Food Quality and Safety, 2019, 3, 265–272
doi:10.1093/fqsafe/fyz026
Research Article
Advance Access publication 7 December 2019

Research Article

Effect of gamma radiation and storage at 4°C on the inactivation of *Listeria monocytogenes, Escherichia coli* and *Salmonella enterica Typhimurium* in Legon-18 pepper (*Capsicum annuum*) powder

Bernard Tawiah Odai*,, Kwaku Tano-Debrah*, Kennedy Kwasi Addo***, Firibu K. Saalia* and Lorenzo Moses Akyeh*****

*Department of Nutrition and Food Science, University of Ghana, Legon, Accra, **Radiation Technology Centre, Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, Kwabenya, Accra, and ***Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra

Correspondence to: Bernard Tawiah Odai, Radiation Technology Centre, Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P.O. Box LG 80, Legon, Accra, Ghana. E-mail: bernardtodai@gmail.com

Received 14 August 2019; Revised 21 August 2019; Editorial decision 11 September 2019.

Abstract

Objectives: Spices are low moisture foods which have been known to be contaminated with various pathogens and sun-dried Legon-18 pepper powder is not left out. Due to its contamination with various pathogens, a study was conducted to determine the effects of gamma irradiation on the decontamination of Legon-18 pepper powder and on some quality parameters.
Methods: Samples were obtained from a local farmer from the Eastern Region of Ghana. Sterility tests were carried out. The samples were inoculated with known cfu/ml of *Escherichia coli, Listeria monocytogenes* and *Salmonella enterica* Typhimurium. Samples were irradiated at 1, 2, 4, and 5 kilogray (kGy). Zero kilogray served as control (unirradiated). All samples were stored at 4°C for 60 days. Enumeration of the various pathogens was done in appropriate media. Some quality parameters were determined after irradiating unsterile samples at 5 kGy and 0 kGy served as control. Capsaicinoids and carotenoids were quantified using a high performance liquid chromatography. The samples were stored at 4°C for 8 weeks.
Results: A dose-dependent effect on the inactivation of the pathogens was observed ($P < 0.05$). Storage time affected the inactivation of the pathogens as well ($P < 0.05$). Complete inactivation of the pathogens was observed at 5 kGy at day 0. Capsaicin, dihydrocapsaicin and total capsaicinoid content of the samples irradiated at 5 kGy increased at 23.64%, 14.7 % and 20.95% respectively as compared with the contents of the unirradiated samples. A gamma irradiation dose of 5 kGy caused losses of 8.11%, 8.67% and 26.54% in capsanthin, beta carotene and beta cryptoxanthin respectively. Quality parameters measured reduced with storage ($P < 0.05$).
Conclusions: Gamma irradiation inactivated pathogens at 5 kGy. Lower doses used during the study could inactivate the pathogens but with time. All quality parameters and carotenoids quantified were affected by gamma irradiation and storage period ($P < 0.05$).

Key words: capsaicinoids; gamma irradiation; pathogens; carotenoids.

Introduction

One of the most widely used spice in the world has been identified as red pepper (*Capsicum annuum*) or chili (Lee *et al.*, 2004). Chili, as a spice is known as the next most important vegetable after tomato (Ochoa-Alejo and Ramirez-Malagon, 2001; Ali, 2006; Liu *et al.*, 2013). It is produced in several countries (Rufino and Penteado, 2006; Pinto *et al.*, 2016). Ripened matured fruits are harvested, and processed into fine powder. The powder is used as a spice, food colourant, a flavouring (Jung *et al.*, 2015) and as a sauce in Ghana, a special sauce known as 'shito' is produced (Doku, 2015).

Processed spices (including chili powder) have been known to be reservoirs of microorganisms (Bhunia, 2018; Kahraman and Ozmen, 2009; DOH/VICTORIA/AU, 2010; Jung *et al.*, 2015) due to the source of these and processing methods (Buckenhuskes and Rendlen, 2004; Jung *et al.*, 2015). Chili has been indicated that pepper can have high microbial contamination of viable counts exceeding 10^7 colony-forming unit (cfu) per gram with most of these being spore formers (Boer *et al.*, 1985; Piggott and Othman, 1993).

Microbial decontamination methods that have been used in spice including chili are the use of fumigants and irradiation techniques (Gregoire *et al.*, 2003; Schweiggert *et al.*, 2007; Rico *et al.*, 2010; Witkowska *et al.*, 2011; Kim *et al.*, 2013; Cheon *et al.*, 2015; Jung *et al.*, 2015), however some of these methods have been identified to adversely affect quality parameters of pepper powder and others as carcinogens (Tainter and Grenis, 2001; Almela *et al.*, 2002; Lilie *et al.*, 2007; Schweiggert *et al.*, 2007). The Joint Food and Agriculture Organization (FAO), the International Atomic Energy Agency (IAEA), and the World Health Organization (WHO) have approved the use of gamma irradiation for the decontamination of foods and it is used in over 51 countries (WHO, 1981). In light of this, a study was conducted to determine the effect of gamma irradiation on the decontamination of notable pathogens in Legon-18 pepper powder, which is one of the most consumed pepper powder in Ghana (MOFA, 2007; Sualihu, 2012; Doku, 2015).

Materials and methods

Microbiological analysis

Source of samples

The samples that were used during the study were collected from a local farmer in the Eastern Region of Ghana. The pepper powder was obtained from matured harvested, dried, and milled samples.

Sterility tests

The samples were used for the experiment were exposed to gamma irradiation dose of 20 kilogray (kGy) at a dose rate of 1.97 $kGyh^{-1}$ at the Radiation Technology Centre located at premises of the Biotechnology and Nuclear Agriculture Research Institute of the Ghana Atomic Energy Commission for the purpose of removing background microflora (Deng *et al.*, 2015). Samples were spread plated on selective media [Cefixime tellurite sorbitol MacConkey (CT-SMAC) Becton Dickinson Diagnostic Systems (BD) for *E. coli*; Palcam *Listeria* Selective (PLS) agar (BD) for *L. monocytogenes* and Xylose Lysine Deoxychocolate (XLD) for *S. enterica* Typhimurium] under sterile conditions to confirm the sterility of the samples (Jeong *et al.*, 2010; Deng *et al.*, 2015). Ten grams of the samples from sterile pouches were weighed into sterile stomacher pouches (Seward, UK) and 90 ml of sterile buffered saline water was added. The samples that were irradiated were homogenized, serially diluted, spread plated, and incubated at 48 hours at 37°C for *L. monocytogenes* and 24 hours for *E. coli* and *S. enterica* Typhimurium.

