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Effect of Gamma Radiation on the Reduction of Aflatoxin in Red Pepper

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Abstract

Nowadays, the reduction of aflatoxin in food commodities using radiation treatment is getting attention. Mycotoxin contaminations in agricultural commodities have significant economic implications. Aflatoxin is a very serious food insecurity issue in developing countries because of climatic conditions, agricultural practices, and storage conditions, which are conducive to fungal proliferation and toxin production. In this study, High-performance liquid chromatography was used for the separation and determination of aflatoxins. The samples were randomly collected from different markets, placed into sealed plastic bags, and prepared for testing and investigation. The samples were exposed to a dose of 2, 4, and 6 kGy of radiation. The samples were irradiated using a Co-60 Gamma-cell research irradiator (GC-220) at a dose rate of 1.5 kGy/h. After irradiation, a promising result has been found that almost 100% reduction of pathogens in each case at each dose, and a 10.96%, 34.25%, and 34.65% aflatoxin reduction in 2, 4, and 6 kGy respectively. From this study, it is clearly shown that irradiation technology is one solution for the food insecurity seen across the globe, and should be recommended for use to stakeholders, policymakers, food storage facility providers, food packaging companies, food preservation facilities providers, warehouse providers, and food item exporters.

Keywords: aflatoxin, contamination, irradiation treatment, radiation dose, red pepper, food preservation

1. Introduction

The problem of mycotoxins is a very serious issue in developing countries because their climatic conditions, agricultural practices, and storage conditions are conducive to fungal proliferation and toxin production (Aziz & Moussa, 2004). Spices are exposed to a wide range of fungal contamination due to poor collection conditions, uncontrolled production processes, and extended drying times, so mycotoxin production can take place at any stage of spice. production from preharvest to storage (Alemayehu et al., 2019; Kerstin & Charity, 2011).

Red peppers are susceptible to fungal growth and very sensitive to aflatoxin contamination, depending on temperature, relative humidity, and drying and processing conditions (Iqbal et al., 2011). Previous research on the effect of irradiation on red pepper powder indicated that a radiation dose of (Alemayehu et al., 2019; Aziz & Moussa, 2004; Iqbal et al., 2011; Kerstin & Charity, 2011) kGy reduced the population of fungi effectively without affecting major quality factors (Lee et al., 2004; Rico et al., 2010).

Food and Agricultural Organization (FAO) estimated that as much as 25% of the world's agricultural commodities are contaminated with mycotoxins, leading to significant economic losses (Kabak et al., 2006). The prevention of aflatoxin contamination prior to harvest or during post-harvest and storage is not always possible, necessitating decontamination before the use of such materials for food and feed use (Bata & Lasztity, 1999; Kabak et al., 2006; Riley & Norred, 1999).

Mycotoxin contaminations in agricultural commodities have significant economic implications. In the case of mycotoxin contamination, losses from rejected shipments and lower prices for inferior quality can devastate developing country export markets (Mulugeta, 2017).

Recently, the problem was magnified as exported agricultural commodities and products failed to meet the minimum quality standards set globally. Some of the exported spices, mostly chili peppers, were shipped back home due to a higher rate of aflatoxin contaminations. Furthermore, locally consumed produces were also confirmed of having a contagion (Birhanu, 2018).

Hot pepper powder worth ten million USD has been returned to Ethiopia from European markets when it was found to have unsafe levels of Aflatoxins and Ochratoxins during testing at entrance laboratories in European countries. In 2017, Ethiopian hot pepper was banned in the UK last fiscal year until it could successfully pass quality control tests and the Ethiopian Embassy in London began working jointly with hot pepper importers to improve the product. Germany also blocked a large amount of hot pepper from entering their borders. Ethiopia harvests the 8^{th} most red pepper in the world (Tesfaye, 2018).

The aim of this present work was to establish the effectiveness of gamma radiation doses on total fungal load, Identification, and aflatoxin concentrations in dry hot peppers packed in polyethylene and the reduction of mold and aflatoxin following gamma radiation of 2, 4, 6 kGy.

