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Comparative effects of gamma rays, low energy electron beam and high energy electron beam on microbial quality of cloves (*Syzygium aromaticum*)

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Abstract

Effects of gamma rays, high energy electron beam (HEEB) and low energy electron beam (LEEB) on the microbial quality of cloves is reported. Cloves were irradiated at 2, 4, 6, 8, and 10 kGy, while un-irradiated samples were used as controls. Treated samples were evaluated for Total Viable Counts (TVC), Total coliform counts, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, yeast and mould, and *Salmonella* spp. The presumptive organisms were isolated and identified. Irradiation reduced the microbial loads (TVC) to below detection limit at 8 kGy and total coliforms were reduced to below detection limit at 4 kGy. The bactericidal action of gamma ray and HEEB was comparable and more effective than that of LEEB. The most dominant isolate was *Bacillus* spp. LEEB has potential for surface microbial decontamination.

Keywords Contamination, Gamma rays, High and low energy electron beams, Pathogens

1 Introduction

Food safety has emerged as a global public health conversation. Foodborne microbial hazards can cause illness and death [1]. The quest to produce and consume foods, food products, and food additives devoid of microbial hazards has necessitated a search for methods capable of controlling or eliminating microbiological contaminants from these products. Food irradiation is the deliberate exposure of food to suitable doses of ionizing radiation to eliminate or reduce the levels of insects, molds, or bacteria in food. Irradiation has been described as a non-thermal and non-chemical treatment that does not lead to a rise in the temperature of the treated product, nor does it leave any residue in the product [2]. Various irradiation methods with varied degrees of penetration have been explored and promoted as capable of decontaminating foods, food products, and food additives, rendering these products practically free from microbial contaminants.



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Gamma radiation is produced from radioisotopes of Cobalt or Caesium. Gamma radiation has a great penetration effect hence it can be used for materials of varying bulk densities [3, 4]. Electron beam radiant energies are produced from linear accelerators and during production, a beam of electrons is released from the accelerators whose traveling speed is increased to 99% the rate of light energy but does not exceed 10 MeV for application in food processing [3–5]. The penetrative power of the electron beam is low compared to gamma rays. As such, they are applied to foods/products with low thickness [3, 4]. HEEB and LEEB have a few advantages over gamma irradiation. Notable ones are that, there is no half-life for HEEB and LEEB, hence no need for purchasing new sources after the equipment has been installed, making them more economically viable to operate than gamma irradiation facilities [3, 6, 7]. HEEB and LEEB are machine sources hence dose-mapping (dose rate) does not change with time as compared with gamma irradiation where dose-mapping is necessary most of the time due to change in half-life which affects the dose rate and dose from the source of irradiation in the irradiation compartment [3, 6, 7]. Due to security threats from radio-active sources, there is a major shift to machine sources [3, 6, 7]. Another type of ionizing radiation, which can be used in food processing is X-ray; which is emitted as a result of interaction of electron beams generated using accelerators with the material of converter. The penetration of X-rays is comparable to gamma rays, but due to low conversion yield, the process is highly expensive.

The risks, cost, and persistent uncertainty associated with the use and supply of gamma radiation sources for future applications [8] dictates that we explore alternative food irradiation methods.

Cloves are the dried buds of a tropical evergreen tree *Eugenia caryophyllata* L. Merr and Perry (Myrtaceae), also known as *Syzygium aromaticum* and *Eugenia aromatica*. They are mostly used for domestic culinary purposes and as a flavouring agent. The main products of cloves are whole or ground clove buds, essential oils produced from clove buds, stem and leaf and clove oleoresins. Besides being a source of natural flavour cloves also contain nutrient such as protein, vitamins, minerals etc. Cloves are also used to treat digestive system disorders and has antioxidant qualities that can counteract free radicals linked to cancer. They can actually support healthy digestion and metabolism in addition to their well-known ability to relieve flatulence. Along with a number of other digestive issues, cloves may also help with vomiting and diarrhea [9, 10]. Cloves may be used as insect repellants [11–13]. Medicinal and aromatic crops such as cloves are natural products and may be burdened with many microorganisms, some with pathogenic potential. Studies have reported that the major problems associated with spice production are high microbial loads and aflatoxin contamination. Bacteria associated with cloves include Enterobacteriaceae, coliforms, *Escherichia coli*, *Salmonella* spp., *Bacillus cereus*, *Pseudomonas* spp [14, 15]. . Cloves could be contaminated with microorganisms during, the preharvest stage during harvesting, drying and during transportation [14]. Pathogens have been reported on black pepper and herbs [16]. Mycotoxins are generally considered a potential hazard to public health due to their toxicity, mutagenicity, teratogenicity, and carcinogenicity [17, 18]. The use of ethylene oxide and methyl bromide as fumigants in spices is no longer acceptable [19]. There is a need to explore techniques or technologies that may effectively and efficiently eliminate or reduce the microbial