Preparation of culture and inoculation

The pathogens were inoculated into the samples according to the procedures of Ducic *et al* (2016) and Cheon *et al.*, (2015) with some modifications.

Listeria monocytogenes inoculum was prepared by transferring 0.1 ml of the stock cultures into 10 ml of tryptic soy broth (TSB) from BD, Maryland, USA. The inoculum was incubated at 37°C for 24 hours. A loop of the pathogen was taken and incubated in TSB in falcon tubes on a shaker for 24 hours at 37°C. The cells were harvested and centrifuged for 10 minutes at 3000 g at a temperature of 4°C. The formed pellets formed were washed and re-suspended in 10 ml of 0.1% PBS and adjusted to yield a final cell concentration of approximately 10^9 CFU/ml.

Stock cultures of *E. coli* and *S. enterica* Typhimurium were stored at −80°C in 0.7 ml of Tryptic Soy Broth (TSB; Difco) and 0.3 ml of 50% glycerol were streaked onto Tryptic Soy Agar (TSA; Difco) and later incubated at 37°C and stored at 4°C for 24 hours. A loop of each of the pathogens was taken, incubated in TSB in falcon tubes on a shaker at 37°C for a 24-hour period. Cells were harvested by centrifugation at 4000 g for 20 minutes at 4°C. These were washed thrice with phosphate-buffered saline (PBS). Final pellets were re-suspended in 10 ml of PBS which was approximately 10^7–10^8 cfu/ml.

Approximately, equal proportions of the various pathogens were used to prepare a cocktail which was used for the inoculation of the pepper powder samples. One millilitre of the cocktail was added to 10 g of the samples in sterile pouches (dimensions of 0.118 m in width and 0.170 m in length) which corresponded to 10^5–10^6 of the various pathogens. Inoculated pepper samples were thoroughly mixed and dried in a biosafety hood for an hour.

Sample irradiation

Inoculated pepper samples in the pouches were irradiated at a dose rate of 2.01 kGy/h at 1, 2, 4, and 5 kGy, and unirradiated (0 kGy) samples were used as control.

Enumeration of pathogens

The pathogens used in the study were enumerated according to the procedures of Jeong *et al.*, (2010), Cheon *et al.* (2015), Deng *et al.* (2015), and Ducic *et al.* (2016) with some modifications. A 90 ml of sterile PBS was poured into 10 g of the inoculated samples in sterile stomacher bags and homogenized. Serial dilutions of the samples were carried out and spread plated under sterile conditions onto PLS, CT-SMAC, and XLD agars (BD) for *L. monocytogenes, E. coli*, and *S.* Typhimurium, respectively. *Listeria monocytogenes* was incubated for 48 hours at 37°C and *E. coli* and *S.* Typhimurium.

Analysis of chemical composition

The chemical composition of the samples analysed were capsaicinoids and carotenoids.

Capsaicinoids

Capsaicinoids analysed in the samples were capsaicin and dihydrocapsaicin, which are the main capsaicinoids responsible for the hotness of chili pepper (Lu *et al.*, 2017).

Source of capsaicinoids

Standards of the capsaicinoids were obtained from Extrasynthese (Lyon, France) and used for the study.

Extraction

Extraction of the capsaicinoids of interest was done according to the procedure of Barbero *et al.* (2006). The pressurized liquid extractor (Fluid Management Systems, USA) was used for the extraction of the two main capsaicinoids. One gram of powdered Legon-18 pepper samples was weighed and contents extracted using accelerated solvent extractor equipped with a 100-ml stainless steel extraction cells. Loaded cells with the pepper samples mixed with inert sea sand which had been homogenized, were filled with 90% ethanol to a pressure of 1500 psi. Heat was applied for the initial period of heat-up time; static extraction was effected after all valves were closed. Contents of the cell were rinsed with the extraction solvent and purged with N_2 gas for 120 seconds. The extracts were collected from the cells after depressurization of the system (Barbero *et al.*, 2006).

Quantification

Sample extracts were filtered through a 0.45-µm Millipore membrane filter and injected into an HPLC PDA (PerkinElmer Flexar), which was equipped with a revered phase column SunFire TM C_{18} (5 µm, 4.6 × 150 mm; Waters) thermostated at 30°C for both qualitative and quantitative analysis. Capsaicinoids were separated by an isocratic mixture of water: acetonitrile 55:44 v/v. Detection wavelength was set at 280 nm (Giuffrida *et al.*, 2013). Calibration curves of the standards were drawn using Microsoft Excel, 2010.

Total capsaicinoids and Scoville Heat Units

Total capsaicinoids and Scoville Heat Units (SHU) were computed according to the procedure of Aguiar *et al.* (2016) and Jung *et al.* (2015) and Orellana-Escobedo *et al.* (2013), respectively. Total capsaicinoids (TC) expressed as

TC = capsaicin + dihydrocapsaicin; and SHU were expressed as SHU = [(% dihydrocapsaicin × 16.1) + (% capsaicin × 16.1)] × 10,000

Carotenoids

The main carotenoids analysed during the study were beta carotene and cryptoxanthin, capsanthin and zeaxanthin.

Table 1. Survival of *E. coli* after gamma irradiation and during storage at 4°C in powdered Legon-18 pepper (*C. annuum*).