The findings of this study which is the first kind in the country will be very helpful in minimizing aflatoxin contamination of pepper products. The study can be used to ensure the safety of pepper products for human consumption as well as to meet the export standard of such products. The data obtained from this study will serve as a basis for setting an aflatoxin reduction mechanism. Researchers and academicians in the field can also use the results of the study as a reference for further study. Generally, the result of this study will highly contribute to strengthening the development of food safety and food security in Ethiopia, and will highly help improve the export trade of food products.

The primary beneficiaries will be farmers who lost the value of their cereal during post-harvest due to food spoilage, the consumers who are the victim of health problems due to exposure to pathogens, and exporters of such cereals and ultimately the government which is economically beneficiary from the hard currency generated. Other beneficiaries will be researchers, technical assistants, extension agents, and food packaging companies.

2. Methods and Materials

2.1 Sampling and Sample Preparation Method

Three samples were collected from the market according to the level of quality of the peppers, we were looking for the poorest quality which arbitrary collected from different places in the market. Each sample representative contains 10 kg (Richard, 2000). Then the samples were let in the sun to dried suitable grinding in a mill to reduce particle size and thoroughly mixed using the electric mixer to homogenize. The three commuted samples were subsampled in to four parts and a total of twelve sample were packed in the polytan plastic bag.

From each three dried hot pepper samples one sample is taken from each and used as control, and the remaining nine sample which are three samples taken from each of the original sample packed in polyethylene bags of consisting 200 gm weight. Then the samples were irradiated at a dose level of 0 (control nonirradiated samples), 2, 4 and 6 kGy in a Cobalt-60 gamma irradiator MDS Nordiaon’s Gamma-cell 220 research irradiator (GC-220) at a dose rate of 1.5 kGy/h at the National Institute for the Control and Eradication of Tsetse and Trypanosomosis (Kality Addis Ababa, Ethiopia). Upon irradiation, the samples were transported to the Bless Agrifoods Laboratory Service PLC under refrigeration ($4^{o}C$), analyzed immediately with in few days.

2.2 Mycological Studies

There were about two plate for the total mold count and two other plates for aspergillus identification and one more plate for control of contamination. For total mold counting the spreading method was used, 10 gm of sample were taken from both control and irradiated samples and thoroughly homogenized with 90 ml buffer first dilution with saline water using a mixer, the second dilution with 10 ml saline water.

Labeling the plates with the same number which it had been given in the samples to identify from which samples they had taken. From the first dilute 1 ml solution was taken and directly poured on the plate and spread on it which contain the media. Similarly, for the second dilution, 1ml of sample was taken and diluted with 9 ml buffer and pour on the second plate and uniformly spread on it. The control plate for all twelve sample were unfolded. For aspergillus test the surface plate method used, there was one plate with media and the leave of peppers is dropped on the plate disk and all the plate put in incubators at $25^{0}C$ for 5 days.

2.3 Determination of Aflatoxins

Aflatoxins were extracted and cleaned up using the method and reference AOAC official method 2005 08 and LCTech sample preparation and analyses method. The principal derivatization of aflatoxins can increase detectability and/or selectivity of response for the High-Performance Liquid Chromatography (HPLC) detector by performing the derivatization photochemical. The derivative structures B2a and G2a are obtained providing the enhanced signals for the B1 and G1 aflatoxin without effect on the B2 and G2 aflatoxin.

20 gm of a sample was taken from 50gm of lab samples and 2 gm of sodium chloride was added. Then extract with 100 ml methanol/water 4/1 v. v) and 50 ml n-Hexane for 45 minutes on the shaker the solution was passed the extract through a filter paper using a vacuum pumper. Then the separatory panel was used to differentiate the organic and the Aquas layers. A 7 ml Aquas layer was taken and diluted with 43 ml of buffer solution the sample volume was filtered employing a syringe filter. 50 ml of diluted extract and passed through the immunoaffinity column. The column is then washed with 10ml of distilled water and residual water is removed and the column is eluted with at least two times of methanol: the first addition of methanol act on the get for 5 min to break the analyte- antibody bond.

