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Short Communication

# Microbial decontamination of dried cassava (*Manihot esculenta*) chips using gamma irradiation

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ABSTRACT

This study presented an evaluation of the impact of gamma irradiation as a decontaminating tool in improving microbial quality of dried cassava chips. Thirteen (13) month old white-flesh cassava roots were purchased from an experimental farm of the Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI) at Pokuase in the Greater Accra Region of Ghana. The cassava roots were cleaned, peeled, washed and processed into chips, and then oven-dried. The dried cassava chips were gamma irradiated to target doses of 2.5, 5.0, 7.5 and 10.0 kGy at a dose rate of 0.75 kGy/h. Un-irradiated chips were used as controls. Total Viable Count (TVC), Total Coliform Count (TCC), Yeast and Mould Count (YMC), *Staphylococcus aureus* count (SAC), *Bacillus* species count (BSC) and *Salmonella* count (SC) of the dried cassava chips were determined using standard methods. Coliform, *Staphylococcus aureus, Bacillus* spp, yeast and moulds were detected in the dried cassava chips, except *Salmonella*. Gamma irradiation significantly reduced the microorganisms recorded on the surfaces of the dried cassava chips in dose-dependent manner. Radiation decontamination has the potential to improve microbial quality of dried cassava chips.

## 1. Introduction

Postharvest handling of cassava roots, particularly during drying could result in insect infestation and contamination through dust and activities of animals (Gacheru et al., 2016). Dried cassava chips have been found to be contaminated with microorganisms, such as bacteria and fungi/mould (Kaaya and Eboku, 2010; Adu-Gyamfi and Appiah, 2012; Gnonlonfin et al., 2012; Chiona et al., 2014; Gacheru et al., 2016; Asaam, 2017). Gacheru et al. (2016) reported unhygienic and poor quality of dried cassava chips sampled from a local market in Nairobi, Kenya. This has been attributed to excessive physical and poor post-harvest handling of the cassava (Gacheru et al., 2016). Chiona et al. (2014) attributed contamination of dried cassava chips by mould and bacteria to unhygienic conditions during production (lack of hand washing areas, lack of protective clothing and drying on dirty surfaces), storage and delayed sun-drying, especially during rainy seasons. Fungal contamination, besides posing major health concern to humans and livestock, can cause discolouration of the chips, mouldy taste and production of off odours (Gwinner et al., 1996).

Quality of cassava chips has been reported to be improved through fermentation (Diop, 1998), parboiling (Balagopalan, 1988), combination of sun-drying and oven-drying (Fakir et al., 2012), and drying in the oven at 70 °C for 48 h immediately after chipping without additional treatment (Chijindu and Boateng, 2008). Irradiation is one of the techniques used in improving hygienic quality of foods. The utilization of irradiation as a food decontaminating tool has been well documented (Farkas, 1988; IAEA, 1992; CAST, 1996; ASTM, 1998). Radiation has been reported to affect DNA directly, causing ionization of the atoms in the DNA molecules of the microorganisms in the food (Jeong and Jeong, 2017). Alternatively, radiation interacts with non-critical target atoms or molecules, usually water, resulting in the production of highly reactive free radicals, which can attack targets such as the DNA of the microorganisms (Jeong and Jeong, 2017). The attack causes the breakage of the single and double bond strands, leading to either the death of the cell or the alteration of the cell gene/genomic instability of the microorganisms (Jeong and Jeong, 2017).

There is little to no data on the use of radiation to decontaminate dried cassava chips. This study therefore aimed at improving microbial

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quality of dried cassava chips using gamma irradiation.

## 2. Materials and methods

### 2.1. Materials

Thirteen (13) month old white-flesh cassava (*Manihot esculenta*) cultivar (*Atakpa genotype*) (400 kg) was obtained fresh from an experimental farm of the Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI) at Pokuase in the Greater Accra Region of Ghana. All chemical reagents used were of analytical grade from MES Equipment, Accra-Ghana.

