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*Research article*

## **Impact of low energy electron beam on black pepper (*Piper nigrum* L.) microbial reduction, quality parameters, and antioxidant activity**

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**Abstract:** Low energy electron beam (e-beam) has the ability to decontaminate or reduce bioburden and enhance the food product's safety with minimal quality loss. The current study aimed to evaluate the efficacy of e-beam on natural microbiota and quality changes in black peppercorns. The black pepper was exposed to e-beam at doses from 6–18 kGy. The microbial quality, physicochemical attributes, total phenolic compounds, and antioxidant activity were evaluated. Results demonstrated the microbial population in black pepper decreased with increasing e-beam treatment doses. Significant inactivation of Total Plate Count (TPC), yeasts, and molds were observed at dose 6 kGy by 2.3, 0.7, and 1.3 log CFU g<sup>-1</sup>, respectively, while at 18 kGy the reduction level was 6, 2.9, and 4.4 log CFU g<sup>-1</sup>, respectively. Similarly, 18 kGy of e-beam yielded a reduction of 3.3 and 3.1 log CFU g<sup>-1</sup> of *Salmonella* Typhimurium and coliform bacteria, respectively. A significant difference ( $p < 0.05$ ) was noted between doses 12, 15, and 18 kGy on *Bacillus cereus* and *Clostridium perfringens* in black pepper. During e-beam doses, the values  $L^*$ ,  $a^*$  and  $b^*$  of black peppercorn were not noticeably altered up to 18 kGy dose. No significant ( $p > 0.05$ ) difference in moisture, volatile oil, and piperine content upon (6–18 kGy) treatments in comparison to the control. A slight difference in the

bioactive compound, retaining >90% of total phenolic compounds and antioxidant activity. Results revealed that e-beam doses  $\geq 18$  kGy were influential for inactivating natural microbes and foodborne pathogens without compromising the physicochemical properties and antioxidant activity of black peppercorns.

**Keywords:** electron beam; inactivation; microbial quality; antioxidant activity; black pepper

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## 1. Introduction

Spices are the most important food additives for enhancing color and flavor since ancient times in Egypt, and different parts of the world. It has many benefits besides seasoning food such as antimicrobials, antioxidants, and anti-inflammatory [1]. Currently, the application of spices occupies an important position in the global market, and its safety during the supply chain is targeted [2]. Egypt is one of the major exporter countries of spices i.e. fennel, basil, parsley, marjoram, cumin, thyme, and anise, but imports black pepper from Vietnam, Indonesia and Brazil, oregano, and sage from Turkey and Albania [3]. While, the USA is the largest importer country of spices from Egypt, India, China, and Europe [4]. During harvest, processing, and handling, the spices may be subjected to significant microbial contamination [5]. Therefore, spices act as a real factor in food contamination when added after cooking and/or ready-to-eat (RTE) foods, leading to serious health risks [6]. Recent studies [7,8] have discovered a link between mortality and consumption of spicy foods due to microbiological contamination and chemical agents.

Black peppercorn (*Piper nigrum* L) is quite used as a spice to enrich the taste and odor of foods. Further, it is beneficial to human health [9]. Black peppercorn liquors contain antioxidant compounds and anti-radical properties [10] and antimicrobial properties [11]. However, black pepper has been found to contain *Clostridium perfringens*, *Bacillus* spp., and *Staphylococcus* spp. [12]. In the United States in 2010, approximately 272 people grew sick as a result of *Salmonella* Montevideo contamination in sliced salami [13]. Black pepper was discovered to be the primary source of pathogen contamination, which was added to salami after it had been pasteurized.

