradiation and electron beam methods. It was evident from the results, that ethylene oxide showed comparatively higher values for ascorbic acid. This increase might be due to the extraction of AsA from the chilli tissues by interacting with ethylene oxide. However, gamma irradiation and electron beam react with ascorbic acid and depict lower values compared to former mentioned treatment. A group of scientists Iqbal, Amjad [30], evaluated the impact of irradiation on functional compounds in dried chillies. They concluded that the applied dose levels of 2, 4 and 6 kGy of gamma irradiation didn't affect the concentration levels of ascorbic acid compared to control samples.

### 3.2.6. Carotenoids content of TRCP

Carotenoids are considered pigments largely distributed in plants, animals, fungi and even bacteria. They are classified as anti-oxidant agents. The total carotenoid content varied in all chilli samples and varied from  $19.16 \pm 0.23$  mg/g in KC to  $11.51 \pm 0.13$  mg/g in HE12. While both varieties also depict different total carotenoid contents *i.e.*  $19.16 \pm 0.23$  mg/g in Kunri while Hybrid has shown less total carotenoid values *i.e.*  $13.19 \pm 0.08$  mg/g (Table 2). The results pertaining to total carotenoids content showed a decreasing trend in all chilli samples being treated with ethylene oxide, gamma irradiation and electron beam. It was evident from the result electron beam affects the total carotenoids content in a more negative way compared to ethylene oxide. The effect of electron beam on the carotenoids content of local chilli varieties was never studied. The results have shown that electron beam decreased the carotenoids more than gamma irradiations however the ethylene oxide showed a higher valued trend concerning the studied parameter. The

results of the current study are in line with previous studies conducted by Iqbal, Amjad [30] who observed the effect of irradiations on functional compounds of chilli samples. They stated that the immediate level of carotenoids decreases due to the effect of irradiations. Moreover, they indicated storage as 2nd most significant determined in the study.

3.2.7. Color ASTA values TRCP

ASTA analytical method 20.1 is frequently used to determine color values as a significant factor in chilli trade. Color ASTA values are classified as the number of extractable carotenoids thus generally stated as extractable color. The results on color ASTA values indicated a range of values where KET10 depicts the maximum attain value i.e. 108.9 ± 3.65 while KG6 showed the lowest values i.e. 94.43 ± 2.86. It is noteworthy that both varieties of chilli samples showed relatable values for the color ASTA such as HC 99.67 ± 5.19 and KC 98.89 ± 5.21. Although no clear visible differences were observed in color values, however, it could be seen in Table 2 that ETO-treated varieties contain higher values compared to irradiation treatment. Besides, among irradiation treatment lower values were observed in gamma treatments compared to the electron beam.

3.2.8. Chemometric analysis on the basis of aflatoxin and phytochemical profile of TRCP

Chemometrics consists of the use of mathematical and statistical techniques to understand the chemical profile along with depicting correlation among physical parameters and quality attributes to analyze data. In the current study chemometric techniques such as Principal Component Analysis (PCA) and Cluster Analysis (CA) were applied to visualize variations in a multivariate dataset. The classification capability of PCA has been depicted in Fig. 2. Assessment of the score plot in Fig. 2 (a) depicts that the variety with treatment causes the least variations among the studied chilli varieties. KG9 and KET 10 are separated from the rest of the varieties along with PC-1 direction and attained the highest values. The distance between locations of different varieties on the score plot is directly proportional to the degree of similarity or differences among them. The correlation loading plot has been shown in Fig. 2 (b), which illustrates the influence of various variables on PCA. It was presented that the first two principal components elucidated 95 % of the variability in which PC-1 explained 78 % of the total variance while PC-2 explained 17 %. The loading plot depicted the information related to the correlation among investigated parameters. The parameters that exist apart are negative while those lying close to each other are positively correlated. Carotenoids and ABTS assay mainly designated PC-2 directions and are slightly positively correlated. Color, flavonoids and TPC also possess positive correlations with them. Besides, AsA content tends to reside on the extreme