### 2.2. Methods

#### 2.2.1. Production of dried cassava chips

The fresh cassava roots (400 kg) were manually peeled with stainless steel knives. The peeled roots were washed thoroughly with clean water in washing containers. The cleaned cassava roots were then chopped by using a locally made cassava chipper into small pieces of dimensions (58 x 56 x 2.7) mm. A 150 kg sample of the wet (fresh) cassava chips was spread out in drying trays and placed in a hot-air oven (Apex dryer, type B35E, London-England). The temperature of the oven was set to 50 °C and drying of the chips was done for 24 h. The oven was put off when the chips were very dried (when the chips crumbled easily). The oven was allowed to cool and the chips removed from the oven and then packaged in a clean low density polyethelene bag and labeled for further treatment.

#### 2.2.2. Irradiation of the dried cassava chips

Irradiation of the dried cassava chips was done at the gamma irradiation facility (GIF) at the Radiation Technology Centre (RTC) of the Biotechnology and Nuclear Agriculture Research Institute (BNARI), Ghana Atomic Energy Commission (GAEC) using Cobalt-60 source (SLL-02/515, Hungary). The packaged dried cassava chips (5 kg) were subjected to the following target doses, 2.5, 5.0, 7.5 and 10 kGy at a dose rate of 0.75 kGy per hour. The un-irradiated dried cassava chips were used as controls. The absorbed dose was determined using ethanol chloro-benzene dosimeter (Kovacs et al., 1985).

#### 2.2.3. Microbial quality of gamma irradiated dried cassava chips

The microbial quality of the gamma-irradiated and the control samples were determined using the ISO 4832–2006 colony count technique (ISO, 2006). Total viable counts (TVC) were determined on Plate Count Agar (Oxoid, England). Yeast and moulds were estimated on Oxytetracyclene glucose yeast extract, *Staphylococcus aureus* counts were estimated on Baird-Parker Agar (Oxoid, England), total coliform counts were determined on Eosine Methylene Blue agar (Oxoid, England), *Salmonella* counts were estimated on XLD agar and *Bacillus cereus* counts were estimated on *Bacillus cereus* selective agar base (Oxoid, England).

From each sample of dried cassava chips, 10g was weighed with an electronic weighing scale (SCIENTECH, USA) Model number SL5000, serial number 12012. This was placed in 90 ml of diluent and agitated for 5 min on an orbit shaker model number M37610-33 and serial number C1861130418486 to obtain the stock. A 1:10 dilution of the stock diluent was prepared by aseptically pouring 1 ml of the stock into 9 ml of plate count agar diluent. Each dilution was agitated in a 28 ml bottle to re-suspend any material that may have settled out during the preparation. Serial dilutions of the diluted stock were made up to $10^6$ to enable counting of the microorganisms. About 1 ml of each diluent was aseptically transferred into sterile well labeled petri dishes (triplicates for each dilution). Plates were poured not more than 15 min after preparation of dilutions. The petri dishes were swirled gently to mix and left to solidify. About 4 ml of molten agar (2nd layer) was added to make sure that the entire sample was covered and left to solidify. The plates were incubated at 37 °C for 48h. The number of colonies in the media were then counted with a colony counter. The detection of *Salmonella* spp. Was done using 25 ml of sample on Xylose Lysine Deoxycholate Agar (Oxoid, England) and confirmatory tests were done according to Harrigan and McCance (1976). Plates with 30–300 colonies were selected for enumeration of colony forming unit per gram (cfu/g) with the aid of a colony counter (Stuart Scientific, UK) (Robinson et al., 2000).

#### 2.2.4. Data analysis

The experiments were done three times and the data obtained converted into $\log_{10}$ cfu. All data were then subjected to analysis of variance (ANOVA) using IBM SPSS statistics version 20 to assess whether the independent variable: radiation doses (2.5, 5, 7.5 and 10 kGy) had significant effects on the dependent variable measured at 95% confidence level. The least significant difference (LSD) at $p \leq 0.05$ was used to separate the means.