Storage	Microbial Count (log cfug^{-1})				
Days	0 kGy	1 kGy	2 kGy	4 kGy	5 kGy
0	6.69 ± 0.08^{Aa}	5.17 ± 0.11^{Ba}	4.59 ± 0.11^{Ca}	3.51 ± 0.11^{Da}	ND
2	6.43 ± 0.09^{Ab}	4.84 ± 0.11^{Bb}	4.24 ± 0.11^{Cb}	3.11 ± 0.11^{Db}	ND
5	6.24 ± 0.08^{Ac}	4.44 ± 0.11^{Bc}	3.94 ± 0.11^{Cc}	2.81 ± 0.15^{Dc}	ND
12	5.67 ± 0.11^{Ad}	4.24 ± 0.11^{Bd}	3.66 ± 0.19^{Cd}	2.41 ± 0.15^{Dd}	ND
21	4.85 ± 0.09^{Ae}	4.07 ± 0.11^{Bd}	3.03 ± 0.09^{Ce}	2.11 ± 0.15^{De}	ND
30	3.53 ± 0.09^{Af}	3.43 ± 0.09^{Ae}	2.87 ± 0.13^{Be}	1.78 ± 0.12^{Cf}	ND
45	ND	ND	ND	ND	ND
60	ND	ND	ND	ND	ND

Least Significant Difference: Means with the same letters (upper cases) in the same row are not significantly (P > 0.05) different from each other and means with the same letters in the same column (lower case, doses per day) are not significantly different (P > 0.05) from each other. Key: ND = not detected.

Table 2. Stepwise regression analysis for the inactivation of *E. coli* in powdered Legon-18 pepper (*C. annuum*) stored at 4°C.

Step	Change	Step (*P* value)	Final (*P* value)	R^2 adjusted (%)
1	Add X_2	0.000*	0.000*	39.309
2	Add X_1	0.000*	0.000*	76.751
3	Add X_1*X_2	0.000*	0.000*	91.302

Key: X_1= storage days; X_2= doses (kGy) of gamma irradiation.

Table 3. Survival of *S.* Typhimurium after gamma irradiation and during storage at 4°C in powdered Legon-18 pepper (*C. annuum*).

Storage Days	Microbial Count (log cfug^{-1})				
	0 kGy	1 kGy	2 kGy	4 kGy	5 kGy
0	6.49 ± 0.08^{Aa}	5.57 ± 0.11^{Ba}	5.37 ± 0.07^{Ca}	4.40 ± 0.09^{Da}	ND
2	6.38 ± 0.08^{Aa}	5.55 ± 0.11^{Ba}	5.18 ± 0.05^{Cb}	3.89 ± 0.08^{Db}	ND
5	5.72 ± 0.11^{Ab}	5.32 ± 0.11^{Bb}	5.00 ± 0.11^{Cc}	3.65 ± 0.08^{Dc}	ND
12	5.62 ± 0.11^{Abc}	5.10 ± 0.11^{Bc}	4.80 ± 0.09^{Cd}	3.44 ± 0.08^{Dd}	ND
21	5.52 ± 0.11^{Ac}	4.90 ± 0.09^{Bd}	4.60 ± 0.09^{Ce}	3.02 ± 0.09^{De}	ND
30	5.12 ± 0.11^{Ad}	4.60 ± 0.09^{Be}	3.65 ± 0.08^{Cf}	2.72 ± 0.11^{Df}	ND
45	4.99 ± 0.11^{Ad}	3.79 ± 0.08^{Bf}	2.88 ± 0.11^{Cg}	1.46 ± 0.13^{Dg}	ND
60	4.65 ± 0.09^{e}	ND	ND	ND	ND

Least Significant Difference: Means with the same letters (upper cases) in the same row are not significantly (P > 0.05) different from each other and means with the same letters in the same column (lower case, doses per day) are not significantly different (P > 0.05) from each other. Key: ND = not detected.

Source

Standards of beta carotene, capsanthin, zeaxanthin, and beta cryptoxanthin were obtained from Extrasynthese (Lyon, France).

Extraction

One gram of the pepper samples was weighed and contents extracted using the accelerated solvent extractor equipped with 100-ml stainless steel extraction cells. The cells were loaded with the homogenized samples mixed with inert sea sand. The cells were filled with 90% ethanol to a pressure of 1500 psi. Heat was applied for the initial period of heat-up time, after which static extraction took place after all the valves were closed. The cells were rinsed with the extraction solvent and purged with N_2 gas for 2 minutes. Extracts from the cells were collected from the cells with 20 ml falcon tubes after depressurization of the system.

Quantification

The carotenoids studied were quantified using an HPLC (Topuz and Odzemir, 2004).

Extracts from the samples were filtered through a 0.45-µm membrane filter (Millipore), into a glass vial (2 ml) and then used for HPLC injection. Each carotenoid was chromatographically separated by an AQUA 5u C_{18} 125A (150 × 4.60, 5 µm) reversed-phase column with a gradient of acetone:water at the beginning 75:25. Microsoft Excel, 2010 was used to draw the calibration curves of the standards.

Data analysis

StatGraphics Centurion XV.I. and Least Significant Difference ($P < 0.05$) were used to analyse the data obtained and means separated, respectively.

Results and discussion

Gamma irradiation has been used for the decontamination of spices and other foods. The effects of gamma irradiation on microorganisms in foods lead to the inactivation of such pathogens (Ban and Kang, 2014; Deng *et al.*, 2015; Yu *et al.*, 2017).

Legon-18 pepper samples were contaminated with pathogens such as *E. coli*, *L. monocytogenes*, and *S.* Typhimurium and exposed to gamma irradiation at various doses to determine its effect on their inactivation.

Table 4. Stepwise regression analysis for the inactivation of *S.* Typhimurium in powdered Legon-18 pepper (*C. annuum*) stored at 4°C.

Step	Change	Step (*P* value)	Final (*P* value)	R^2 Adjusted (%)
1	Add X_2	0.000*	0.000*	60.892
2	Add X_1	0.000*	0.000*	80.360
3	Add $(X_2)^2$	0.000*	0.000*	82.376
4	Add $(X_1)^2$	0.003*	0.003*	83.556
5	Add X_1*X_2	0.011*	0.011*	84.334

Key: X_1= storage days; X_2= doses (kGy) of gamma irradiation.