Microbial food safety of dried spices is a substantial concern with trade and health implications. According to a study by Van Doren, Neil [14], three outbreaks from the spices in the United States from (2007 to 2010) were reported. An estimated 457 illness cases, 68 hospitalizations, and one death. Most illness cases are caused by serotypes of *Salmonella enterica* subspecies enterica (87%) and *Bacillus* spp. (13%). According to the CDC estimates one million people get sick in the USA from food contaminated with *Salmonella* each year [15,16]. Other studies confirmed that spices were contaminated with different foodborne pathogens i.e. fecal coliform, *E. coli*, *Listeria monocytogenes*, *B. cereus*, and *Salmonella* Typhimurium [17,18]. Spices associated with the outbreaks were red pepper (*Capsicum* spp.), black peppercorn (*Piper nigrum*), anise seed (*Pimpinella anisum*), fennel seed (*Foeniculum vulgare*), thyme powder (*Thymus vulgaris*), sage powder (*Salvia officinalis*), and oregano powder (*Rosmarinus officinalis* L.) [14]. As seen, many spice contaminants with undesirable bacteria and pathogens, whereas enzymatic and biochemical deterioration also occur [6].

One way to control and reducing of microbial contamination in these spices, thereby improving quality and safety, is using ethylene oxide (EO) and/or steam sterilization (SS) [19]. Although EO is

effective for the reduction of microbial population and killing insects in spices, but has health hazards and is carcinogenic [20]. EO is permitted in the USA with less than 50 ppm, while not permitted in European countries [21]. The SS is safe and effective against microbial growth as well, not chemical residue but a loss of essential oil [22]. One novel approach to overcome these disadvantages of prior methods is the use of irradiation by electron beam low energy [23].

Food irradiation was utilized on a commercial scale in the 1950s, and since then it's well known to be a safe and effective way of decontamination and inactivating foodborne pathogens of spices [24]. Nowadays, more than 60 countries permit irradiation of spices and other food commodities, around 24 countries recommended a dose of ~10 kGy, and other countries, including the USA allow up to 30 kGy [25,26]. The main sources of  $\gamma$ -rays (nuclear energy) are  $^{137}\text{Cs}$  or  $^{60}\text{Co}$  of shorter half-life [27]. Recently, electron beams (e-beam) and also called  $\beta$ - rays are linear and/or circular electron accelerators, the electrons generated from an electrical machine with an energy level of 10 MeV [23]. E-beam has benefits, i.e. chemical-free, nonthermal, no nuclear waste, and more effective against foodborne pathogens, fungi, and molds comparatively with Ethylene oxide (ETO), Propylene oxide (PPO), and steam sterilization technologies [28]. *In vitro* studies found that e-beam with energy of 80 and 200 keV was effective against *E. coli*, *Bacillus pumilus*, and viruses [29–31]. The impact of e-beam (low energy) was checked for tainted spices [32]. Another study demonstrated the effectiveness of e-beam at an energy of 300 keV against foodborne pathogens in white and black pepper [23]. Also, found that e-beam irradiation had positive impacts on Fuzhuan brick-tea for improving sensory quality and concentration of polyphenolic compounds [33]. The current study aimed to assess the impact of e-beam irradiation on microbial decontamination, physicochemical properties, and antioxidant ability of black peppercorns.

## 2. Materials and methods

### 2.1. Materials

Whole black peppercorn (*Piper nigrum* L.) was obtained from Vietnam, in August 2020. Foreign materials and impurities were mechanically removed. The pure samples were packed in bags (~ 50 pounds).

### 2.2. Electron beam irradiation

The electron beam treatment of whole black peppercorn samples was done at  $20 \pm 2$  °C according to Woldemariam, Kießling [34]. The system consists of two low-energy electron beam lamps in a face-to-face configuration. The black peppercorn free falls between them in a thin layer. The lamps emit electrons with low electron energy, defined as having a maximum kinetic energy of 300 keV. The processing unit conveys the material to the treatment zone and then out of the system, where the tested material is collected. The system ramps up in seconds and only allows the conveying of the foodstuff into the treatment zone once the electron beams are stable at the facility in New Jersey, USA. The accelerator could generate between 248 and 250 keV energy levels. The e-beam can deliver dose ranges up to 18 kGy at different levels of doses (6, 9, 12, 15, and 18 kGy) were applied. A radio-chromic film (B3) is utilized for dose measurements and is calibrated to international standards (NPL). On-the-product Dosimetry is not possible so, a product model combined with a Monte Carlo simulation

is used to estimate the dose delivered to the black peppercorns. B3 radiochromic film Dosimetry systems are commonly used in industrial irradiation processing. The Dosimetry system is calibrated by RISO HDRL with traceability to The NPL national standard and measures  $D_{\mu}$ , the dose to the first micron of the dosimeter film [35]. The B3 Dosimeters are measured using the RisoScan software provided by RISO HDRL.