## 3. Results and discussion

High counts of microorganisms were recorded by the un-irradiated dried cassava chips (Table 1). The high numbers of microorganisms on the surface of the un-irradiated dried cassava chips (control) could partially be attributed to deficiencies in production protocol (Adu-Gyamfi and Appiah, 2012), such as inadequate washing of the cassava roots and proper drying of chips. In general, gamma irradiation doses of 2.5–7.5 kGy significantly ($p \leq 0.05$) reduced the microbial load on the dried cassava chips by 1–6 log cycles. Gamma irradiation has been reported to cause loss of reproductive capability in proliferating cells (Hall and Garcia, 2006).

Total viable count (TVC) generally acts as good hygienic quality indicator (Adu-Gyamfi and Appiah, 2012), therefore samples, especially food with high numbers of this parameter raises food safety concerns. TVC recorded in the present study for un-irradiated dried cassava chips (control) was higher than the maximum permissible TVC for edible dried cassava chips ($1.0 \times 10^4$ cfu/g) set by the Ghana Standards Authority (GSA) (GS 300:2017). Gamma irradiation significantly decreased ($p \leq 0.05$) the TVC of the dried cassava chips in a dose-dependent manner (Table 1).

Adu-Gyamfi and Appiah (2012) also reported a decrease in TVC with increasing gamma irradiation dose in some irradiated Ghanaian food products. Gamma irradiation dose of 7.5 kGy and 10.0 kGy completely eliminated all the viable microorganisms from the dried chips (Table 1).

The mean Total Coliform counts (TCC) ranged from below detection limit (<1 cfu/g) (ND) to 4.66 $\log_{10}$ cfu/g (Table 1). The presence of coliforms on the un-irradiated dried cassava chips is an indication of poor sanitary conditions (Halkman and Halkman, 2014), as they may have originated from either faecal or non-faecal contamination (Yousef and Carlstrom, 2003). This could be partially attributed to inadequate washing with portable water (Ibekwe et al., 2008). The mean populations of coliforms on the un-irradiated dried cassava chips (control) were above the maximum acceptable level of coliforms ($5.0 \times 10^1$ cfu/g) on edible dried cassava chips set by the Ghana Standards Authority (GS 300:2017). Gamma irradiation caused a significant reduction ($p \leq 0.05$) in the coliform population on the control dried cassava chips in a dose-dependent manner. Gamma irradiation of 7.5 kGy and 10.0 kGy completely eliminated all the coliform counts (Table 1).

The presence of *Staphylococcus aureus* on the un-irradiated dried cassava chips could be attributed to post-processing handling (Obadina et al., 2008). *Staphylococcus aureus* has been reported to produce enterotoxins which are not easily destroyed, and their presence may cause food poisoning (Gacheru et al., 2016). In the present study, the mean *Staphylococcus aureus* counts ranged from not detected (ND) to 4.41 $\log_{10}$ cfu/g for the dried cassava chips (Table 1). According to the Ghana Standards Authority (GSA) standards (GS 300:2017), there shall be no *Staphylococcus aureus* on edible dried cassava chips. Guthrie (1983)

**Table 1**
Microbial quality of radiation decontaminated dried cassava chips.

| Dose (kGy) | Microbial Characteristics ($log_{10}$ cfu/g) | | | | | |
|---|---|---|---|---|---|---|
| | TVC | TCC | *Staphylococcus aureus* | Yeasts& Moulds | *Bacillus* Spp | *Salmonella* |
| Control | $6.00 \pm 0.00^{a}$ | $4.66 \pm 0.05^{a}$ | $4.41 \pm 0.05^{a}$ | $3.97 \pm 0.02^{a}$ | $1.05 \pm 0.06^{b}$ | ND |
| 2.5 | $5.10 \pm 0.09^{b}$ | $3.54 \pm 0.05^{b}$ | $3.68 \pm 0.01^{b}$ | ND | ND | ND |
| 5.0 | $3.26 \pm 0.24^{c}$ | $2.10 \pm 0.17^{c}$ | $1.00 \pm 0.00^{c}$ | ND | ND | ND |
| 7.5 | $1.00 \pm 0.00^{d}$ | ND | ND | ND | ND | ND |
| 10.0 | $1.00 \pm 0.00^{d}$ | ND | ND | ND | ND | ND |

