

# Efficiency of Gamma Irradiation to Inactivate Growth and Fumonisin Production of *Fusarium moniliforme* on Corn Grains

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The efficiency of gamma irradiation (0, 1, 5, 10, 15, 20, and 30 kGy) as a sterilization method of corn samples (30 g) artificially contaminated with *Fusarium moniliforme* stored at normal condition (25°C with approximate relative humidity (RH) of 55%) and optimal condition (25°C with a controlled RH of 97%) was studied. The results showed that the fungal growth and the amount of fumonisin were decreased as the dose of gamma irradiation increased. Gamma irradiation at 1–5 kGy treatment significantly inhibited the growth of *F. moniliforme* by 1–2 log reduction on corn samples ( $P < 0.05$ ). Sublethal effect of gamma irradiation was observed at 10–20 kGy doses after storage, and a complete inactivation required 30 kGy. Fungal growth and fumonisin production increased with higher humidity and longer storage time in all corn samples. This study also demonstrated that there was no strict correlation between fungal growth and fumonisin production. Storage at normal condition significantly resulted in lower growth and fumonisin production of *F. moniliforme* as compared with those stored at optimal condition ( $P < 0.05$ ). Gamma irradiation with the dose of  $\geq 5$  kGy followed by storage at normal condition successfully prolonged the shelf life of irradiated corns, intended for human and animal consumptions, up to 7 weeks.

**Keywords:** Corn, *Fusarium moniliforme*, growth, fumonisin, gamma irradiation, inactivation

## Introduction

Corn (*Zea mays L.*) is an important source of food and feed products for industry. Nowadays, there is a dichotomy through the demands of the first world populations for foods with no chemical residues and the desperate needs of third world populations to protect their agricultural products from damages by fungal contamination in order to maintain a minimum level of food safety [8].

Stored corn is a man-made ecosystem in which quality and nutritive changes occur because of fungal spoilage, and mycotoxin contamination is a major concern. Fungal infection results in mycotoxin contamination during the growing, harvesting, storage, transport, and processing [8]. Fumonisin are naturally occurring mycotoxins produced by a limited number of *Fusarium* species such as *Fusarium moniliforme* (*F. verticillioides*) and *F. proliferatum*, which are the most important fungi as they infect corn crops around

the world [5, 11]. From the 53 known fumonisins, fumonisin B<sub>1</sub> (FB<sub>1</sub>) and fumonisin B<sub>2</sub> (FB<sub>2</sub>) are generally the most common members of this class of fumonisins [13, 17]. The implications of fumonisin toxicity in foods and feeds are serious [27, 38]. Therefore, the US Food and Drug Administration (FDA) has released guidance levels of total fumonisin in corn and corn-based products at 2–4 µg/g for foods and 5–100 µg/g for animal feeds [12]. In addition, The European Union has also recently regulated fumonisin (B<sub>1</sub>+B<sub>2</sub>): the maximum levels are 1 mg/kg for corn-based foods and 4 mg/kg for unprocessed corn [9].

Gamma irradiation can be an effective alternative technology in controlling microorganisms in postharvest food and feed products [10]. Low levels of gamma irradiation (up to 10 kGy) do not cause toxicological hazards and significant nutritional losses; and the losses caused by higher doses (above 10 kGy) are of minor importance or comparable to those observed during the heating process [14, 44]. In

recent years, there has been increasing interests in the scientific community for the use of gamma irradiation to improve the quality and to extend the shelf life of food and feed products [4, 11, 16, 28, 32, 39, 42, 45]. However, contamination of corn by toxigenic fungi and mycotoxins can occur during storage and at any time until consumption. Environmental conditions play an important role on the growth of *F. verticillioides* and fumonisin production before and after harvest [26, 31]. Although the average dose of 10 kGy irradiation significantly reduced or completely killed bacterial populations in corn grains, higher doses of irradiation followed by controlling of humidity during storage are required for ensuring food safety. So far, less studies have been reported on the presence of fungi and mycotoxin in corn grains treated by higher doses of gamma radiation after storage for longer periods. Therefore, the purpose of this study was to evaluate the efficacy of various doses of gamma irradiation up to 30 kGy on the inhibition of growth and production of fumonisins B<sub>1</sub> and B<sub>2</sub> of *F. moniliforme* inoculated on corn grains. Nevertheless, it was also essential to obtain a better understanding of the relationships between growth and fumonisin production as influenced by their optimal moisture and temperature during storage in order to prolong the shelf life of irradiated corn intended for human and animal consumptions.