burden in or on cloves. Gamma rays and e-beams have been utilized for the removal or elimination of microbes from foods [20, 21].

This study sought to compare the effects of three food irradiation techniques, gamma rays, HEEB and LEEB on the microbiological quality of cloves.

2 Methodology

2.1 Source of samples

Whole cloves were purchased from one of the local markets in Accra, Ghana. The clove samples were sorted, all extraneous materials were removed, and packaged in 13 × 20 cm zip-lock bags.

2.2 Irradiation of samples

Irradiation was performed using the method described by Ocloo et al. [20] with some modifications. Packaged cloves samples were shipped to the Institute of Nuclear Chemistry and Technology in Warsaw, Poland for irradiation. The samples shipped were irradiated separately with gamma rays, HEEB and LEEB. Targeted irradiation doses were 2, 4, 6, 8, and 10 kGy. Un-irradiated cloves served as control. Briefly, packed cloves (150 g) were irradiated with HEEB (ELEKTRONIKA 10–10 accelerator with 9 MeV electrons energy), at a dose rate of 10^3 Gy/s. Also, a mass of 150 g packed cloves was irradiated with gamma rays (Gamma Chamber 5000 ^{60}Co source), at a dose rate of ca. 2 kGy/h. Alanine pellets (Aerial) were used to determine the doses delivered for samples irradiated with HEEB and gamma rays, and were measured at the INCT Laboratory for Measurements of Technological Doses, with an uncertainty of 5%. All dose measurements were traceable to the NPL standard. The conditions of irradiations were determined to ensure that the dose uniformity ratio (DUR), determined as the ratio of maximum and minimum dose in the sample, was 1.1.

For LEEB, a mass of 150 g cloves was placed in a cylinder (Fig. 1) and irradiated using an ILU-6 accelerator at a surface dose rate of 2.5 kGy/min. For dose measurements and determination of penetration ability of electrons, B3 dosimetric foil with RISOSCAN software was used. Dose rate was determined by irradiating rolled B3 foil and cloves samples together. The B3 foil is an 18- μm thick foil, which limits the dose gradient in a dosimeter for LEEB irradiation. The dose rate was determined as the average dose



Fig. 1 Irradiation of the cloves with LEEB in rotating cylinder

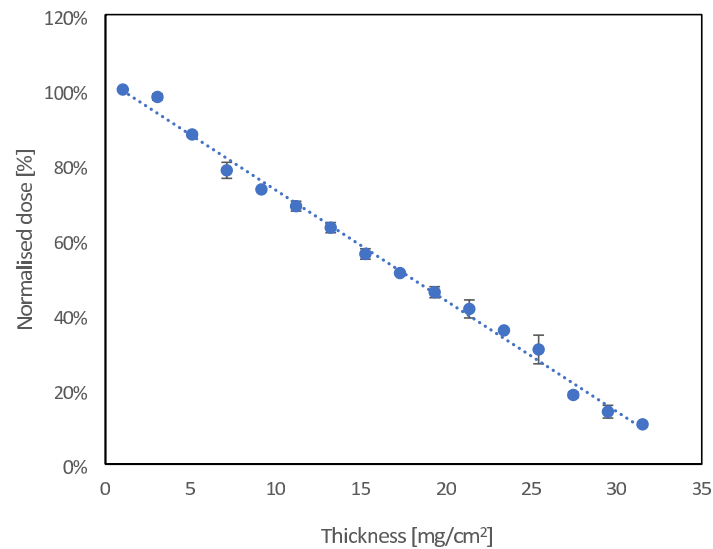


Fig. 2 The depth dose curve for electron of energy 330 keV determined using B3 dosimetric film