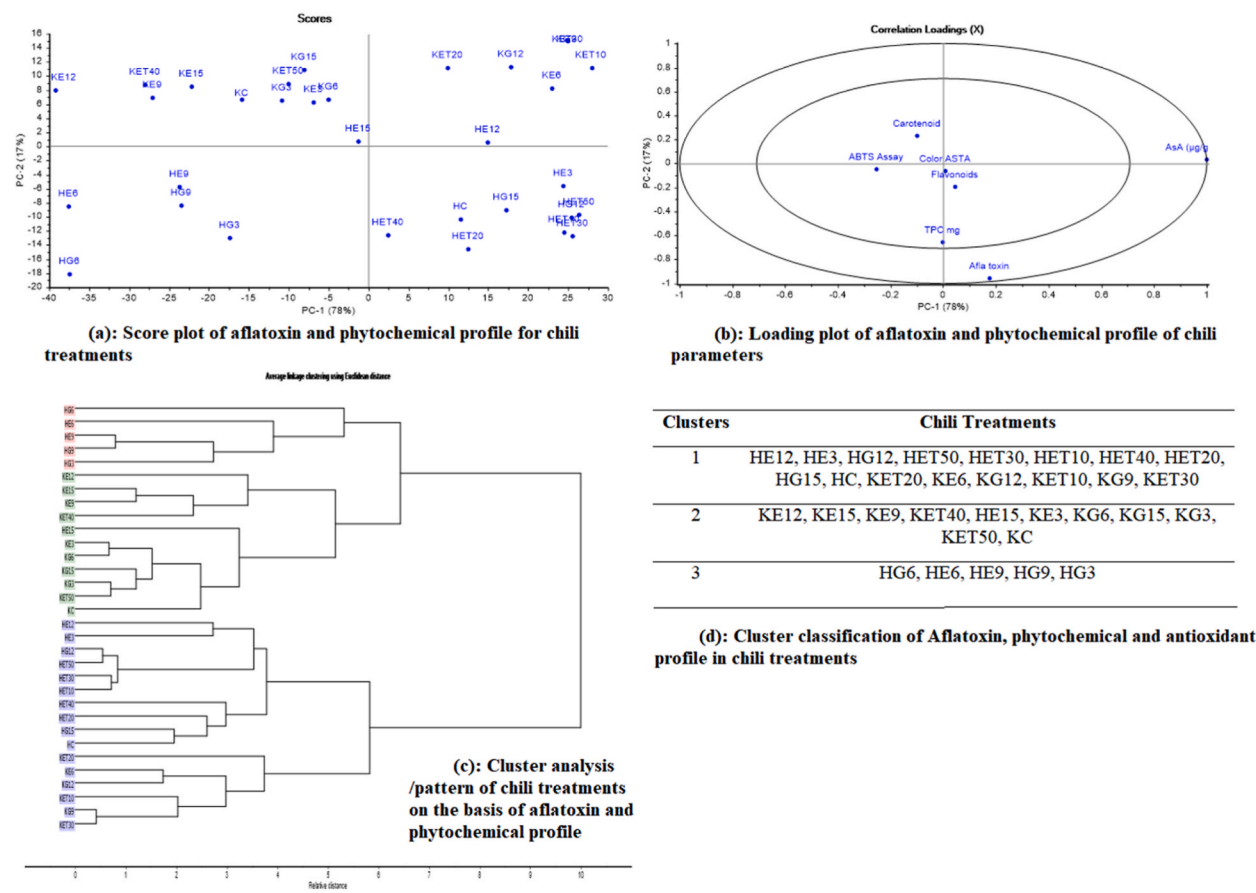


Fig. 2. Chemometric analysis based on Aflatoxin and Phytochemical profile of TRCP.