## Materials and Methods

### Samples Collection

The corn (*Zea mays L.*) grain samples used for this experiment were obtained from the National Agricultural Products Quality Management Service, Republic of Korea. Corn samples (500 g) were used in triplicates and individually packaged in plastic bags, and then wrapped in paper bags and sealed. The samples were irradiated to 30 kGy to eliminate the natural microflora.

### Humidity and Temperature Control

The corn samples in sterile petri dishes were incubated at 25°C with a controlled relative humidity (RH) of 97% and at 25°C (with approximate RH of 55%) for 7 weeks. The 97% RH was made by using a method established by Rockland [35]. Humidity and temperature during storage were controlled with a thermohygrometer.

### Fungal Strain and Inoculation

The strain of *Fusarium moniliforme* NRRL 13569 used in all experiments was obtained from Department of Biotechnology, Korea University. The inoculum was prepared by using a sterile inoculation loop to scrape off sporulating mycelia from the surfaces of potato dextrose agar (PDA, Oxoid) with the addition of a 10% lactic acid solution (pH 2.8). The lactic acid solution was

added to avoid the growth of yeast and bacteria. The inoculum was then cultured first by incubating the petri plates for 7 days at 30°C. After culturing, the inoculum was diluted in a phosphate buffer solution (8 g/l NaCl; 0.2 g/l KCl; 0.12 g/l KH<sub>2</sub>PO<sub>4</sub>; 0.9 g/l Na<sub>2</sub>HPO<sub>4</sub>), and then mixed with 0.05% of Tween 80. The final conidial suspension concentration was adjusted to ~10<sup>4</sup> CFU/ml using a hemocytometer. Five hundred grams of corn sample was placed in sterilized beaker glass flasks and immersed with 2 L of conidial suspension concentration of the fungus *F. moniliforme* for 14 h (stirred every 4 h) [43]. The inoculated corn samples were then dried on sterilized paper towels and weighed (30 g) in a sterile petri dish for each gamma irradiation treatment.

### Gamma Irradiation

Inoculated samples (30 g) in triplicates were sealed in sterile petri dishes. Samples were irradiated at different doses of 0, 1, 5, 10, 15, 20, and 30 kGy at room temperature in a <sup>60</sup>Co gamma irradiator (Greenpia Technology Inc., Yeosu, Gyeonggi, Republic of Korea). After each treatment, samples were kept in plastic containers. The control sample was not irradiated. Both irradiated and un-irradiated corn samples were divided into two groups: (i) un-irradiated and irradiated samples stored in a 25°C incubator with a controlled 97% RH, and (ii) un-irradiated and irradiated samples stored in a 25°C incubator (with approximate RH of 55%), for 7 weeks. Samples were evaluated at zero time of storage and at 1, 3, 5, and 7 weeks for the fungal growth (log CFU/g) and levels of fumonisin B<sub>1</sub> and fumonisin B<sub>2</sub> (µg/g).

### Plate Counting

Thirty grams of the samples (treated or untreated) was ground, thoroughly mixed with solution containing 0.05% Tween 80, and blended using a Waring blender for 10 min. Counting of fungal population was performed by the plate count technique on potato dextrose agar (PDA, Oxoid) using each suspension in a serial dilution. After incubation at each interval of storage time, the counting was performed according to Nelson *et al.* [30], and the results are expressed in log colony forming units per gram of corn samples (log CFU/g).

### Determination of Fumonisins in Corn Samples

The stock solutions of fumonisins B<sub>1</sub> and B<sub>2</sub> were prepared by transferring the contents of the vial (5 mg) to a 10 ml volumetric flask and made to volume with acetonitrile/water (20:80 (v/v)) to give 500 µg/ml of stock solution. The stock solutions were used for serial dilutions. Dilutions made with acetonitrile/water (20:80 (v/v)) were 10, 5, 3, 1, 0.5, and 0.3 µg/ml. The FB<sub>1</sub> and FB<sub>2</sub> were extracted according to a modified technique originally described by Thakur and Smith [40].

### Statistical Analysis

Samples were analyzed in triplicates and the results were presented as means ± standard deviations. All data were subjected to one-way ANOVA in SPSS v.13.0 (Statistical Package for the

Social Sciences, Chicago, IL, USA). Tukey's multiple range tests were used to determine the significant difference at  $P < 0.05$ .