Table 5. Survival of *L. monocytogenes* after gamma irradiation and during storage at 4°C in powdered Legon-18 pepper (*C. annuum*).

Storage	Microbial Count (log cfug^{-1})				
Days	0 kGy	1 kGy	2 kGy	4 kGy	5 kGy
0	6.26 ± 0.08^{Aa}	5.87 ± 0.11^{Ba}	4.97 ± 0.11^{Ca}	4.83 ± 0.12^{Da}	ND
2	6.15 ± 0.08^{Aa}	5.42 ± 0.11^{Bb}	4.46 ± 0.09^{Cb}	3.81 ± 0.11^{Db}	ND
5	6.04 ± 0.08^{Aa}	5.28 ± 0.11^{Bb}	4.26 ± 0.09^{Cc}	3.12 ± 0.11^{Dc}	ND
12	5.78 ± 0.11^{Ab}	5.02 ± 0.11^{Bc}	4.10 ± 0.09^{Cc}	2.86 ± 0.11^{Dd}	ND
21	5.42 ± 0.11^{Ac}	4.90 ± 0.09^{Bc}	3.84 ± 0.09^{Cd}	2.66 ± 0.13^{Dde}	ND
30	5.18 ± 0.11^{Ad}	4.30 ± 0.09^{Bd}	3.15 ± 0.08^{Ce}	2.46 ± 0.13^{De}	ND
45	5.07 ± 0.11^{Ad}	3.19 ± 0.08^{Be}	2.47 ± 0.11^{Cf}	1.96 ± 0.13^{Df}	ND
60	4.85 ± 0.09^{Ae}	2.22 ± 0.11^{Bf}	ND	ND	ND

Least Significant Difference: Means with the same letters (upper cases) in the same row are not significantly ($P > 0.05$) different from each other and means with the same letters in the same column (lower case, doses per day) are not significantly different ($P > 0.05$) from each other. Key: ND = not detected.

Table 6. Stepwise regression analysis for the inactivation of *L. monocytogenes* in powdered Legon-18 pepper (*C. annuum*) stored at 4°C.

Step	Change	Step (*P* value)	Final (*P* value)	R^2 Adjusted (%)
1	Add X_2	0.000*	0.000*	68.864
2	Add X_1	0.000*	0.000*	84.500
3	Add $(X_2)^2$	0.011	0.011*	85.223
4	Add X_2*X_2	0.053**	0.053**	85.579

Key: X_1= storage days; X_2= doses (kGy) of gamma irradiation.

Sterility of samples

There was no detection of the pathogens after the samples were exposed to sterility dose. This observation was similar to the study of Deng *et al.* (2015).

Inactivation of *E. coli*

Table 1 indicates the effect of gamma irradiation on the survival of *E. coli* after exposure to gamma irradiation. There was a general reduction in the count (log cfu/g) of *E. coli* in the pepper samples at all the doses of gamma irradiation in the pepper samples during the period of study ($P < 0.05$). Inactivation of the pathogen was dose-dependent which is similar to the observations of Deng *et al.* (2015), Waje *et al.* (2008), Ban and Kang (2014), Carcel *et al.*, 2015, and Jeong and Kang (2017). This observation may be attributed to the inability of the pathogen to recover from the injuries caused by gamma irradiation, the inability of the injured cells to adopt to their environment (Wu *et al.*, 2008).

A stepwise regression analysis (Table 2) was used to determine the effect of gamma irradiation and storage ($P < 0.05$; $R^2 = 91.52\%$ or 0.9152) on the inactivation of *E. coli*.

A model computed from the regression analysis is indicated as

$$\text{Inactivation of } E.\ coli \text{ (log cfu/g)} = 6.021 - 0.1078\ X1 - 1.1255\ X2 + 0.0200\ X1 * X2 \quad (1)$$

Where X_1 = storage days
X_2 = doses (kGy) of gamma irradiation

Inactivation of *S.* Typhimurium

Inactivation of *S.* Typhimurium is indicated in Table 3. A reduction in the microbial load (log cfu/g) was observed ($P < 0.05$) from the first day to the end of the study. Gamma irradiation and storage affected the microbial count ($P < 0.05$; Table 4) of the pathogen. Jeong and Kang (2017) and Ban and Kang (2014) had indicated the effect of gamma irradiation on the inactivation of *S.* Typhimurium which was dose dependent. In this study, a dose-dependent effect was observed. This observation may be due to the production of free radicals, reactive oxygen species, and other products from gamma irradiation which might have interacted with cellular composition, leading to inactivation (Yong *et al.*, 2015). There was no detection at 5 kGy.

The effect of gamma irradiation and storage on the inactivation of *S.* Typhimurium was determined using the stepwise regression (Table 4) analysis ($P < 0.05$; $R^2 = 0.8500$ or 85.00%).

The model (2) expressed the inactivation of the pathogen.

$$\text{Inactivation of } S.\ Typhimurium \text{ (log cfu/g)} = 6.294 - 0.0196X_1 - 0.379X_2 - 0.0001(X_1)^2 - 0.1375(X_2)^2 + 0.0056X_1 * X_2 \quad (2)$$

Inactivation of *L. monocytogenes*

The microbial count of the *L. monocytogenes* in the samples ranged from no detection to about 6 log cfu/g (Table 5). A general reduction in the count of *L. monocytogenes* in the pepper samples from the onset to the end of the study. There was a dose-dependent effect of gamma irradiation on the inactivation of the pathogen is similar to literature (Rico *et al.*, 2010; Mukhopadhyay *et al.*, 2013; Jeong and Kang, 2017). Inactivation was completely achieved at a dose of 5 kGy (no detection). Jeong and Kang (2017) and Waje *et al.* (2008) had indicated the effect of gamma irradiation on the inactivation

Table 7. Effect of gamma irradiation and storage on some quality parameters of Legon-18 pepper (*C. annuum*) powder at 4°C.