**Table 3**  
Microbiological analysis of treated red chili powder.

Treatment	Coliform	EB	Staph	E.coli	Aspergillus flavus	Aspergillus parasiticus	TPC	Y & M
KC	6018 ± 86 <sub>bc</sub>	12727 ± 759 <sup>d</sup>	4040 ± 57 <sup>d</sup>	1080 ± 80 <sup>ef</sup>	10000 ± 391 <sup>d</sup>	16363 ± 552 <sup>cd</sup>	1181818 ± 112678 <sub>bcd</sub>	41818 ± 981 <sup>a</sup>
HC	6574 ± 35 <sup>a</sup>	18790 ± 233 <sup>a</sup>	6010 ± 64 <sup>a</sup>	1550 ± 54 <sup>b</sup>	15450 ± 945 <sup>a</sup>	21818 ± 1172 <sup>a</sup>	1545454 ± 275829 <sup>a</sup>	33636 ± 2028
KET10	5153 ± 70 <sup>d</sup>	10507 ± 671 <sup>e</sup>	3080 ± 16 <sup>e</sup>	1000 ± 92 <sup>efg</sup>	7834 ± 60 <sup>f</sup>	14370 ± 874 <sup>def</sup>	1014885 ± 90742 <sub>cde</sub>	38500 ± 829 <sup>a-d</sup>
KET20	5019 ± 86 <sup>d</sup>	10800 ± 108 <sup>e</sup>	2400 ± 110 <sup>f</sup>	90 ± 28 <sup>i</sup>	5050 ± 36 <sup>h</sup>	8720 ± 89 <sup>ij</sup>	809800 ± 21435 <sup>ef</sup>	39754 ± 1164 <sub>abc</sub>
KET30	2020 ± 91 <sup>ij</sup>	5630 ± 73 <sup>g</sup>	1010 ± 72 <sup>h</sup>	0 ± 0 <sup>i</sup>	6100 ± 109 <sup>g</sup>	11390 ± 541 <sup>gh</sup>	509765 ± 11348 <sup>ghi</sup>	24356 ± 611 <sub>ijk</sub>
KET40	1866 ± 82 <sup>j</sup>	2087 ± 17 <sup>hij</sup>	90 ± 15 <sup>k</sup>	0 ± 0 <sup>i</sup>	2190 ± 66 <sup>l</sup>	600 ± 122 <sup>n</sup>	380450 ± 14688 <sup>hij</sup>	6530 ± 88 <sup>no</sup>
KET50	750 ± 41 <sup>no</sup>	1120 ± 32 <sup>jkl</sup>	10 ± 4 <sup>k</sup>	0 ± 0 <sup>i</sup>	10 ± 5 <sup>o</sup>	50 ± 13 <sup>n</sup>	222670 ± 18631 <sub>ijklm</sub>	3000 ± 79 <sup>opq</sup>
HET10	5854 ± 68 <sup>c</sup>	16800 ± 702 <sub>bc</sub>	4120 ± 45 <sup>d</sup>	800 ± 116 <sup>g</sup>	12400 ± 211 <sup>c</sup>	17890 ± 574 <sub>bc</sub>	1212730 ± 136587 <sub>bc</sub>	37340 ± 779 <sup>b-e</sup>
HET20	5820 ± 86 <sup>c</sup>	12320 ± 280 <sup>d</sup>	2890 ± 34 <sup>e</sup>	90 ± 18 <sup>i</sup>	7860 ± 100 <sup>f</sup>	14560 ± 798 <sup>def</sup>	1190876 ± 89092 <sub>bc</sub>	36590 ± 1022 <sub>cde</sub>
HET30	3210 ± 64 <sup>g</sup>	6543 ± 41 <sup>fg</sup>	2200 ± 61 <sub>fg</sub>	10 ± 3 <sup>i</sup>	3000 ± 26 <sub>ijkl</sub>	11230 ± 746 <sup>gh</sup>	780930 ± 15772 <sup>efg</sup>	25400 ± 1263 <sub>ij</sub>