## Results

### Growth of *Fusarium moniliforme* on Irradiated Corn Stored at Normal and Optimal Conditions

Effects of gamma irradiation on the reduction of *F. moniliforme* population (with the initial inoculum of  $\sim 10^4$  CFU/g) on corn stored at normal (25°C with approximate RH of 55%) and optimal (25°C with a controlled 97% RH) conditions for 7 weeks are presented in Table 1.

At 0 day of storage, the 1 and 5 kGy gamma irradiation treatments reduced significantly about 1 and 2 log CFU/g of *F. moniliforme* in treated corn samples as compared with control ( $P < 0.05$ ), respectively, and *F. moniliforme* growth was not detected at 10–20 kGy treatments. However, after 1 week of storage, the population of *F. moniliforme* was increased up to  $5.48 \pm 0.18$  log CFU/g in 10 kGy,  $1.70 \pm 0.11$  log CFU/g in 20 kGy at normal condition and  $6.90 \pm 0.10$  log CFU/g in 10 kGy and  $1.85 \pm 0.15$  log CFU/g in 20 kGy at optimal condition. At the same doses of gamma irradiation, the populations of *F. moniliforme* on corn with 1–20 kGy treatments stored at optimal condition were significantly higher than those stored at normal condition after 7 weeks of culture period ( $P < 0.05$ ). Moreover, the populations of *F. moniliforme* on corn samples irradiated with the doses of

1–10 kGy followed by storage at optimal condition were significantly higher compared with untreated samples stored at normal condition after 7 weeks ( $P < 0.05$ ), and there was no significant difference in 20 kGy treatment stored at optimal storage condition compared with control at normal storage condition ( $P > 0.05$ ). In the meantime, *F. moniliforme* was completely inactivated at 30 kGy treatment irrespective of culture conditions after 7 weeks.

### Fumonisin Production on Irradiated Corn Stored at Normal and Optimal Conditions

Effects of storage at normal (25°C with approximate RH of 55%) and optimal (25°C with a controlled 97% RH) conditions on fumonisins B<sub>1</sub> and B<sub>2</sub> produced by *F. moniliforme* on gamma-irradiated corn samples stored for 7 weeks were also investigated (Tables 2 and 3, respectively).

Tables 2 and 3 show that there were no fumonisins productions on corn samples having initial inoculum of *F. moniliforme* with approximately  $\sim 10^4$  CFU/g at 0 day of storage. Fumonisin production in all corn samples was significantly influenced by RH and storage time ( $P < 0.05$ ). The different levels of fumonisins B<sub>1</sub> and B<sub>2</sub> in un-irradiated corn samples stored at optimal condition after 1 week were  $\pm 0.35$  µg/g and  $\pm 0.62$  µg/g, significantly higher than those stored at normal condition ( $P < 0.05$ ), respectively. After 7 weeks of storage, the different levels of fumonisins B<sub>1</sub> and B<sub>2</sub> increased significantly up to  $\pm 21$  µg/g and  $\pm 5$  µg/g

**Table 1.** Effects of gamma irradiation on the reduction of *F. moniliforme* populations (mean  $\pm$  standard deviation; log CFU/g) for 7 weeks at normal (25°C with approximate RH of 55%) and optimal (25°C with a controlled RH of 97%) conditions.