Storage	Capsaicin (mg/100 g)		Dihydrocapsaicin (mg/100 g)		Total capsaicinoids (mg/100 g)		SHU (×10,000)	
Weeks	0 kGy	5 kGy	0 kGy	5 kGy	0 kGy	5 kGy	0 kGy	5 kGy
0	178.74 ± 1.30^{Ia}	221.00 ± 2.29^{Hb}	77.12 ± 1.84^{Ga}	88.48 ± 1.11^{Hb}	255.87 ± 2.57^{Ia}	309.47 ± 3.32^{Ib}	4119.46 ± 41.33^{Ia}	4982.51 ± 53.49^{Ib}
1	177.18 ± 0.28^{Ha}	217.35 ± 0.37^{Hb}	75.48 ± 0.02^{Fa}	88.76 ± 0.04^{Hb}	252.65 ± 0.39^{Ha}	306.12 ± 0.36^{Hb}	4067.74 ± 62.26^{Ha}	4928.46 ± 57.45^{Hb}
2	173.25 ± 0.00^{Ga}	216.88 ± 0.11^{Gb}	74.29 ± 0.05^{Ea}	87.21 ± 0.00^{Gb}	247.54 ± 0.30^{Ga}	304.09 ± 0.11^{Gb}	3985.45 ± 36.96^{Ga}	4865.9118.19^{Gb}
3	165.73 ± 0.77^{Fa}	213.72 ± 0.17^{Fb}	73.35 ± 0.12^{Ea}	86.38 ± 0.37^{Fb}	239.08 ± 1.06^{Fa}	300.10 ± 0.47^{Fb}	3849.15 ± 17.01^{Fa}	4831.62 ± 76.45^{Fb}
4	161.51 ± 0.15^{Ea}	210.53 ± 0.00^{Eb}	66.15 ± 0.00^{Da}	85.32 ± 0.15^{Eb}	227.66 ± 0.21^{Ea}	295.85 ± 0.15^{Eb}	3665.39 ± 33.07^{Ea}	4763.26 ± 23.72^{Eb}
5	154.40 ± 1.22^{Da}	207.31 ± 0.39^{Db}	64.46 ± 0.63^{Ca}	83.56 ± 0.51^{Db}	218.86 ± 1.14^{Da}	290.86 ± 0.20^{Db}	3523.64 ± 18.43^{Da}	4682.88 ± 32.45^{Db}
6	146.93 ± 0.00^{Ca}	204.44 ± 0.73^{Cb}	63.82 ± 0.58^{Ca}	82.62 ± 0.39^{Cb}	210.75 ± 0.76^{Ca}	287.06 ± 0.34^{Cb}	3393.05 ± 12.30^{Ca}	4621.74 ± 54.33^{Cb}
7	144.74 ± 0.00^{Ba}	198.95 ± 0.09^{Bb}	61.97 ± 0.00^{Ba}	80.84 ± 0.10^{Bb}	206.70 ± 0.05^{Ba}	279.79 ± 0.17^{Bb}	3327.91 ± 0.78^{Ba}	4504.64 ± 27.11^{Bb}
8	140.34 ± 0.00^{Aa}	190.57 ± 0.11^{Ab}	59.80 ± 0.15^{Aa}	80.05 ± 0.18^{Ab}	200.14 ± 0.42^{Aa}	270.61 ± 0.14^{Ab}	3222.23 ± 67.96^{Aa}	4356.87 ± 22.17^{Ab}

Least Significant Difference: Means with the same letters (upper case, within the same column) are not significantly ($P > 0.05$) different from each other and means with the same letters in the same row (lower case, doses within a particular week) are not significantly different ($P > 0.05$) from each other.

Table 8. Effect of gamma irradiation and storage on some carotenoids in Legon-18 pepper (*C. annuum*) powder at 4°C.

Storage	Capsanthin (mg/100 g)		Beta carotene (mg/100 g)		Beta cryptoxanthin (mg/100 g)	
weeks	0 kGy	5 kGy	0 kGy	5 kGy	0 kGy	5 kGy
0	1.48 ± 0.00^{Fb}	1.36 ± 0.02^{Ga}	10.27 ± 0.00^{Ib}	9.38 ± 0.00^{Ia}	2.11 ± 0.02^{Hb}	1.55 ± 0.01^{Da}
1	1.46 ± 0.01^{Eb}	1.35 ± 0.01^{Ga}	10.04 ± 0.00^{Hb}	9.24 ± 0.00^{Ha}	2.05 ± 0.00^{Gb}	1.59 ± 0.00^{Da}
2	1.42 ± 0.00^{Db}	1.33 ± 0.00^{GFa}	9.92 ± 0.00^{Gb}	8.78 ± 0.00^{Ga}	2.03 ± 0.02^{Gb}	1.56 ± 0.03^{Da}
3	1.41 ± 0.00^{Db}	1.30 ± 0.00^{EFa}	9.43 ± 0.00^{Fb}	8.48 ± 0.00^{Fa}	1.95 ± 0.00^{Fb}	1.48 ± 0.01^{Ca}
4	1.41 ± 0.00^{Db}	1.27 ± 0.00^{DEa}	9.20 ± 0.00^{Eb}	8.23 ± 0.02^{Ea}	1.87 ± 0.00^{Eb}	1.43 ± 0.02^{BCa}
5	1.41 ± 0.00^{Db}	1.26 ± 0.00^{DCa}	8.91 ± 0.02^{Db}	8.21 ± 0.00^{Da}	1.80 ± 0.01^{Db}	1.40 ± 0.00^{Ba}
6	1.38 ± 0.02^{Cb}	1.23 ± 0.00^{Ca}	8.69 ± 0.07^{Cb}	7.91 ± 0.00C^{a}	1.71 ± 0.00^{Cb}	1.30 ± 0.00^{Aa}
7	1.35 ± 0.02^{Bb}	1.20 ± 0.00^{Ba}	8.48 ± 0.00^{Bb}	7.26 ± 0.00^{Ba}	1.66 ± 0.00^{Bb}	1.24 ± 0.00^{Aa}
8	1.32 ± 0.02^{Ab}	1.14 ± 0.04^{Aa}	8.17 ± 0.00^{Ab}	6.01 ± 0.00^{Aa}	1.50 ± 0.03^{Ab}	1.24 ± 0.11^{Aa}

Least Significant Difference: Means with the same letters (upper case, within the same column) are not significantly ($P > 0.05$) different from each other and means with the same letters in the same row (lower case, doses within a particular week) are not significantly different ($P > 0.05$) from each other.

of *L. monocytogenes*. The observed pattern is similar to the observation of Yu *et al.* (2017), Jeong and Kang (2017), and Waje *et al.* (2008). The irradiation effects might have led to injuries to cells as well as the effects of gamma irradiation on the cellular components (Yong *et al.*, 2015).