HET40	2141 ± 60 <sup>i</sup>	2980 ± 50 <sup>hi</sup>	1070 ± 52 <sup>h</sup>	0 ± 0 <sup>i</sup>	4010 ± 68 <sup>ij</sup>	3560 ± 120 <sup>lm</sup>	450870 ± 7958 <sup>hi</sup>	8568 ± 198 <sup>mn</sup>
HET50	920 ± 79 <sup>mn</sup>	990 ± 62 <sup>jkl</sup>	1080 ± 72 <sup>h</sup>	0 ± 0 <sup>i</sup>	1040 ± 61 <sup>mno</sup>	1090 ± 30 <sup>n</sup>	240000 ± 7789 <sup>j-m</sup>	4040 ± 173 <sup>op</sup>
KG3	4153 ± 41 <sup>e</sup>	11530 ± 924 <sub>de</sub>	4000 ± 153 <sup>d</sup>	400 ± 93 <sup>h</sup>	9710 ± 212 <sup>de</sup>	15200 ± 864 <sup>de</sup>	1115885 ± 97903 <sub>cd</sub>	40370 ± 894 <sup>ab</sup>
KG6	3856 ± 48 <sup>f</sup>	7820 ± 86 <sup>f</sup>	2020 ± 51 <sup>g</sup>	10 ± 5 <sup>i</sup>	7800 ± 79 <sup>f</sup>	9870 ± 85 <sup>hi</sup>	1054609 ± 87067 <sub>cde</sub>	35600 ± 1216 <sub>def</sub>
KG9	2134 ± 64 <sup>i</sup>	3207 ± 67 <sup>h</sup>	1090 ± 39 <sup>h</sup>	0 ± 0 <sup>i</sup>	2030 ± 66 <sup>lm</sup>	6590 ± 57 <sup>jk</sup>	809765 ± 11918 <sup>ef</sup>	37540 ± 1153 <sub>bcd</sub>
KG12	1010 ± 40 <sub>lm</sub>	1020 ± 55 <sup>jkl</sup>	520 ± 26 <sup>i</sup>	0 ± 0 <sup>i</sup>	980 ± 27 <sup>no</sup>	1300 ± 127 <sup>mn</sup>	367450 ± 18049 <sup>hij</sup>	10700 ± 1043 <sub>lm</sub>
KG15	502 ± 15 <sup>p</sup>	490 ± 28 <sup>kl</sup>	210 ± 9 <sup>jk</sup>	0 ± 0 <sup>i</sup>	450 ± 38 <sup>no</sup>	1120 ± 70 <sup>n</sup>	120876 ± 7337 <sup>jk</sup>	1650 ± 368 <sup>pq</sup>
HG3	6143 ± 45 <sup>b</sup>	17700 ± 442 <sub>ab</sub>	5080 ± 59 <sup>b</sup>	1390 ± 64 <sub>bc</sub>	13890 ± 286 <sup>b</sup>	19870 ± 1344 <sup>ab</sup>	1512720 ± 81678 <sup>a</sup>	30700 ± 1055 <sub>gh</sub>
HG6	5823 ± 51 <sup>c</sup>	15667 ± 110 <sup>c</sup>	5000 ± 87 <sup>b</sup>	1010 ± 81 <sub>de</sub>	14300 ± 852 <sup>b</sup>	12560 ± 924 <sup>fg</sup>	1530030 ± 86426 <sup>a</sup>	24530 ± 1243 <sub>ijk</sub>
HG9	2825 ± 44 <sup>h</sup>	7890 ± 100 <sup>f</sup>	3090 ± 76 <sup>e</sup>	2090 ± 98 <sup>a</sup>	8790 ± 128 <sup>ef</sup>	6540 ± 92 <sup>jk</sup>	890234 ± 16127 <sup>def</sup>	27580 ± 1143 <sub>hi</sub>
HG12	1200 ± 35 <sub>kl</sub>	1790 ± 18 <sup>ijk</sup>	1060 ± 69 <sup>h</sup>	60 ± 13 <sup>i</sup>	3287 ± 58 <sup>jk</sup>	2300 ± 160 <sup>mn</sup>	510000 ± 16330 <sup>ghi</sup>	13890 ± 265 <sup>l</sup>