Irradiation doses (kGy)	Storage (weeks)					
	0	1	3	5	7	
A*	0	4.02 $\pm$ 0.12 <sup>Ab</sup>	8.48 $\pm$ 0.11 <sup>Db</sup>	7.30 $\pm$ 0.13 <sup>Be</sup>	8.00 $\pm$ 0.41 <sup>Cd</sup>	8.60 $\pm$ 0.10 <sup>Dc</sup>
	1	3.33 $\pm$ 0.15 <sup>Ac</sup>	6.64 $\pm$ 0.28 <sup>Bf</sup>	7.60 $\pm$ 0.10 <sup>Cd</sup>	6.91 $\pm$ 0.13 <sup>Be</sup>	8.00 $\pm$ 0.39 <sup>Dd</sup>
	5	2.60 $\pm$ 0.09 <sup>Ad</sup>	6.60 $\pm$ 0.22 <sup>BCf</sup>	6.30 $\pm$ 0.11 <sup>Bg</sup>	6.30 $\pm$ 0.14 <sup>Bf</sup>	6.48 $\pm$ 0.17 <sup>Be</sup>
	10	ND	5.48 $\pm$ 0.18 <sup>ABg</sup>	5.25 $\pm$ 0.18 <sup>Ah</sup>	5.30 $\pm$ 0.16 <sup>Ag</sup>	6.30 $\pm$ 0.13 <sup>Ce</sup>
	20	ND	1.70 $\pm$ 0.11 <sup>Ah</sup>	3.00 $\pm$ 0.17 <sup>Bi</sup>	2.91 $\pm$ 0.11 <sup>Bh</sup>	3.70 $\pm$ 0.23 <sup>Cf</sup>
	30	ND	ND	ND	ND	ND
	0	4.58 $\pm$ 0.13 <sup>Aa</sup>	9.41 $\pm$ 0.14 <sup>Ba</sup>	9.50 $\pm$ 0.17 <sup>Ba</sup>	9.40 $\pm$ 0.12 <sup>Ba</sup>	9.50 $\pm$ 0.09 <sup>Ba</sup>
B*	1	3.33 $\pm$ 0.16 <sup>Ac</sup>	7.85 $\pm$ 0.16 <sup>Bc</sup>	8.83 $\pm$ 0.09 <sup>Cb</sup>	9.00 $\pm$ 0.10 <sup>CDb</sup>	9.42 $\pm$ 0.11 <sup>Ea</sup>
	5	2.68 $\pm$ 0.11 <sup>Ad</sup>	7.48 $\pm$ 0.14 <sup>Bd</sup>	8.73 $\pm$ 0.19 <sup>Cb</sup>	8.85 $\pm$ 0.20 <sup>Cbc</sup>	9.38 $\pm$ 0.08 <sup>Da</sup>
	10	ND	6.90 $\pm$ 0.10 <sup>Ae</sup>	8.39 $\pm$ 0.12 <sup>Bc</sup>	8.62 $\pm$ 0.14 <sup>BCc</sup>	9.00 $\pm$ 0.10 <sup>Db</sup>
	20	ND	1.85 $\pm$ 0.15 <sup>Ah</sup>	6.91 $\pm$ 0.15 <sup>Bf</sup>	8.02 $\pm$ 0.10 <sup>Cd</sup>	8.45 $\pm$ 0.16 <sup>Dc</sup>
	30	ND	ND	ND	ND	ND

<sup>A-E</sup>Numbers within each row followed by different capital letters are significantly different ( $P < 0.05$ ).

<sup>a-i</sup>Numbers within each column followed by different small letters are significantly different ( $P < 0.05$ ).

\*Normal condition (A); Optimal condition (B).

ND: Not detected.

**Table 2.** Effects of gamma irradiation on the production of fumonisin B<sub>1</sub> by *F. moniliforme* (mean ± standard deviation; µg/g) for 7 weeks at normal (25°C with approximate RH of 55%) and optimal (25°C with a controlled RH of 97%) conditions.