A significant effect ($P < 0.05$) of gamma irradiation and storage was observed from the stepwise regression analysis (Table 6), which had an R^2 = 86.07% or 0.8607.

Model (3) from the regression analysis for the inactivation is expressed as

$$\text{Inactivation of } L.monocytogenes\,(\log \text{cfu/g}) = 6.470 - 0.0514X_1 - 0.645X_2 - 0.0820(X_2)^2 + 0.0039X_1 * X_2$$

$$\text{Where } X_1 = \text{storage days}$$
$$X_2 = \text{doses (kGy) of gamma irradiation}$$

Chemical composition

Effect of gamma irradiation on capsaicinoids during storage

The capsaicinoids analysed in the study were capsaicin and dihydrocapsaicin (Table 7). Total capsaicinoids and SHU were computed as described earlier. Samples that were irradiated at 5 kGy had higher values as compared to the samples that were not irradiated. Samples irradiated at 5 kGy recorded percentage increase of 23.64%, 14.7%, and 20.95% in capsaicin, dihydrocapsaicin, and total capsaicinoids, respectively as compared with the control. There was a general reduction in the capsaicinoid content of the samples during storage. Since TC and SHU were computed from the capsaicinoids analysed, similar patterns of were observed. Giuffrida *et al.* (2014), Yu *et al.* (2017), and Topuz and Odzemir (2004) in a previous indicated that gamma and storage caused a reduction in the content of capsaicinoids, however, Byun *et al.* (1996) and Lee *et al.* (2004) indicated the stability of capsaicinoids below 15kGy. The observed higher contents in the samples irradiated are similar to the observations of Giuffrida *et al.* (2014), Yu *et al.* (2017), and Topuz and Odzemir (2004) and this may be attributed to the induction of water deficit by gamma irradiation (Farkas, 2006). The general reduction in the capsaicinoids, TC, and SHU during storage may be due to the effect of milling and residue enzymatic action (Wang *et al.*, 2009).

Effect of gamma irradiation on carotenoids during storage

The carotenoids in the pepper samples analysed were capsanthin, beta carotene, beta cryptoxanthin, and zeaxanthin. Zeaxanthin was below the detection point hence no data on this. The other carotenoid contents (Table 8) reduced after exposure to gamma irradiation at 5 kGy as compared to the contents of the samples that were not irradiated ($P < 0.05$). Gamma irradiation at 5kGy caused losses 8.11%, 8.67%, and 26.54% in capsanthin, beta carotene, and beta cryptoxanthin, respectively in the pepper samples as compared with the unirradiated samples. There was a general reduction in the carotenoid content during storage ($P < 0.05$). These observations have been reported elsewhere in literature (Pérez-Gálvez and Mínguez-Mosquera, 2001; Topuz and Ozemir, 2003; Kim *et al.*, 2004; Kim *et al.*, 2006; Rico *et al.*, 2010; Giuffrida *et al.*, 2014; Guadarrama-Lezama *et al.*, 2014; Jung *et al.*, 2015). The reduced content after irradiation and during storage may due to the secondary effects of gamma irradiation, component oxidation, form of the milled sun-dried pepper samples, and the structure of the carotenoids.

Conclusions

Gamma irradiation and storage had significant effect ($P < 0.05$) on the inactivation and quality parameters determined in Legon-18 pepper powder. Gamma irradiation can be used for the decontamination of pathogens such *E. coli*, *L. monocytogenes*, and *S.* Typhimurium in Legon-18 pepper powder. A dose of 5 kGy can inactivate all the pathogens immediately after exposure to gamma irradiation, not withstanding, doses such as 2 and 4 kGy can also be used for the inactivation of the pathogens used in the study but subject to storage time of 60 days. *E. coli* could be inactivated at a dose of 1 kGy at day 30. Samples irradiated had lower carotenoid contents as compared to the unirradiated. Zeaxanthin was below the detection limit. Higher values of hotness indices were observed in the irradiated but not in the unirradiated samples.

Acknowledgements

Our profound gratitude goes to the technologists at the Department of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra-Ghana for the assistance rendered during the microbiology aspect, the Radiation Technology Centre (BNARI-GAEC) for carrying out dosimetry for dose delivery and Central Laboratory of KNUST for the analysis of capsaicinoids and carotenoids in the samples.

Funding

Funds were obtained from the authors from the collection of data, its analysis, and interpretation.

Conflict of interest

Authors hereby certify that there are no conflicts of interest with any organization, financial, or other regarding the material discussed in the manuscript

References

Aguiar, A. C., Silva, L. P. S., de Rezende, C. A., Barbero, G. F., Martinez, J. (2016). Encapsulation of pepper oleoresin by supercritical fluid extraction of emulsions. *The Journal of Supercritical Fluids*, 112:37–43.

Ali, M. (ed.). (2006). *Chilli (Capsicum spp) Food Chain Analysis: Setting Research Priorities in Asia.* AVRDC-The World Vegetable Centre, Technical Bulletin No. 38, AVRDC Publication, Shanhua, Taiwan, pp. 1–253.

Almela, L., Nieto-Sandoval, J. M., Fernández López, J. A. (2002). Microbial inactivation of paprika by a high-temperature short-X time treatment. Influence on color properties. *Journal of Agricultural and Food Chemistry,* 50: 1435–1440.