HG15	1050 ± 31 <sub>lm</sub>	1040 ± 64 <sup>jkl</sup>	430 ± 39 <sup>ij</sup>	0 ± 0 <sup>i</sup>	1040 ± 73 <sup>mno</sup>	810 ± 74 <sup>n</sup>	154890 ± 9934 <sup>jk</sup>	3400 ± 228 <sup>opq</sup>
HE3	4321 ± 42 <sup>e</sup>	16542 ± 646 <sub>bc</sub>	4490 ± 89 <sup>c</sup>	1200 ± 105 <sub>de</sub>	13500 ± 356 <sup>b</sup>	18760 ± 981 <sup>b</sup>	1422822 ± 70679 <sub>ab</sub>	31250 ± 1364 <sub>gh</sub>
HE6	2010 ± 39 <sup>ij</sup>	12380 ± 509 <sup>d</sup>	3030 ± 70 <sup>e</sup>	803 ± 26 <sup>f</sup>	7890 ± 59 <sup>f</sup>	013470 ± 859 <sup>efg</sup>	1139036 ± 120525 <sub>bcd</sub>	23600 ± 1314 <sub>jk</sub>
HE9	1320 ± 34 <sup>k</sup>	6920 ± 72 <sup>fg</sup>	2100 ± 71 <sup>g</sup>	1080 ± 31 <sup>ef</sup>	4560 ± 62 <sup>hi</sup>	7658 ± 215 <sup>ij</sup>	780453 ± 22762 <sup>efg</sup>	10500 ± 1101 <sub>lm</sub>
HE12	600 ± 20 <sup>op</sup>	1323 ± 56 <sup>jkl</sup>	1080 ± 49 <sup>h</sup>	9 ± 5 <sup>i</sup>	1050 ± 76 <sup>mn</sup>	2300 ± 84 <sup>mn</sup>	385891 ± 12756 <sup>hij</sup>	4040 ± 122 <sup>op</sup>
HE15	10 ± 2 <sup>q</sup>	90 ± 5 <sup>l</sup>	60 ± 6 <sup>k</sup>	0 ± 0 <sup>i</sup>	210 ± 14 <sup>no</sup>	90 ± 8 <sup>n</sup>	98000 ± 816 <sup>jk</sup>	1020 ± 128 <sup>pq</sup>
KE3	3916 ± 31 <sup>f</sup>	10850 ± 542 <sup>e</sup>	2380 ± 103 <sup>f</sup>	970 ± 51 <sup>fg</sup>	8640 ± 82 <sup>f</sup>	14568 ± 904 <sup>def</sup>	980870 ± 8530 <sup>cde</sup>	32760 ± 1282 <sub>fg</sub>
KE6	2923 ± 24 <sup>h</sup>	7590 ± 36 <sup>f</sup>	1050 ± 98 <sup>h</sup>	10 ± 5 <sup>i</sup>	6300 ± 47 <sup>g</sup>	11570 ± 700 <sup>gh</sup>	619820 ± 2251 <sup>fgh</sup>	21340 ± 1575 <sup>k</sup>
KE9	1200 ± 34 <sub>kl</sub>	2330 ± 48 <sup>hij</sup>	90 ± 8 <sup>k</sup>	0 ± 0 <sup>i</sup>	2910 ± 83 <sup>kl</sup>	5000 ± 89 <sup>kl</sup>	340670 ± 8196 <sup>hij</sup>	7800 ± 158 <sup>mn</sup>
KE12	70 ± 5 <sup>q</sup>	1570 ± 42 <sup>jk</sup>	0 ± 0 <sup>k</sup>	0 ± 0 <sup>i</sup>	1010 ± 43 <sup>mno</sup>	1200 ± 81 <sup>n</sup>	98040 ± 2157 <sup>jk</sup>	1860 ± 115 <sup>pq</sup>
KE15	0 ± 0 <sup>q</sup>	7 ± 3 <sup>l</sup>	0 ± 0 <sup>k</sup>	0 ± 0 <sup>i</sup>	10 ± 5 <sup>o</sup>	70 ± 7 <sup>n</sup>	10220 ± 574 <sup>k</sup>	90 ± 12 <sup>q</sup>