Irradiation doses (kGy)	Storage (weeks)					
	0	1	3	5	7	
A*	0	ND	2.15 ± 0.11 <sup>Ac</sup>	10.19 ± 0.10 <sup>Be</sup>	12.98 ± 0.17 <sup>Ce</sup>	13.16 ± 0.11 <sup>Cf</sup>
	1	ND	1.80 ± 0.11 <sup>Ad</sup>	9.62 ± 0.08 <sup>Bf</sup>	11.30 ± 0.12 <sup>Cg</sup>	13.29 ± 0.11 <sup>Df</sup>
	5	ND	1.35 ± 0.04 <sup>Ae</sup>	1.81 ± 0.06 <sup>Bg</sup>	1.46 ± 0.14 <sup>Ah</sup>	1.97 ± 0.09 <sup>Bg</sup>
	10	ND	1.15 ± 0.07 <sup>Af</sup>	1.40 ± 0.15 <sup>Bh</sup>	1.45 ± 0.14 <sup>Bh</sup>	1.50 ± 0.07 <sup>Bh</sup>
	20	ND	0.02 ± 0.01 <sup>Ah</sup>	0.06 ± 0.01 <sup>Bi</sup>	0.05 ± 0.01 <sup>Bi</sup>	0.08 ± 0.01 <sup>Bi</sup>
	30	ND	ND	ND	ND	ND
	B*	0	ND	2.50 ± 0.08 <sup>Aa</sup>	12.60 ± 0.04 <sup>Ba</sup>	16.86 ± 0.08 <sup>Ca</sup>
1		ND	2.31 ± 0.10 <sup>Ab</sup>	12.52 ± 0.04 <sup>Ba</sup>	16.58 ± 0.10 <sup>Cb</sup>	31.04 ± 0.03 <sup>Db</sup>
5		ND	2.08 ± 0.06 <sup>Ac</sup>	12.27 ± 0.09 <sup>Bb</sup>	16.16 ± 0.09 <sup>Cc</sup>	28.26 ± 0.12 <sup>Dc</sup>
10		ND	0.51 ± 0.03 <sup>Ag</sup>	12.01 ± 0.07 <sup>Bc</sup>	15.01 ± 0.05 <sup>Cd</sup>	27.12 ± 0.08 <sup>Dd</sup>
20		ND	0.13 ± 0.02 <sup>Ah</sup>	10.97 ± 0.10 <sup>Bd</sup>	12.34 ± 0.14 <sup>Cf</sup>	24.69 ± 0.03 <sup>De</sup>
30		ND	ND	ND	ND	ND

<sup>A-D</sup>Numbers within each row followed by different capital letters are significantly different ( $p < 0.05$ ).

<sup>a-i</sup>Numbers within each column followed by different small letters are significantly different ( $p < 0.05$ ).

\*Normal condition (A); Optimal condition (B).

ND: Not detected.

( $p < 0.05$ ), respectively. The fumonisin production in all treated corn samples were significantly higher than control after storage for 7 weeks ( $p < 0.05$ ), except the FB<sub>1</sub> production on treated samples at 1 kGy stored at normal condition. Among the irradiated samples, the levels of fumonisins

B<sub>1</sub> and B<sub>2</sub> on corn samples at 5 kGy treatment stored for 7 weeks at optimal condition were roughly 14 times and 5 times higher than those stored at normal condition ( $p < 0.05$ ), respectively. Production of fumonisins B<sub>1</sub> and B<sub>2</sub> was almost completely inactivated at 20 kGy treatments for

**Table 3.** Effects of gamma irradiation on the production of fumonisin B<sub>2</sub> by *F. moniliforme* (mean ± standard deviation; µg/g) for 7 weeks at normal (25°C with approximate RH of 55%) and optimal (25°C with a controlled RH of 97%) conditions.

Irradiation doses (kGy)	Storage (weeks)					
	0	1	3	5	7	
A*	0	ND	0.34 ± 0.06 <sup>Ad</sup>	2.25 ± 0.09 <sup>Bb</sup>	2.98 ± 0.10 <sup>Cc</sup>	3.12 ± 0.10 <sup>Ce</sup>
	1	ND	0.28 ± 0.06 <sup>Ad</sup>	0.65 ± 0.11 <sup>Be</sup>	0.71 ± 0.08 <sup>Bf</sup>	2.06 ± 0.01 <sup>Cf</sup>
	5	ND	0.15 ± 0.05 <sup>Ae</sup>	0.60 ± 0.10 <sup>Be</sup>	0.63 ± 0.09 <sup>Bf</sup>	0.81 ± 0.08 <sup>Cg</sup>
	10	ND	0.02 ± 0.01 <sup>Af</sup>	0.21 ± 0.06 <sup>Bg</sup>	0.26 ± 0.03 <sup>Bg</sup>	0.58 ± 0.06 <sup>Ch</sup>
	20	ND	0.02 ± 0.01 <sup>Af</sup>	0.03 ± 0.004 <sup>Bh</sup>	0.02 ± 0.003 <sup>Ah</sup>	ND
	30	ND	ND	ND	ND	ND
	B*	0	ND	0.96 ± 0.06 <sup>Aa</sup>	2.80 ± 0.10 <sup>Ba</sup>	4.29 ± 0.02 <sup>Ca</sup>
1		ND	0.60 ± 0.07 <sup>Ab</sup>	2.77 ± 0.10 <sup>Ba</sup>	4.12 ± 0.08 <sup>Cb</sup>	5.21 ± 0.07 <sup>Db</sup>
5		ND	0.51 ± 0.03 <sup>Ac</sup>	1.53 ± 0.04 <sup>Bc</sup>	2.82 ± 0.14 <sup>Cd</sup>	4.41 ± 0.08 <sup>Dc</sup>
10		ND	0.48 ± 0.08 <sup>Ac</sup>	1.31 ± 0.08 <sup>Bd</sup>	2.18 ± 0.06 <sup>Ce</sup>	3.56 ± 0.12 <sup>Dd</sup>
20		ND	0.06 ± 0.01 <sup>Af</sup>	0.40 ± 0.03 <sup>Bf</sup>	0.59 ± 0.07 <sup>Cf</sup>	3.07 ± 0.06 <sup>De</sup>
30		ND	ND	ND	ND	ND