Ban, G. H., Kang, D. H. (2014). Effects of gamma irradiation for inactivating Salmonella Typhimurium in peanut butter product during storage. *International Journal of Food Microbiology,* 171: 48–53.

Barbero, G. F., Palma, M., Barroso, C. G. (2006). Pressurized liquid extraction of capsaicinoids from peppers. *Journal of Agricultural and Food Chemistry,* 54: 3231–3236.

Bhunia, A. K. (2018). *Foodborne Microbial Pathogens. Mechanisms and Pathogenesis*. Springer Science+Business Media, LLC, New York, USA, pp. 12–22.

Boer, E. D., Spiegelenberg, W., Jansen, F. (1985). Microbiology of Spices and herbs. *Antonie van Leeuwenhoek*, 51:435–438.

Buckenhuskes, H. J., Rendlen, M. (2004). Hygienic problems of phytogenic raw material for food production with special emphasis on herbs and spices. *Food Science and Biotechnology*, 13:262–268.

Byun, M. W., Yook, H. S., Kwon, J. H., Kim, J. O. (1996). Improvement of hygiene quality and long-term storage of dried red pepper by gamma-irradiation. *Korean Society of Food Science and Technology*, 28: 482–489.

Carcel, J. A., Benedito, J., Cambero, M. I., Cabeza, M. C., Ordonez, J. A. (2015). Modelling and optimization of the E-beam treatment of chicken steaks and hamburgers, considering food safety, shelf-life and sensory quality. *Food and Bioproducts Processing*, 96:133–144.

Cheon, H-L., Shin, J-S., Park, K-H., Chung, M-S., Kang, D-H. (2015). Inactivation of pathogens in red pepper powder (*Capsicum annuum* L.) using combined UV-C irradiation and mild heat. *Food Control*, 50:441–445.

Deng, W., Wu, G., Guo, L., Long, M., Li, B., Liu, S., Cheng, L., Pan, X., Zou, L. (2015). Effect of gamma irradiation on *Escherichia coli*, *Salmonella* Enterica Typhimurium and *Aspergillus niger* in peppers. *Food Science and Technology Research*, 21: 241–245.

Department of Health, State Government of Victoria, Australia (DOH/Victoria/AU). 2010. Report on a survey of spices for the presence of pathogens - 2007. http://www.health.vic.gov.au/archive/archive2011/foodsafety/archive/microbiological.htm. Accessed 26 September 2017.

Doku, S. K. (2015). Genetic diversity studies. In: *Twenty Accessions of Hot Pepper (Capsicum Spp L.) In Ghana*. A thesis presented to the Department of Nuclear Agriculture and Radiation Processing School of Nuclear and Allied Sciences University of Ghana, Kwabenya-Accra, Ghana.

Ducic, M., Klisara, N., Markov, S., Blagojevic, B., Vidakovic, A. (2016). The fate and pasteurization of-based inactivation of *Escherichia coli* O157, *Salmonella* Typhimurium and Listeria monocytogenes in dry, fermented sausages. *Food Control*, 59:400–406.

Farkas, J. (2006). Irradiation for better foods. *Trends in Food Science Technology*, 17:148–152.

Farkas, J., Andrassy, E. (1988). Comparative analysis of spices decontaminated by ethylene oxide or gamma-radiation. *Acta Alimentaria*, 17: 77–94.

Giuffrida, D., Dugo, P., Torre, G., Bignardi, C., Cavazza, A., Corrandi, C., Dugo, G. (2013). Characterization of 12 Capsicum varieties by evaluation of their carotenoid and pungency determination. *Food Chemistry*, 140:794–802.

Giuffrida, D., Dugo, P., Torre, G., Bignardi, C., Cavazza, A., Corrandi, C., Dugo, G. (2014). Evaluation of carotenoid and capsaicinoid and capsaicinoid contents in powder of red chili peppers during one year of storage. *Food Research International*, 65: 163–170.

Gregoire, O., Cleland, M. R., Mittendorfer, J., Dababneh, S., Ehlermann, D. A. E., Fan, X., *et al*. (2003). Radiological safety of food irradiation with high energy X-rays: theoretical expectations and experimental evidence. *Radiation Physics and Chemistry*, 67: 169–183.

Guadarrama-Lezama, A. Y., Jaramillo-Flores, E., Gutierrez-Lopez, G. F., Perez-Alonso, C., Dornates-Alvarez, L., Alamilla-Beltran, L. (2014). Effects of storage temperature and water activity on the degradation of carotenoids contained in microencapsulated chili extract. *Drying Technology*, 32:1435–1447.

Jeong, A-R., Jo, M. J., Koo, M., Ku, K-H., Park, J-B., Kim, H. J. (2010). Microbial contamination of fresh-red pepper and packaged-red pepper powder in South Korea. *Journal of Food Science and Nutrition*, 15:233–238.

Jeong, S-G., Kang, D-H. (2017). Inactivation of *Escherichia coli O157:H7, Salmonella Typhimurium,* and *Listeria monocytogenes* in ready-to-bake cookie dough by gamma and electron beam irradiation. *Food Microbiology*, 64:172–176.

Jung, K., Song, B-S., Kim, M. J., Moon, B-G., Go, S-M., Kim, J-K., Lee, Y-J., Park, J-H. (2015). Effect of X-ray, gamma ray, and electron beam irradiation on the hygienic and physicochemical qualities of red pepper powder. *LWT-Food Science and Technology*, 63:846–851.

Kahraman, T., Ozmen, G. (2009). Quality of selected spices and herbs consumed in Turkey. *Archiv für Lebensmittel hygiene*, 60:185–191.

Kim, S., Lee, K. W., Park, J., Lee, J., Hwang I. K. (2006). Effect of drying in antioxidant activity and changes of ascorbic acid and colour by different drying and storage in Korean red pepper (*Capsicum annuum* L.). *International Journal of Food Science and Technology*, 41:90–95.

Kim, S., Park, J. B. and Hwang, I. K. (2004). Composition of main carotenoids in Korean red pepper (*Capsicum annuum*, L.) and the changes of pigment stability during drying and storage process. *Journal of Food Science*, 69:FCT39–44.