### 2.3. Microbiological assay

Microbial quality (naturally contaminated) of black peppercorn samples irradiated and control was carried out. The samples were handled aseptically in a filtered bag (Bethlehem, PA., USA) containing 25 mL buffered peptone water (BPW; Difco; 0.1%), homogenised (Seward 400 Stomacher, West Sussex, England). Serial dilutions were done and 100  $\mu$ L spread plated onto Plate Count Agar (TBC, Difco) for the total bacterial count, Xylose Lysine Deoxycholate Agar (XLD, Difco) for *Salmonella* Typhimurium, Violet Red Bile Agar (VRBA, Difco) for coliform, Bacara agar (chromogenic) for *Bacillus cereus*, Tryptone-sulfite-neomycin (TSN) agar for *Clostridium perfringens*, and YM Agar and YM Broth for mold and yeast to ascertain the presence of any microbial cells. The colonies were counted after 24 to 48 hours of incubation at 37 °C, while molds and yeasts were counted after 5 days at 7 °C at 25 °C, the populations expressed as  $\log_{10}$  CFU/g [36].

### 2.4. Antioxidants activity

The ability of black pepper to scavenge DPPH radicals was done according to Liu, Ardo [37]. In brief, 200  $\mu$ g of black pepper was blended with a 3.8 mL DPPH solution and placed at 25 °C in a dark condition for 1 h. At 517 nm, the absorbance of the mixture was measured. Results were expressed as  $IC_{50}$  ( $\mu$ g  $mL^{-1}$ ) using a standardized ascorbic acid solution [38].

### 2.5. Color

The color value of irradiated and control samples was measured by a spectrophotometer CM-508d (Minolta Corp., Ramsey, U.S.A.) [39]. The values of  $L^*$ ,  $a^*$ , and  $b^*$  were measured. A standard white tile was used to calibrate the instrument.

### 2.6. Total phenolic (TP)

The TP content of black peppercorn was determined using the Folin-Ciocalteu method at 750 nm [40]. In brief, 200  $\mu$ g of the sample and 3 mL distilled water, mixed gently with Folin-Ciocalteu reagent (0.5 mL/3 min), and then 2 mL sodium carbonate (20%;  $w/v$ ) were added. The mixture was placed in the dark for 60 minutes before the absorbance was at 750 nm. TP was expressed as mg of Gallic acid equivalent (GAE) per 100 g of black peppercorn.

### 2.7. Volatile oil, moisture, and piperine

The volatile matter in black pepper was determined using the distillation method by the Clevenger instrument [41]. The moisture content in the sample was performed according to AOAC [41]. The piperine content was determined using a UV-Visible spectrophotometer at wavelength 342–345 nm [41].