Means carrying different letters in a column are statistically significant ( $p < 0.01$ ); ND means not detected.

Kunri control (KC), Hybrid Control (HC), Kunri ethylene oxide treated (KET10), Kunri ethylene oxide treated (KET20), Kunri ethylene oxide treated (KET30), Kunri ethylene oxide treated (KET40), Kunri ethylene oxide treated (KET50), Hybrid ethylene oxide treated (HET10), Hybrid ethylene oxide treated (HET20), Hybrid ethylene oxide treated (HET30), Hybrid ethylene oxide treated (HET40), Hybrid ethylene oxide treated (HET50), Kunri gamma treated (KG3), Kunri gamma treated (KG6), Kunri gamma treated (KG9), Kunri gamma treated (KG12), Kunri gamma treated (KG15), Hybrid gamma treated (HG3), Hybrid gamma treated (HG6), Hybrid gamma treated (HG9), Hybrid gamma treated (HG12), Hybrid gamma treated (HG15), Hybrid E-beam treated (HE3), Hybrid E-beam treated (HE6), Hybrid E-beam treated (HE9), Hybrid E-beam treated (HE12), Hybrid E-beam treated

(HE15), Kunri E-beam treated (KE3), Kunri E-beam treated (KE6), Kunri E-beam treated (KE9), Kunri E-beam treated (KE12), Kunri E-beam treated (KE15)

right of the loading plot identifying that it has an impact on variation in studied chilli varieties. The chilli varieties which received different irradiation techniques such as electron beam and gamma rays were classified into different groups while applying Hierarchical Cluster Analysis (HCA) on the basis of their physicochemical compositional profile. The chilli treatments were grouped into three main clusters as depicted in Fig. 2 (c). All three clusters could be recognized in the dendrogram obtained by using the centroid clustering technique as shown in Fig. 2 (d). It was evident that chilli treatments such as HE12, HE3, HG12, HET50, HET30, HET10, HET40, HET20, HG15, HC, KET20, KE6, KG12, KET10, KG9, KET30 were grouped in cluster 1. Cluster 3 indicated that five chilli treatments *i.e.* HG6, HE6, HE9, HG9 and HG3 were placed in separate groups depicting their distinct characteristics compared to other treatments.

### 3.3. Microbial count in TRCP

The microbial count in chilli treatments being treated with ethylene oxide, gamma irradiation and electron beam methods showed significant variations among the results. It was evident from the results that both varieties behaved differently in terms of microbial colonies and treatments. For instance, the Kunri variety presented lower microbial numbers for all tested microorganisms except general yeast and mold count compared to the Hybrid variety. The data showed that ethylene oxide is more effective against *E. coli* in higher concentrations in both chilli varieties. Moreover, gamma irradiations showed effectiveness against all microbial loads compared to ETO. However, the maximum effectiveness against all types of microbial colonies was shown by the electron beam. Precisely, 12 and 15 kGy dosage levels were more lethal as compared to other levels especially in the case of *Aspergillus* species which are responsible for the production of aflatoxins (possesses the maximum problem in the case of chillies). The electron beam was also lethal to other pathogenic bacterial species such as *Coliforms*, *E. coli*, *Enterobacteriaceae* and *Staphylococci* results listed in Table 3. The present study is in line with the findings of Song, Sung [46], who worked on the effect of irradiations on pathogenic bacteria such as *E. coli* and *Salmonella*. They described that 5 kGy is a level at which *E. coli* 3.8 log cycle reduction was observed in red chilli samples.

## 4. Conclusion

It was evident from the study that the Kunri chilli variety depicts higher nutritional and responsive values compared to the Hybrid variety. A close overview of the degradation pattern depicts that ETO has proven more detrimental to the protein and other functional constituents compared to irradiation techniques, however, surprisingly electron beam irradiation technique has proven effective in preserving the protein and antioxidant potential of the chilli samples compared to gamma. That is probably due to the fact that an electron beam is less likely to disintegrate the larger molecules compared to gamma irradiations. In conclusion, it is recommended to use an electron beam as a novel method for controlling aflatoxins, mold and pathogenic microbial growth while saving the Physicochemical, and phytochemical profile values of the chilli spice. Moreover, gamma irradiations also shown good results and potential application by replacing ethylene oxide which is considered as likely carcinogenic molecule. So applying electron beam or gamma irradiations on chilli samples with respect to the local variety is recommended. Further, investigation is required to determine the particle size dependency, product characteristics and efficacy studies for optimization of the whole process of irradiation technology. Finally, the induction and resuscitation of injured cells in chilli upon the exposure of irradiations need to be studied.

## 5. Data availability statement

Data will be furnished upon request.

## Additional information

No additional information is available for this paper.

## CRediT authorship contribution statement

**Muzzammal Ahmed Muzzafar:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Shinawar Waseem Ali:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Munawar Iqbal:** Software, Methodology, Formal analysis. **Maryam Saeed:** Investigation. **Mateen Ahmad:** Investigation. **Muhammad Rizwan Tariq:** Resources, Methodology. **Abdikhaliq Mursal Yusuf:** Resources, Investigation. **Ayesha Murtaza:** Resources, Methodology. **Aftab Ahmed:** Resources. **Shazia Yaqub:** Resources. **Muhammad Riaz:** Formal analysis.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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