Fig. 3 Surface and cross-sectional view

measured for one layer of B3 foil, and the dose variability was about 15%. The irradiation using LEEB was performed in a batch mode. For every batch the same mass of samples was placed in a cylinder, having diameter 7 cm and length 8.5 cm and rotated with a constant speed 150 rpm. The cylinder was then placed in an irradiation zone. The dose delivered to the samples was controlled by controlling of irradiation time. The system for loose samples irradiation with LEEB [22] ensures uniformity of dose distribution on the surface of each food item and allows for controlling dose by the irradiating time. The energy of the electrons was 330 keV, and the penetration ability, determined using B3 film is presented in Fig. 2. The experimental conditions allowed for the irradiation of a layer about 60 μm thick under the surface of each clove. The structure of the samples was observed using USB microscope. In the cross-section of samples, as shown in Figs. 3 and 4. The thickness of layers (cover and internal part of cloves) was measured to calculate the most probable penetration depth of LEEB. Irradiation was performed in triplicates. After the irradiation, the cloves were shipped back to Ghana for microbial analysis.



Fig. 4 The structure of cloves samples observed using USB microscope

3 Microbiological analysis

3.1 Microbial enumeration

Microbial contamination (culturing and enumeration) levels of the various samples (10, 8, 6, 4, 2 kGy and control) were determined according to the procedures of [23] and [24]. Ten grams of the clove samples were homogenized separately in 90 mL of sterile peptone water, resulting in a 1:10 dilution. Serial dilutions were performed up to 10^{-6} . From each dilution, 1 mL of the sample was transferred into sterile Petri dishes, followed by the addition of molten agar (cooled to 50 °C). The plates were gently swirled to distribute the inoculum within the agar evenly and the agar was allowed to solidify. The inoculated plates were incubated at 37 °C for 48 h. For the enumeration of total viable count (TVC) and total coliform counts, Plate Count Agar (PCA) and Violet Red Bile Agar (VRBA) were used. For the detection and enumeration of *Salmonella* spp. *Staphylococcus aureus* and *Bacillus cereus*, (XLD) agar, Baired-Parker agar and *Bacillus cereus* agar with supplement added were used. For Yeast and mold analysis Oxytetracycline Glucose Yeast Extract-(OGYE) agar with added supplement was used and plates were incubated for 72 h.

3.1.1 Isolation and identification of presumptive bacteria

Isolates from the microbial analysis were identified using MALDI-TOF (Bruker MALDI Biotyper, Germany) equipment at the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, after conducting preliminary biochemical tests such as Grams test, catalase test and sugar fermentation (data not added).

3.2 Data analysis

Data obtained from the study were analyzed using Minitab 20 (PA-Minitab, LLC, USA) to determine significant differences and separation of the means at $p < 0.05$ by Fisher's Least Significant Difference.

4 Results

The effects of gamma radiation, HEEB and LEEB on the microbiological quality of cloves are illustrated in Table 1. For the TVC, a gamma radiation dose of 2 kGy reduced the initial microbial load from 2.4 log to 1.5. In contrast, gamma irradiation doses 4 kGy and 6 kGy achieved a log reduction of 1.0. A dose of 8 kGy and 10 kGy completely inactivated the TVC, reducing the initial bioburden below the detection limit. For the HEEB, a dose of 2 kGy was able to reduce the initial microbial load by 0.6 log. Gamma ray and HEEB doses of 4 and 6 kGy were able to reduce the initial microbial load to 1.0 log CFU/g and doses of 8 kGy and 10 kGy reduced the TVC populations to below the detection limit. For LEEB, a dose of 2 kGy gave a 0.4 log reduction, with a 4 kGy dose giving a 0.6 log reduction and 6 kGy dose delivering 1.2 log reduction of the TVC.