## 2.8. Data analysis

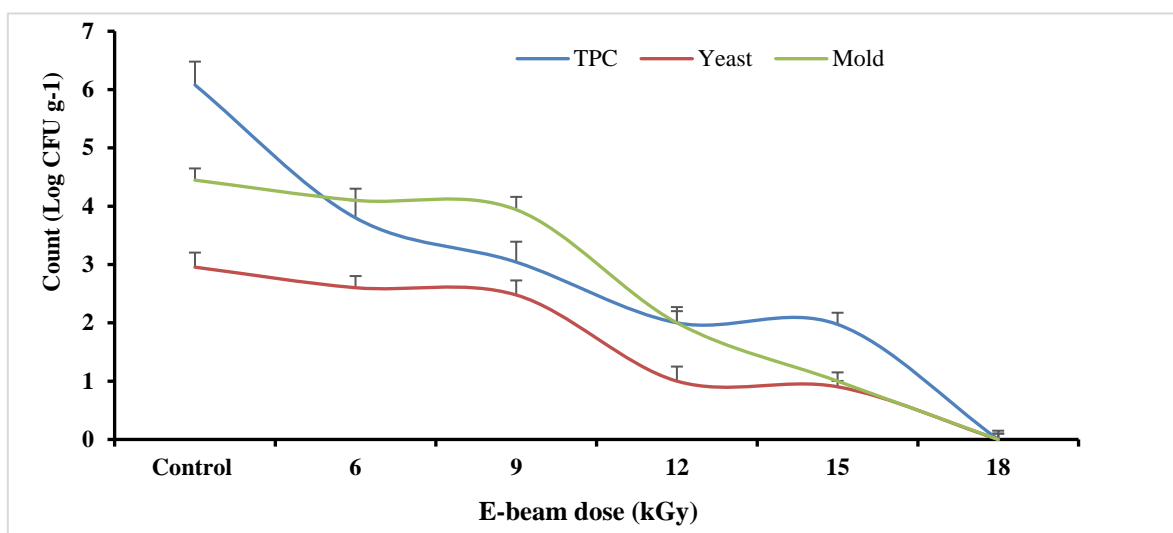
Results were statically analyzed using one-way ANOVA with a significance level of ( $p \leq 0.05$ ) using SPSS software (version 19; Chicago, IL, U.S.A.). The physicochemical properties and microbiological assay variance were analyzed as a completely randomized design according to Steel and Torrie [42]. All experiments and tests were carried out in triplicate. Multiple comparisons were made, using the least significant difference (LSD).

## 3. Results and discussion

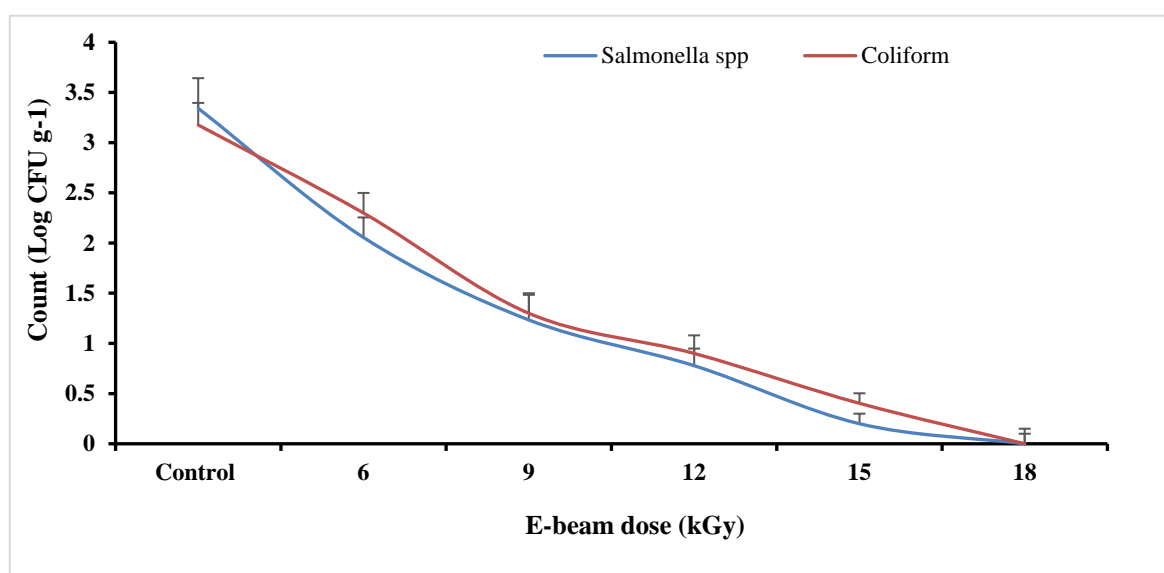
### 3.1. Inactivation of natural microbial load in black peppercorn using e-beam