Lee, J. H., Sung, T. H., Lee, K. T., Kim, M. R. (2004). Effect of gamma-irradiation on colour, pungency and volatiles of Korean red pepper powder. *Journal of Food Science*, 69:C585–C5591.

Lilie, M., Hein, S., Wilhelm, P., Müller, U. (2007). Decontamination of spices by combining mechanical and thermal effects – an alternative approach for quality retention. *International Journal of Food Science and Technology*, 42:190–193.

Liu, W. Y., Kang, W-H., Kang, B-C. (2013). Basic information o pepper. In: *Genetics, Genomics and Breeding of Peppers and Eggplants*. Kang, B-C., Kole, C. (eds.) CRC Press. Taylor and Francis group. LLC, Boca Raton, pp. 1–13.

Lu, M., Ho, C. T., Huang, Q. (2017). Extraction, bioavailability, and bioefficacy of capsaicinoids. *Journal of Food and Drug Analysis,* 25: 27–36.

Ministry of Food and Agriculture (MOFA). (2007). *Chilli Pepper Production Guide*. Horticulture Export Industry Initiative, MOFA, Accra-Ghana, pp. 9.

Mukhopadhyay, S., Ukuku, D., Fan, X., Juneja, V. K. (2013). Efficacy of integrated treatment of UV light and low-dose gamma irradiation on inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* on grape tomatoes. *Journal of Food Science*, 78: M1049–M1056.

Ochoa-Alejo, N., Ramirez-Malagon, R. 2001. Invited review: in vitro chilli pepper biotechnology. *In Vitro Cellular and Developmental Biology-Plant*, 37:701–729.

Orellana-Escobedo, L., Garcia-Amezquita, L.E., Olivas, G. I., Ornelas-Paz, J. J., Sepulveda, D. R. (2013). Capsaicinoids content and proximate composition of Mexican chili peppers (Capsicum spp.) cultivated in the State of Chihuahua. *CytA-Journal of Food*, 11: 179–184.

Pérez-Gálvez, A., Mínguez-Mosquera, M. I. (2001). Structure-reactivity relationship in the oxidation of carotenoid pigments of the pepper (*Cap-*

sicum annuum L.). *Journal of Agricultural and Food Chemistry,* 49: 4864–4869.

Piggott, J., Othman, Z. (1993). Effect of irradiation on volatile oils of black pepper. *Food Chemistry*, 46:115–119.

Pinto, C. M. F., dos Santos, I. C., de Araujo, F. F., da Silva, T. P. (2016). Pepper importance and growth. In: do Rêgo, E. R., do Rêgo, M. M., Finger, F. L, eds. *Production and Breeding of Chilli Peppers (Capsicum spp.)*. Springer International Publishing, Switzerland, pp. 503–545.

Rico, C. W., Kim, G-R., Ahn, J-J., Kim, H-K., Furuta, M., Kwon, J-H. (2010). The comparative effect of steaming and irradiation on the physicochemical and microbiological properties of dried red pepper (*Capsicum annum* L.). *Food Chemistry,* 119: 1012–1016.

Rufino, J. L. S., Penteado, D. C. S. (2006) *Importância econômica, perspectivas e potencialidades do Mercado para pimenta*. Informe Agropecuário, Belo Horizonte 27:7–15

Schweiggert, U., Carle, R., Schieber. A., (2007). Conventional and alternative processes for spice production-a review. *Trends in Food Science and Technology*, 18: 260–268.

Sualihu, A. (2012). *Effect of Three Pre-Drying Treatments and Two Drying Methods on the Quality of Scotch Bonnet (capsicum chinense) Grown in the Tolon/Kumbungu District of Northern Ghana*. A thesis submitted to Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Tainter, D. R., Grenis, A. T. (2001). *Spices and Seasonings—A Food Technology Handbook*. 2nd edn. VCH, New York Wiley.

Topuz, A., Odzemir, F. (2004). Influences of gamma irradiation and storage on the carotenoids of sun-dried and dehydrated paprika. *Food Chemistry*, 86: 509–515.

Topuz, A., Ozdemir, F. (2003). Influences of gamma-irradiation and storage on the carotenoids of sun-dried and dehydrated paprika. *Journal of Agricultural and Food Chemistry*, 51: 4972–4977.

Waje, C. K., Kim, H. K., Kim, K. S., Todoriki, S., Kwon, J. H. (2008). Physicochemical and microbiological qualities of steamed and irradiated ground black pepper (*Piper nigrum* L.). *Journal of Agricultural and Food Chemistry,* 56: 4592–4596.

Wang, Y., Xia, Y, Wang, J., Luo, F., Huang, Y. (2009). Carotenoids in chili pepper (*Capsicum annuum*, L.) powder as affected by heating and storage methods. *American Society of Agricultural and Biological Engineers*, 52: 2007–2010.

WHO. (1981). *Wholesomeness of Irradiated Food. Report of a Joint FAO/IAEA/WHO Expert Committee, Technical Report Series 659*. World Health Organization, Geneva, Switzerland. p. 33.

Witkowska, A. M., Hickey, D. K., Alonso-Gomez, M., Wilkinson, M. G. (2011). The microbiological quality of commercial herb and spice preparations used in the formulation of a chicken supreme ready meal and microbial survival following a simulated industrial heating process. *Food Control*, 22: 616–625.

Wu, V. C. (2008). A review of microbial injury and recovery methods in food. *Food Microbiology,* 25: 735–744.

Yong, H. I., Kim, H-J., Nam, K. C., Kwon, J. H., Jo, C. (2015). Radiation sensitivity of foodborne pathogens in meat byproducts with different packaging. *Radiation Physics and Chemistry*, 115: 155–157.

Yu, W-J., Liu, H-P., Zhang, X-W., Dong, D., Jiang, Y., Sun, N-X., Liu, Y-K., Yuan, J-F. (2017). Postirradiation changes of the microbiological quality, aflatoxin, capsaicinoids, volatile oils, and the color of red pepper powder. *Journal of Food Processing and Preservation*, 1352:1–9.