Each value is a mean of three experiments ± standard deviations. Values with different letters in a column are significantly different at $p \leq 0.05$. ND = Not detected, Detection limit = 1 cfu/g, TVC = Total viable count, TCC = Total coliform count.

reported that very large numbers of *Staphylococcus* spp. Cells (5.56–6.69 $log_{10}$ cfu/g) are required for expression of disease symptoms, hence the levels reported in the present study (ND -4.50$log_{10}$ cfu/g) (Table 1) may pose low risk of causing disease if not eliminated. Gamma irradiation significantly ($p \leq 0.05$) decreased the *Staphylococcus aureus* counts with increasing irradiation dose. Gamma radiation dose of 7.5 kGy destroyed all *Staphylococcus aureus* on the dried cassava chips. Adu-Gyamfi and Appiah (2012) also reported that gamma radiation dose of 7.5 kGy destroyed all *Staphylococcus aureus* from milled dried cassava chips (*kokonte* powder).

The mean mould and yeast counts (MYC) recorded for the dried cassava chips in the present study ranged from not detected (ND) to 3.97 $log_{10}$ cfu/g (Table 1). The values for the un-irradiated dried cassava chips (control) were high compared to the acceptable MYC limit of $1.0 \times 10^{3}$ $cfug^{-1}$ for edible dried cassava chips set by the GSA (GS 300:2017). Gamma irradiation significantly ($p \leq 0.05$) decreased the MYC with increasing irradiation dose. A dose of 2.5 kGy was enough to totally eliminate MYC from the dried cassava chips. Similar trends have been reported by Adu-Gyamfi and Appiah (2012) for some irradiated Ghanaian food products.

*Bacillus* species are aerobic, sporulating and rod-shaped bacteria that can be found everywhere in the environment, but their primary habitat is the soil (Tajkarimi, 2007). *Bacillus* spp is part of the fermentation microflora of cassava (Adu-Gyamfi and Appiah, 2012). A minimum dose of 2.5 kGy eliminated *Bacillus* spp completely from the dried cassava chips, thus eliminating the risk of contraction of *Bacillus*-related food borne disease associated with the consumption of the dried cassava chips. *Salmonella* species were not detected in the control dried cassava chips (Table 1).

The above data suggest that irradiation with 7.5 kGy would be effective for decontamination of dried cassava chips for enhanced hygienic quality. However, Ghana Standards specify a dose of 5 kGy for dry foods in Ghana (Ghana Standards Board-GSB-GS 210:1998).

## 4. Conclusion

The dried cassava chips were found to be naturally contaminated with various microorganisms, such as *Staphylococcus aureus*, mould and yeasts, *Bacillus* spp. and coliforms whose population exceeded national and internationally accepted limits, exposing potential consumers to health hazards. Gamma irradiation dose of 7.5 kGy completely eliminated all the examined microorganisms and improved the hygienic quality of the dried cassava chips, hence eliminating the risks of health hazard associated with the consumption of the chips. It also eliminated the risk of microorganism-related spoilage with the potential of extending the shelf-life of the dried cassava chips. Radiation decontamination can be used in reducing and eliminating the examined microorganisms in dried cassava chips.

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## Author contributions

JOA Investigation, Methodology, Formal analysis, Writing- Original draft preparation; FCKO Conceptualization, Data curation, Resources, Supervision, Writing – Review and Editing; VO Resources, Supervision, Writing – Review and Editing.

## 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.

## Data availability

Data will be made available on request.

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