Black peppercorn (*Piper nigrum* L.) is a crucial spice and most consumed worldwide. The impact of e-beam doses on the decontamination of microbes presented in black peppercorns was evaluated. Results revealed that the control sample had a preliminary microbial count of 6, 3.3, 3.1, 2.9, 4.4, 2.1, and 1.7 log CFU/g of TPC, *Salmonella*, Coliform, yeasts, molds, *Bacillus cereus*, and *Clostridium perfringens*, respectively. Generally, a significant reduction ( $p < 0.05$ ) was observed in microbial counts in irradiated samples compared to the control. At dose six kGy, the number of TPCs that were initially assigned was reduced by 2.3 log CFU g<sup>-1</sup>, yeasts 0.7 log CFU g<sup>-1</sup>, and molds 1.3 log CFU g<sup>-1</sup> (Figure 1). While at 12 kGy the reduction level was 4, 1.9, and 2.4 log CFU g<sup>-1</sup> of TPC, yeasts, and molds, respectively. Moreover, an irradiation dose of 18 kGy was required for decontamination of the initial count of TPC, yeasts, and molds by 6, 2.9, and 4.4 log CFU g<sup>-1</sup>, respectively. One study by Esmaeili, Barzegar [43] found that e-beam dose treatment at 5 kGy decreased the count of yeasts and molds in turmeric by 4 log CFU g<sup>-1</sup>. Another study by Byun, Cho [44] revealed that e-beam treatment with a dose of 3.5 kGy reduced *Aspergillus flavus* (>4 log) in red pepper. The effectiveness of e-beam reduction of TPC in black peppercorn samples increased significantly with dose ( $p < 0.05$ ). For example, 2.3, 4.2, and 6 log reduction of TPC in black pepper from an initial count of 6 log CFU g<sup>-1</sup> was accomplished at doses of 6, 12, and 18 kGy (Figure 1). A significant difference ( $p < 0.05$ ) was verified in TPC between the 6, 12 and 18 kGy treatments. A similar investigation demonstrated that  $\gamma$ -irradiation at 10 and 30 kGy inactivated the TPC by 4–5 and 5–7 logs, respectively [45,46]. This remarkable decrease in microbial load may be due to the direct impact of e-beam, as a result of the disintegration of links in the RNA/DNA molecule of microbial cells and death [47]. The dose levels used in our study were within the FDA's maximum allowable limit (30 kGy) for spice irradiation [48].

As shown in (Figure 2), the impact of e-beam on *Salmonella* spp. and coliform bacteria in black pepper was investigated. Results demonstrated that the potentially pathogenic bacteria were reduced in the samples treated with e-beam at different doses. At dose 6 kGy, the initial count of *Salmonella* and coliform was reduced by 1.2 and 0.9 log CFU g<sup>-1</sup>, respectively. While the samples were treated with a high dose of 18 kGy, the reduction level was 3.3 and 3.1 log CFU g<sup>-1</sup> of *Salmonella* and coliform, respectively. Lee, Ameer [49] showed that e-beam irradiation at dose 7 kGy reduced coliform 2.5 log CFU g<sup>-1</sup> in dried laver products. Another study by Kundu, Gill [50] demonstrated that e-beam irradiation more against *E. coli* O157:H7 and *Salmonella* serovars.