<sup>A-D</sup>Numbers within each row followed by different capital letters are significantly different ( $P < 0.05$ ).

<sup>a-h</sup>Numbers within each column followed by different small letters are significantly different ( $P < 0.05$ ).

\*Normal condition (A); Optimal condition (B); ND: Not detected.

the corn samples stored at normal condition. A completely inactivated production of fumonisins B<sub>1</sub> and B<sub>2</sub> on corn was observed at the dose of 30 kGy at both storage conditions, with no cell growth and fumonisin production until 7 weeks of storage.

## Discussion

The effects of gamma irradiation on corn-based food and feed products have been reported by several investigators all around the world [4, 11, 28, 32]. In this study, the results showed that the population of *F. moniliforme* in treated corn samples decreased significantly with increasing doses of gamma irradiation as compared with control ( $p < 0.05$ ). Aziz *et al.* [3] found that gamma irradiation delays the mycelial growths of fungi and the total viable populations of fungi decreased significantly with increasing gamma irradiation doses. In the present study, *F. moniliforme* showed resistance up to 5 kGy dose of gamma irradiation. Rizk and Botros [33] reported that *Aspergillus candidus*, *Aspergillus granulosis*, and *Curvularia geniculata* are the most sensitive species in gamma irradiation of 1.5 kGy on food crops, whereas the most resistant species were *F. moniliforme*, *F. oxysporum*, and *F. solani*, which showed resistances at up to 6.5 kGy gamma irradiation.

*F. moniliforme* growth was not detected in corn samples with the dose of 10–20 kGy immediately after treatment. Similar results were reported in previous studies that showed elimination of *F. verticillioides* on corn [11], natural occurring *F. moniliforme* on cereal grains [4], total aerobic bacteria, yeast, and mold on soybean seeds [45] at 10 kGy treatment, and almost complete elimination ( $< 1 \log \text{CFU/g}$ ) of total aerobic bacteria on aniseeds (*Pimpinella anisum*) [1]. Moreover, low levels of radiation (up to 10 kGy) do not cause toxicological hazards and significant nutritional losses, and the losses caused by higher doses (above 10 kGy) are of minor importance or comparable to those observed during the heating process [14, 44]. However, higher storage humidity and longer storage period resulted in higher growth rates and infections of *F. moniliforme* on both treated and untreated corn samples ( $p < 0.05$ ), except the corn samples treated with the dose of 30 kGy. Therefore, gamma irradiation with the dose of 10–20 kGy was considered as the sublethal dose. A similar finding was reported by the World Health Organization [44], in the case of high-dose irradiation to decontaminate dry commodities with doses up to 30 kGy. Low numbers of irradiation-resistant microbial cells may survive at 30 kGy treatment, but these survivors cannot grow in the low-water

environments of dry spices or dried vegetables.

Different humidity rates between environment and corn samples might lead to moisture migration, which is water diffusing from the “wet” phase (incubator) to the “dry” phase (corn samples), leading to irreversible changes in the organoleptic and microbiological qualities of the samples, and reducing their shelf life [18, 34] as it causes the increase of water activity in the corn samples. Therefore, results from this study also confirm similar findings reported by Marin *et al.* [21, 22, 23], Velluti *et al.* [41], and Samapundo *et al.* [36], which reported that higher water activity results in higher growth rates and infections of *Fusarium* on corn grain samples.