For the total coliform counts, gamma irradiation doses of 2, 4, 6, 8 and 10 kGy reduced the coliform counts by 2.1 log. *Salmonella* spp., *E. coli*, and yeast and molds were not detected in the control samples; hence were absent in the irradiated samples. A 2 kGy gamma ray, HEEB and LEEB dose reduced the *S. aureus* counts by 1.9 log, 0.5 log, and 0.4 log respectively. Doses of 4, 6, 8 and 10 kGy reduced the counts below the detection

Table 1 Effects of gamma rays, high energy electron beam and low energy electron beam on some microbes in cloves

Microbial parameter (log ₁₀ CFU/g)	Irradiation Source	Doses of irradiation (kGy)					
		0	2	4	6	8	10
Total Viable counts	Gamma rays	2.4 ± 0.0 ^{Aa}	1.5 ± 0.1 ^{Ab}	1.0 ± 0.0 ^{Ac}	1.00 ± 0.0 ^{Ac}	<1	<1
	HEEB		1.8 ± 0.0 ^{Bb}	1.0 ± 0.0 ^{Ac}	1.00 ± 0.0 ^{Ac}	<1	<1
	LEEB		2.0 ± 0.1 ^{Bb}	1.8 ± 0.1 ^{Bb}	1.2 ± 0.2 ^{Ac}	<1	<1
Total coliform counts	Gamma rays	2.1 ± 0.1 ^a	<1	<1	<1	<1	<1
	HEEB		1.5 ± 0.0 ^{Bb}	<1	<1	<1	<1
	LEEB		1.6 ± 0.1 ^{Bb}	<1	<1	<1	<1
<i>Salmonella</i> spp.	Gamma rays	<1	<1	<1	<1	<1	<1
	HEEB	<1	<1	<1	<1	<1	<1
	LEEB	<1	<1	<1	<1	<1	<1
<i>Staphylococcus aureus</i>	Gamma rays	1.9 ± 0.0 ^{Aa}	<1	<1	<1	<1	<1
	HEEB		1.4 ± 0.1 ^{Bb}	<1	<1	<1	<1
	LEEB		1.5 ± 0.0 ^B	<1	<1	<1	<1
<i>Escherichia coli</i>	Gamma rays	<1	<1	<1	<1	<1	<1
	HEEB	<1	<1	<1	<1	<1	<1
	LEEB	<1	<1	<1	<1	<1	<1
<i>Bacillus cereus</i>	Gamma rays	1.5 ± 0.0 ^a	<1	<1	<1	<1	<1
	HEEB		<1	<1	<1	<1	<1
	LEEB		1.2 ± 0.2 ^b	<1	<1	<1	<1
Yeast and Molds	Gamma rays	<1	<1	<1	<1	<1	<1
	HEEB		<1	<1	<1	<1	<1
	LEEB		<1	<1	<1	<1	<1

LSD: Means with different superscripts in the same row (in relation to irradiation dose for a particular microbial parameter (microbe) -upper case) are significantly different ($p < 0.05$) from each other and means in the same column (in relation to irradiation sources for each microbial parameter-lower case) with different superscripts (upper case) are significantly different ($p < 0.05$) from each other. Detection limit: $< \log_{10}$ CFU/g ($< \log 1$)

HEEB: High energy electron beam; LEEB: Low energy electron beam

Table 2 High confidence identification (HCI) of bacteria isolates by MALDI-TOF MS

Organisms	Number of Isolates identified	Score Range	Colour	Interpretation
<i>Acinetobacter baumannii</i>	3	2.35-2.4		HCI
<i>Alkalihalobacillus clausii</i>	1	2.09		HCI
<i>Bacillus cereus</i>	3	2.02-2.11		HCI
<i>Bacillus endophyticus</i>	5	2.00-2.56		HCI
<i>Bacillus megaterium</i>	5	2.00-2.27		HCI
<i>Bacillus subtilis</i>	2	2.00-2.06		HCI
<i>Brachybacterium conglomeratum</i>	1	2.02		HCI
<i>Enterobacter bugandensis</i>	1	2.19		HCI
<i>Enterobacter clocae</i>	1	2.24		HCI
<i>Staphylococcus epidermidis</i>	1	2.06		HCI
<i>Staphylococcus hominis</i>	1	2.05		HCI
<i>Terribacillus halophilus</i>	2	2.00-2.14		HCI