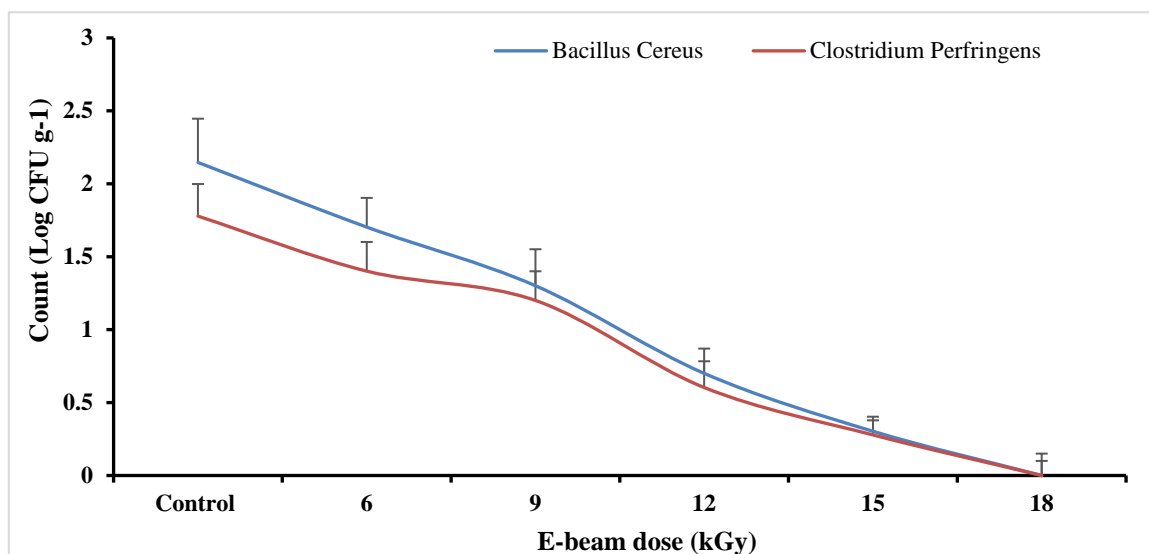


**Figure 1.** Effect of irradiation (e-beam) at different doses on total bacterial count, yeasts, and molds. Error bars represent standard deviation, (n = 3).



**Figure 2.** Effect of irradiation (e-beam) at different doses on *Salmonella* spp. and coliform bacteria. Error bars represent standard deviation, (n = 3).

Also, the e-beam affected on spore-forming bacteria such as *Bacillus cereus* and *Clostridium perfringens* in black pepper samples (Figure 3). Data showed that the e-beam was more effective at high doses against *Bacillus cereus* and *Clostridium perfringens*. No significant ( $p > 0.05$ ) difference between doses 6 and 9 kGy on *Bacillus cereus* and *Clostridium perfringens*, however, a significant ( $p < 0.05$ ) difference was noted between doses 12, 15, and 18 kGy on spore-forming bacteria. At 18 kGy the reduction level was 2.1 and 1.7 log CFU g<sup>-1</sup> of *Bacillus cereus* and *Clostridium perfringens*, respectively. The findings corroborate those reported by Fertey, Bayer [29] who discovered that an e-beam at 200 keV was successful in the inactivation of spore-forming i.e. *Bacillus pumilus* spores [29].



**Figure 3.** Effect of irradiation (e-beam) at different doses on sporeforming bacteria i.e. *Bacillus cereus* and *Clostridium perfringens*. Error bars represent standard deviation, (n = 3).

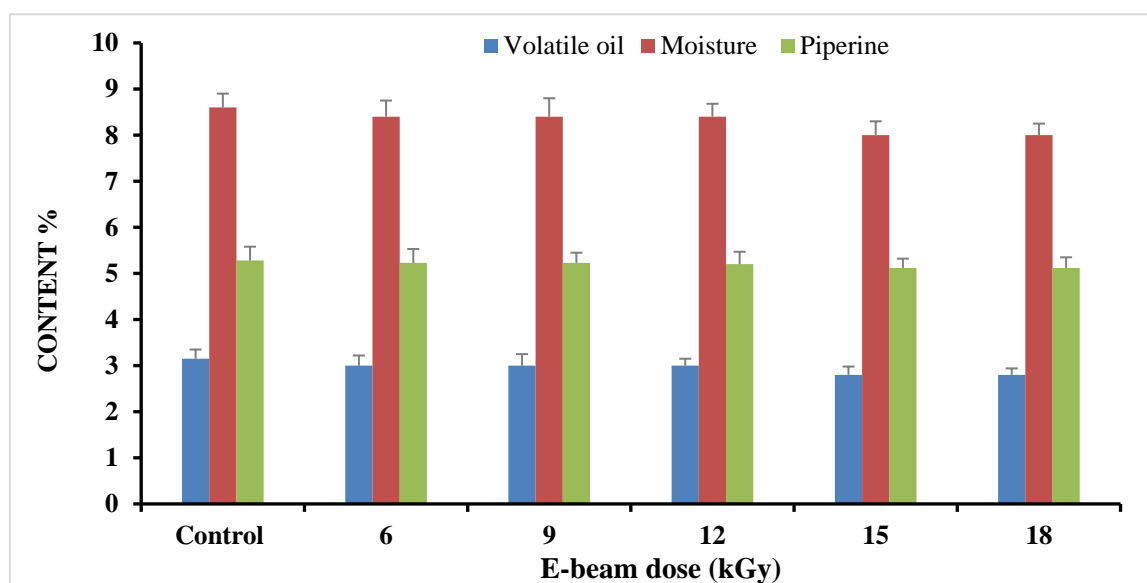
### 3.2. Impact of e-beam on physicochemical qualities of black pepper