The HPLC system was let using the given condition: mobile solvent Acetonitrile: method water (15,25,60); flowrate: 1.2 ml/min; column: stainless steel 5 µm reversed phase c18; and column over temperature: 39^{o}C. fluorescence detection with the excitation wavelength of 365, an emission wavelength of 440 nm gain of 10 and attenuation of 4. The derivation is achieved by hydroxylation from the water of the mobile phase under UV light at 265 nm wavelength so that aflatoxin B2a and G2a are formed and flourished well for easy detection, unlike the parent B1 and G1. A critical-based approach whereby a set of performance criteria is established with which the analytical method used should comply is appropriate before injection of the known standard solution of aflatoxins and sample to the HPLC.

3. Results and Discussion

Table 1. Responses of Total Mold Counts, Aspergillus Identified, and Total Aflatoxins in Three Hot Pepper Samples as Affected by Gamma Radiation

Dose in kGy	Total mold counts (CFU/g)	% Reduction Mold	Aspergillus Identified	Total aflatoxins (ppb)	Average aflatoxins (ppb)	% Reduction aflatoxins
0 (control)	$2.7x10^4$	-	ND	48.67	97.48	
0 (control)	$4.1x10^4$	-	ND	31.78		
0 (control)	$1.6x10^6$	-	80%	212.00		
2	$2.2x10^2$	99.20%	ND	46.29	86.79	10.96%
2	$3.0x10^3$	92.50%	ND	29.13		
2	$1.3x10^3$	99.90%	ND	184.96		
4	$1.1x10^2$	99.59%	ND	20.15	64.09	34.25%
4	$1.1x10^2$	99.73%	ND	20.30		
4	$1.1x10^2$	99.95%	ND	151.86		
6	ND	100%	ND	18.90	63.7	34.65%
6	ND	100%	ND	16.20		
6	ND	100%	ND	156.51		

ND: Not Detected

3.1 Total Mold Counts

The initial mold counts before irradiation were highest for the third control sample ($1.6x10^6$ CFU/g), followed by the second control sample ($4.1x10^4$ CFU/g) and the first control sample ($2.7x10^4$ CFU/g). After irradiation of the three samples at 2 kGy the mold counts significantly decreased by 99.20%, 92.50% and 99.90% for the first, second and third samples respectively. At 4 kGy radiation dose, the mold decreases further to 99.59%, 99.73% and 99.95% respectively.

The mold population decreased to nondetectable levels immediately after treatment at a radiation dose of 6 kGy in the three hot pepper samples. As expected, significant differences were observed in total mold counts between untreated hot peppers and those irradiated. These results indicated that the mold populations very sensitive to gamma radiation at 6 kGy.

Other researchers have reported reductions similar to ours in total mold counts by ionizing irradiation with gamma rays. Thus Iqbal et al. (2011) concluded that radiation at 4 kGy reduced total mold and Aspergillus counts by 99 and 97%, and Aziz and Mahrous (2004) reported that a dose rate of 4 to 6 kGy was required for complete elimination of fungi in crop seeds. Aziz et al. (2006) achieved significant reductions in total mold counts in grains irradiated at 2 to 4 kGy, and molds were eliminated at 6 kGy.

Similarly, Kumar et al. (2010) found that a gamma radiation dose of up to 10 kGy was sufficient for complete microbial control of commonly used herbal products. The behavior observed for total mold counts in our study was also in agreement with that reported for mold and aflatoxin reduction by gamma radiation of packed hot peppers.

3.2 Aspergillus Identification

In the three samples collected from the market, the Aspergillus parasiticus was detected in one sample which covered 80% of the content of the sample. Aspergillus is a high load of aflatoxin-producing species and is a major concern because the toxin can be produced in favorable packaging materials and storage conditions of hot peppers (Kumar et al., 2010). The Aspergillus percentage in irradiated samples immediately after treatment dropped to a non-detectable level. Consistent with the observed effect on total mold counts immediately after treatment at a radiation dose of 2, 4 and 6 kGy doses. It shows significant differences in Aspergillus detection between untreated hot peppers and those irradiated.