The role of humidity in fumonisin production of the fungus was also clearly important. As the optimal storage was more suitable for growth, the fumonisin production increased more rapidly compared with those stored at normal conditions. The optimal storage condition became a significant factor for fumonisin production as well as longer storage time ( $p < 0.05$ ). Shelby *et al.* [37] reported the occurrence of high levels of fumonisin when the hot and dry weathers are followed by periods of high humidity. Hennigen *et al.* [15] also found high levels of fumonisin on corns to be associated with relatively high humidity rates in Argentina. Another result from Atukwase *et al.* [2] also confirmed that corns from high altitude zones (12–24°C, relative humidity of 86–90%) have significantly higher total fumonisin content (4.93 mg/kg) than corn from the mid altitude-moist (17–34°C, relative humidity of 68–75%) (4.53 mg/kg) and mid altitude-dry (17–34°C, relative humidity of 60–65%) zones (4.50 mg/kg) ( $p < 0.05$ ).

The success of decontamination methods will depend on many factors, including the moisture contents of the product, the degrees of contamination, and the distribution of toxin in the product [6]. Therefore, carefully selected gamma-irradiation methods can potentially result in lower fumonisin levels in corn or corn-based products if followed by controlling moisture or humidity under optimal condition of fungal growths during storage.

In this study, there was no fumonisin production in all corn samples immediately before storage. However, as storage time increased, more fumonisin was produced in all samples ( $p < 0.05$ ), but the fumonisin production was significantly lower as the dose of gamma irradiation increased ( $p < 0.05$ ). The results also showed that fumonisin production had no strict correlation with the growth of *F. moniliforme*. Although a higher cell number of *F. moniliforme* produced more fumonisin, cells in more prolonged stationary phase produced more fumonisin than cells in

early stationary phase with similar cell populations. These results showed that more fumonisin was produced owing to higher relative humidity and longer storage time in all samples, which was similar to the reports from Marin *et al.* [24] and Menniti *et al.* [25]. Generally, total fumonisins B<sub>1</sub> and B<sub>2</sub> produced after 7 weeks of incubation at optimal condition were significantly higher than those stored at normal conditions in all corn grain samples ( $P < 0.05$ ). It was also found that the levels of fumonisin B<sub>1</sub> (FB<sub>1</sub>) from both storage conditions were higher than FB<sub>2</sub>. Munkvold and Desjardins [29] found that FB<sub>1</sub>, FB<sub>2</sub>, and FB<sub>3</sub> are encountered predominantly in maize kernels, with FB<sub>1</sub> occurring at the highest level. FB<sub>1</sub> typically accounts for 70 to 80% of the total fumonisin produced, whereas FB<sub>2</sub> usually makes up 15 to 25% and FB<sub>3</sub> usually makes up from 3 to 8% when cultured on corn or rice or in liquid medium [7, 19, 20].

Elimination of the growth of toxigenic *Fusarium* species is very important to prevent the subsequent production of fumonisin and dry matter loss in foods used for human consumption [26]. The US FDA [12] reported that total fumonisin in corn and corn-based products for foods and animal feeds are 2.0–4.0 µg/g and 5.0–100 µg/g, respectively, whereas the maximum levels of fumonisin by EU are 1 µg/g for corn-based foods, and 4 µg/g for unprocessed corn grains [9]. The fumonisin levels on irradiated corn samples stored at normal condition in this study were lower than the levels allowed by the FDA and EU for foods and animal feeds, and fumonisin production in gamma-irradiated corn samples stored at optimal condition increased more rapidly and reached the unsafe levels of fumonisin for foods and animal feeds after 1 week and 7 weeks of culture period, respectively. Corn grains were gamma irradiated with doses up to 5–30 kGy followed by storage at normal condition; thus, they do not require testing for the presence of fumonisin and growth of *F. moniliforme*.

In conclusion, the fungal growth and the amount of fumonisin were decreased as the dose of gamma irradiation increased. Gamma irradiation with the dose of 1–5 kGy effectively inhibited the growth of *F. moniliforme* by 1–2 log reductions in corn grains. A sublethal effect of gamma irradiation was observed at 10–20 kGy doses after storage and a complete inactivation required 30 kGy. Fungal growth and fumonisin production increased with higher humidity and longer storage time in all corn samples. This study also demonstrated that there was no strict correlation between fungal growth and fumonisin production. Thus, gamma irradiation with the dose of  $\geq 5$  kGy followed by storage at proper condition could be good tools to reduce the fungal

population and enhance the shelf-life of corn in microbial safety perspectives. However, further studies related to the effects of these methods on sensory quality of irradiated corn grains are still necessary.

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