#### 3.2.1. Moisture content

The impact of e-beam on moisture content in black pepper is shown in Figure 4. The initial moisture content was 8.6%. There was no statistically significant ( $p > 0.05$ ) difference in moisture contents between treatment doses 6–12 kGy. However, a significant ( $p < 0.05$ ) difference was noted in high doses. A study by Nieto-Sandoval, Almela [51] reported that a quite high dose of 12.5 kGy not affected the moisture of red paprika. Duncan, Moberg [52] found water activity ( $a_w$ ) to be significantly lower of irradiated peppercorn and cumin seeds. Another study by Kotilainen, Meneses [53] revealed that black peppercorn and coriander had a slight reduction in moisture content. A reason for this slight reduction of moisture may be due to the low energy of the e-beam, which affects water distribution and content.

#### 3.2.2. Volatile oil and piperine

The volatile oil and piperine content in black pepper was 3.15 and 5.28%, respectively (Figure 4). No significant ( $p > 0.05$ ) difference in volatile oil and piperine content upon (6-18 kGy) treatments as compared with the control. Sádecká, Kolek [54] found no remarkable changes in volatile oil in irradiated black pepper (up to 30 kGy), while the most important changes were a threefold increase in caryophyllene oxide concentration and a parallel decrease of sesquiterpene caryophyllene, comparison to control. Another study by Rahman, Islam [55] confirmed that irradiation has not any negative impact on volatile compounds and sensory evaluation.



**Figure 4.** Effect of irradiation using e-beam at different doses on chemical compounds of black pepper i.e. moisture, volatile oil, and piperine. Error bars represent standard deviation, (n = 3).

### 3.2.3. Color parameters

Table 1 demonstrates the color of the black peppercorn sample changes with varying e-beam doses. The lightness ( $L^*$ ) parameter of samples increased significantly with dose gradient-wise. While the positive  $a^*$  values indicated, the redness was not changed ( $p > 0.05$ ) at different irradiation dose treatments. The positive  $b^*$  values indicating yellowness of the sample were increased significantly ( $p < 0.05$ ) upon treatments. However, color differences could not be seen with the naked eye. Song, Sung [56] showed that different doses of  $\gamma$ -irradiation (2, 3, and 5 kGy) had no impact on the color values of black and red peppercorns. Bambirra, Junqueira [57] reported remarkably lower  $a^*$  and  $b^*$  values in turmeric with increased of irradiation doses. In the current work, all samples exposed to e-beam treatment doses ranging from 6 to 18 kGy showed slight variations in color when in comparison to the control.

### 3.3. Impact of e-beam on total phenolic compounds (TP)

The influence of different doses of e-beam treatment on the TP content of black pepper was evaluated. The content of total phenolic compounds (TP) in black peppercorn was between 24.88 and 26.56 g GAE  $g^{-1}$  (Table 1). A significant reduction ( $p < 0.05$ ) in the TP (6.3%) upon the highest dose of e-beam treatment (18 kGy). Koseki, Villavicencio [58] found that rosemary irradiated with 10–30 kGy had a lower TP content. Jamshidi, Barzegar [59] reported no significant ( $p > 0.05$ ) reduction of TP content in *Cinnamomum zeylanicum* up to 15 kGy dose. On the other hand, [60] found a significant increase ( $p < 0.05$ ) in TP contents of clove and nutmeg after  $\gamma$ -irradiation.