Table 3 Low confidence identification (LCI) of bacteria isolates by MALDI-TOF

Organisms	Number of Isolates identified	Score Range	Colour	Interpretation
<i>Bacillus altitudinis</i>	2	1.77		LCI
<i>Bacillus amyloliquefaciens</i>	1	1.78		LCI
<i>Bacillus cereus</i>	1	1.84		LCI
<i>Bacillus endophyticus</i>	3	1.86-1.96		LCI
<i>Bacillus megaterium</i>	5	1.74-1.99		LCI
<i>Bacillus safensis</i>	1	1.82		LCI
<i>Bacillus pumilus</i>	4	1.77-1.86		LCI
<i>Bacillus subtilis</i>	3	1.83-1.92		LCI
<i>Roseomonas gilardii</i>	1	1.77		LCI
<i>Staphylococcus hominis</i>	1	1.96		LCI

limit. For *B. cereus*, gamma ray and HEEB doses of 2 kGy reduced the counts below the detection limit, whilst LEEB at 2 kGy reduced counts to 1.2. Gamma ray, HEEB and LEEB doses of 4, 6, 8 and 10 kGy reduced the *B. cereus* counts below the detection limit.

The presumptive organisms isolated, purified, and subjected to biochemical tests (Gram, catalase and sugar fermentation) (Results not shown) and identified and confirmed by MALDI-TOF MS are shown in Tables 2 and 3.

Three (3) *Acinetobacter baumannii*, an *Alkalihalobacillus clausii*, 3 *Bacillus cereus* strains, 5 *Bacillus endophyticus*, 5 *Bacillus endophyticus*, 2 *Bacillus subtilis* strains, a *Brachybacterium conglomeratum* strain, an *Enterobacter bugandensis*, an *Enterobacter clocae*, a *Roseomonas gilardii*, a *Staphylococcus epidermidis*, *Staphylococcus hominis*, and 2 *Terribacillus halophilus* strains. The confidence score attached to the identified bacteria strains are shown in the Table 2.

The organisms in Table 3 were identified with a low degree of confidence. They include 2 *Bacillus altitudinis* strains, a *Bacillus amyloliquefaciens* and *Bacillus cereus*, 3 *Bacillus endophyticus* strains, 5 *Bacillus megaterium* strains, a *Bacillus safensis* strain, 4 *Bacillus pumilus* strains, 3 *Bacillus subtilis* strains, a *Bacillus subtilis*, a *Roseomonas gilardii* and a *Staphylococcus hominis*, strain.

5 Discussion

Microbial contamination often results in either food spoilage, foodborne infection or intoxication [25, 26]. Several researchers have exploited gamma rays, HEEB, and LEEB to inactivate the microbial populations on produce [20, 27, 28]. Generally, inactivation of the microbial bioburden by the radiation techniques in the current study was expected, as it resonates with the literature [16, 20, 29, 30]. The ability of radiation techniques to inactivate microorganisms is mainly associated with the destruction inflicted on microbial DNA, and the induced damage corresponds to the applied dose [31]. TVCs were inactivated by low gamma irradiation and HEEB doses, compared with LEEB, which required a higher dose to inactivate the TVCs. The current result compares with an earlier study by [32] who reported that HEEB and gamma rays significantly reduced the microbial bioburden on African nutmeg. A study [20] reported that a gamma dose of 6 kGy inactivated the TVC on sorghum grains, while 8 kGy HEEB and LEEB doses were able to inactivate the TVC on sorghum grains. Also a study by [33] reported that LEEB doses of (3, 5, 8 and 10 kGy) reduced the total microbial load by 4 log CFU/g on dried red chili pepper. The differences in inactivation achieved in this study can likely be attributed to the depth of penetration of the three radiation sources used [16, 20, 22]. The low TVC obtained in this study could be attributed to the fact that the cloves samples were not highly contaminated. Additionally, cloves contains essential oils and this may have prevented the infection, colonization and growth [34] of high numbers of bacteria. It is possible that cloves from other sources may contain higher counts of microorganisms than values recorded in the current study. Although the initial total bacteria count on the cloves samples were unusually low, the ability of the three irradiation techniques to reduce or inactivate the low bacteria counts at the doses used is a testament that when the microbial count is high, irradiation can be used to decontaminate cloves or any other produce.