3.3 Total Aflatoxins

Table 2. Aflatoxin Reduction of AG1, AG2, AFB1 and AFB2

Dose (kGy)	AG2	% Deviation of AG2	AG1	% Deviation of AG1	AB2	% Deviation of AB2	AB1	% Deviation of AB1	Total aflatoxin
0	0.605726	-	30.53	--	1.72432	-	15.81	-	48.67
0		-		--		-		-	
0	35.94786	-	9.23	-	166.8233	--	0	-	212.007
2									
2	0.368579		10.48		1.12		17.16		29.13
2	15.6646	56.42	10.18	-10.30	159.12	4.61	0	-	184.97
4	0.27572	54.48	5.72	81.24	0.82	52.52	13.336	15.64	20.15
4	0.340007		7.16		0.98		11.826		20.30
4	13.8945636	61.35	4.40	52.31	133.57	19.93	0	-	151.87
6	0.5200104	14.15	9.68	68.30	0.47	72.65	8.246	47.88	18.91
6	0.192861		6.23		0.48		9.296		16.20
6	15.7517436	56.18	3.83	58.52	137.14	17.79	0	-	156.72

On average, the initial aflatoxin concentration in control samples was 97.48 ppb, while aflatoxins in irradiated samples immediately after treatment decreased to 86.53 ppb (2 kGy), 64.09 ppb (4 kGy), and 63.7 ppb (6 kGy), respectively, showing an aflatoxin decline of 34.65% at the highest radiation dose of 6 kGy (as shown in Table 1). This decline in aflatoxin levels was not statistically significant, indicating that this mycotoxin is resistant to radiation doses up to 6 kGy in whole dried peppers.

Gamma radiation has been studied for its efficiency in destroying mycotoxins in agricultural produce and processed foods. Jalili et al. (2010) observed that the reductions in AFB1, AFB2, AFG1, and AFG2 were 43, 24, 40, and 36%, respectively, in black pepper irradiated at 60 kGy.

Hot peppers, used as natural flavoring and coloring agents, are usually irradiated in the prepacked form to prevent recontamination and to extend shelf life. Gamma radiation proved to be effective in reducing total mold and Aspergillus percentage in the sample of whole dried hot peppers packed in polyethylene bags.

From our results, it can be concluded that radiation at 4 kGy reduced total mold by 99.75%, and Aspergillus is not detected at this dose level, while radiation doses at 6 kGy eliminated both fungal groups. In contrast, irradiated samples of hot peppers showed a 34.65% decline in aflatoxin levels compared with the levels in nonirradiated samples.

The distinct effectiveness of gamma radiation for molds can be explained by the target theory of food irradiation, which states that the likelihood of a microorganism being inactivated by gamma rays increases as its size increases.

4. Conclusion

Food security is achieved when the food pillars: food availability, food access, food utilization, and food stability are at levels that allow all people at all times to have physical and economic access to affordable, safe, and nutritious food to meet the requirement for an active and healthy life. When one of these four pillars weakens, then a society undermines its food security. Factors related to food insecurity and malnutrition not only influence human health and welfare, but also affect social, economic, and political aspects of society.

In this study, the treatment of pathogens and aflatoxin contaminations in red pepper products was investigated using irradiation technology. From this study, it is clearly shown that irradiation technology is the best solution for the food insecurity seen across the globe, and should be recommended for use to stakeholders, policymakers, food storage facility providers, food packaging companies, food preservation facilities providers, warehouse providers, and food item exporters.

The study can be used to ensure the safety of red pepper products for human consumption as well as to meet the export standard of such products. The data obtained from this study will serve as a basis for setting an aflatoxin reduction mechanism since it is the first in its kind in Ethiopia. Researchers and academicians in the field can also use the results of the study as a reference for further investigation.

Generally, the result of this study will highly contribute to strengthening the development of food safety and food security in Ethiopia by enhancing food preservation, and will highly help improve the export trade of food products as a whole.

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