### 3.4. Impact of e-beam on antioxidants capacity

Table 1 demonstrates black pepper's antioxidant activity at various treatment doses. The antioxidant capacity of the untreated sample (control) was 62.44%. It was noted only a significant ( $p < 0.05$ ) decrease



in the antioxidant capacity (7.5%) with the highest treatment e-beam (12, 15, and 18 kGy) dose. The findings are consistent with those previously reported by Sajilata and Singhal [61] who discovered a decrease in the free radical scavenging activity of plant extracts following irradiation. This decrease could be attributed to the oxidation or radiolysis processes that occur after irradiation [62].

**Table 1.** Total phenolic compounds, antioxidant activity, and color values of black pepper at various levels of e-beam treatment doses (mean  $\pm$  SD).

Dose (kGy)	Total phenols [mg g <sup>-1</sup> dw]	Scavenging activity [%]	Color values		
			<i>L</i> *	<i>a</i> *	<i>b</i> *
Control	26.56 $\pm$ 0.65 <sup>a</sup>	62.44 $\pm$ 1.28 <sup>a</sup>	31.2 $\pm$ 0.45 <sup>c</sup>	6.3 $\pm$ 0.15 <sup>b</sup>	9.5 $\pm$ 0.18 <sup>d</sup>
6	26.38 $\pm$ 0.44 <sup>a</sup>	61.86 $\pm$ 1.05 <sup>a</sup>	31.8 $\pm$ 0.32 <sup>c</sup>	6.2 $\pm$ 0.19 <sup>b</sup>	9.9 $\pm$ 0.22 <sup>d</sup>
9	26.29 $\pm$ 0.72 <sup>a</sup>	61.32 $\pm$ 0.94 <sup>a</sup>	32.1 $\pm$ 0.28 <sup>b</sup>	6.6 $\pm$ 0.12 <sup>a</sup>	10.4 $\pm$ 0.14 <sup>c</sup>
12	26.18 $\pm$ 0.38 <sup>b</sup>	60.65 $\pm$ 1.14 <sup>b</sup>	33.8 $\pm$ 0.56 <sup>a</sup>	6.5 $\pm$ 0.25 <sup>a</sup>	12.2 $\pm$ 0.28 <sup>a</sup>
15	25.12 $\pm$ 0.62 <sup>c</sup>	58.24 $\pm$ 0.85 <sup>c</sup>	33.9 $\pm$ 0.62 <sup>a</sup>	6.4 $\pm$ 0.22 <sup>a</sup>	11.1 $\pm$ 0.24 <sup>b</sup>
18	24.88 $\pm$ 0.26 <sup>d</sup>	57.78 $\pm$ 0.68 <sup>d</sup>	34.2 $\pm$ 0.40 <sup>a</sup>	6.4 $\pm$ 0.18 <sup>a</sup>	12 $\pm$ 0.16 <sup>a</sup>

Note: <sup>abc</sup> There is no significant differences between any two means 'in the same column' have the same superscript small letter ( $p > 0.05$ ).

#### 4. Conclusions

The e-beam treatment was found to be effective in inactivating the microbiota naturally present in black peppercorn, where the degree of reduction is heavily influenced based on doses. The results support e-beam treatment within the ranges (6–18 kGy) of investigated doses was efficient for decontamination of TPC, yeasts, molds, *Salmonella* spp., and coliform bacteria. A significant ( $p < 0.05$ ) decrease was noted at doses 12, 15, and 18 kGy on *Bacillus cereus* and *Clostridium perfringens* in black pepper. While, *L*\*, *a*\* and *b*\* values of black peppercorn were not significantly altered up until 18 kGy dose. Slightly decrease in moisture, volatile oil, and piperine content upon (6–18 kGy) doses as compared to the control. A significant decrease in the bioactive compounds, retaining >90% of total phenolic compounds and antioxidant activity. According to the current study's approach, e-beam treatments up to 12 kGy had a positive impact on the inactivation of natural microbiota and could improve the safety of black peppercorns without compromising their qualities or antioxidant activity.

#### Acknowledgment

The authors express their sincere appreciation for Kalustyan Corporation for provided black pepper and plant to conduct this project.

#### Conflict of interest

The authors declare that they have no conflict of interest.

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