The inactivation of the total coliform count achieved in the current study concurs with [20] who reported that gamma radiation, HEEB, and LEEB doses up to 4 kGy inactivated the total coliform load on sorghum samples and [33] reported LEEB doses of (3, 5, 8 and 10 kGy) reduced the total coliform count load by 4 log CFU/g on dried red chili pepper. Total coliforms were completely inactivated on saffron by gamma and e-beam radiation doses of 2 kGy and above [35].

In this study gamma ray dose of 2 kGy inactivated *S. aureus* in the clove samples compared to HEEB and LEEB dose of 4 kGy which was required to inactivate *S. aureus*. Studies by [36] reported that 2 kGy e-beams dose inactivated *S. aureus* in suspension culture and 4 kGy inactivated *S. aureus* cells in biofilms. For *B. cereus* low doses of gamma ray and HEEB were enough to inactivate *B. cereus* load, while a high LEEB dose was needed to inactivate the *B. cereus* population to below the detection limit. An investigation by [35] reported that no yeast and molds, or any microorganism, were detected on saffron when gamma and e-beam radiation doses of 2 kGy and above were applied. *Salmonella* spp., *E. coli* yeast and molds were undetected in the clove samples. It is possible that these microorganisms were entirely absent, or they may be present but below the limit of detection, hence were not picked up by the analytical method used.

To effectively control microbial contamination, there is a need to isolate and identify the bacteria responsible for the contamination. Various researchers have adopted varied approaches to identify and confirm the presence of specific groups or species of bacteria

present in or on food. Several studies have reported that MALDI-TOF MS is capable of distinguishing between closely related bacterial species with a high degree of confidence [37–39].

The peaks formed by ribosomal proteins dominate the bacterial cell spectra [40]. The ribosomal protein peaks remain unaltered during the process of evolution and are practically unchanged during culture conditions, hence the bacteria cell spectra when generated can be highly characteristic of a particular species or strain. This feature of the ribosomal protein spectra allows their use for the identification of bacteria by the MALDI-TOF MS system. MALDI-TOF MS has been proposed and employed for quick and dependable identification of bacteria at the genus, species, and in some cases, strain levels [41]. This study used MALDI-TOF MS system to confirm the bacterial isolates that were obtained during the microbial analysis. Table 2 gave a high-confidence identification of the isolates compared to Table 3, which gave a low-confidence identification of the isolates. A high proportion of the isolates were in the genus *Bacillus*, an indication that most of the microorganisms identified on the cloves in the current study could be spore-forming *Bacillus* bacteria. Their occurrence in food should be regarded with concern.

6 Conclusion

This study aimed to compare the effect of three irradiation techniques on the microbiological quality of cloves. The results show that each technique was able to reduce or inactivate the microbial populations at increasing doses. Gamma irradiation and HEEB were the most effective methods compared to LEEB. The initial microbial load on the cloves samples were low, and this could have impaired the ability of the study to fully demonstrate the efficacy of some of the treatments. This raises the need for further studies and a challenge test the samples. Notwithstanding limitations, the study is still relevant as it confirms the use of irradiation to decontaminate the microbial load on spices. The use of HEEB as alternative to gamma rays is proposed.

There is also the need for further studies on surface decontamination of cloves using LEEB regarding surface dose and energy of electrons required to achieve the desired effectiveness of the decontamination process.

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Author contributions

Conceptualization, Data curation, Resources, Writing – Review and Editing (FCKO); Investigation, Methodology, Formal analysis, Writing- Original draft preparation (BTO, BD, WT, IDK, JOA, EAA, IA, JB, UG, SB, and JAA).

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Data availability

Data will be made available on request.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

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