DEGRADATION OF AFLATOXIN IN MAIZE USING FERULIC ACID (PHYDROXY-3-METHYL CINNAMIC ACID) CATALYZED BY HYDROGEN PEROXIDE

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Degradation of aflatoxin in maize using Ferulic acid (phydroxy-3-methyl cinnamic acid) catalyzed by Hydrogen peroxide

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Abstract

Purpose: The study aimed to determine the rate of degradation of aflatoxin in contaminated maize using ferulic acid catalyzed by hydrogen peroxide

Methodology: 100 g of dried maize grain was grounded using a laboratory hammer mill and divided into 2 portions of 50 g each. 20 g sample was taken per portion and treated with 100 mL solution of methanol and deionized water in the ration of 8:1, 50 mL of Acetonitrile, 1 g NaCl and 4 g of anhydrous magnesium sulphate, then blended at 120 RPM for 30 min. Aflatoxin content in each extract was analysed using enzyme-linked immunoassay test kits and confirmed using high performance liquid chromatography (HPLC) coupled with fluorescence detector. Further experiments tested the effect of coating, size, concentration, catalyst and reaction time on degradation of aflatoxin in maize. Data analysis was conducted using SPSS and Microsoft excel.

Findings: Four-hour treatment of contaminated maize with 0.5 mM ferulic acid reduced aflatoxin by 91.0% for whole maize, 90.5% for dehulled maize and 90.9% for ground maize. Addition of 20 mL of 0.5% hydrogen peroxide to the reaction mixtures increased degradation of aflatoxin load to 99.0% for whole maize, 99.1% for dehulled maize and 99.1% for ground maize within 4-hour reaction time. The rate of decontamination followed first order kinetics with R^2 values of 0.919, 0.916 and 0.930 for the whole maize, dehulled maize, ground maize, respectively and achieved degradation half-lives of 43.59, 41.26 and 39.84 minutes in the same order.

Unique contribution to theory, practice and policy: Ferulic acid combined with hydrogen peroxide is an effective degrader of aflatoxin in maize. The rate degradation is dependent on the nature of maize pre-treatment, the concentration of ferulic acid, and the catalyst. Ferulic acid and hydrogen peroxide reacted with the lactone ring of the coumarin moity of aflatoxin. **Recommendations;** Further studies on degradation of aflatoxin in maize should elucidate the

pathways and metabolites formed in the ferulic acid degradation process and determine their toxicities.

Key words: *Aflatoxin, rate, decontamination, maize, ferulic acid.*

1.0 INTRODUCTION

Maize is a global food crop and an important staple food for majority of people in sub-Saharan African countries. There is high significance attached to maize as food for human consumption, raw material for livestock feed and as an industrial raw material for bio-ethanol fuel production (Omotayo *et al.,* 2019). Maize grain is rich in starch and therefore prone to fungal attacks (Zhang *et al.,* 2012) leading to production of a myriad of mycotoxins that are known to cause serious health risks to humans and animals, particularly in developing countries (Munkvold *et al.,* 2019). Aflatoxins are among the most common mycotoxins of concern in maize, due to their high toxicity to humans (Gruber-Dorninger, Jenkins & Schatzmayr, 2019). Mycotoxins are secondary metabolites produced primarily by Aspergillus flavus and A. parasiticus, found in soil and air (Abbas *et al*., 2009). Aspergillus genera grow to produce the aflatoxins, at temperatures between 22 and 35 ℃, and water activity (aW) in the range of 0.95 - 0.98 (Reiter *et al.,* 2009). The most frequently occurring aflatoxin in food and feed varieties like maize, peanuts, dried fruits, spices and tree nuts are B1, B2, G1 and G2. These toxins resist most conditions during maize growth, harvesting, processing and storage (Cheli *et al*., 2017). In almost all agricultural produce, the toxins accumulate to dangerously high levels under suitable abiotic, biotic and environmental conditions (Peng, Marchal & van der Poel., 2018). This pose a serious problem in many tropical and subtropical countries, especially in the developing regions.

From 1960s, different scientists proposed ways to prevent and reduce fungal growth in cereals and other food commodities (Agriopoulou, Stamatelopoulou & Varzakas, 2020). Towards this effort, global organizations have instituted guidelines and codes of practice fsuch as Good Agricultural Practices (GAP) and Good Storage and Manufacturing Practices (GSP and GMP), targeting to control field and storage contamination (Coloma *et al.,* 2019; Mahuku *et al.,* 2019). However, challenges are still encountered in most developing countries due to favourable climatic conditions for fungal growth, poor storage conditions and high levels of poverty among the farmers. Whereas the codes and guidelines are easily adopted by financially endowed farmers, the small- scale farmers in developing countries, who are the major maize producers are still grappling with adopting the guidelines and codes of practice. Cconsequences significant economic losses caused by mycotoxin related factors are still experienced by many small-scale maize farmers. According to FAO, (2015) and David & Gray, (2012), up to 25% of global agricultural markets and food supplies are lost to mycotoxins contamination annually. The associated monetary loss due to mycotoxin contamination is approximately 1 billion dollars globally (Mamo *et al.,* 2020). In addition, aflatoxins health effects are of global concern owing to associated acute and chronic toxicity.

Benkerroum (2020) reported high levels of aflatoxin contamination on maize, groundnuts and sorghum from China, Thailand, Vietnam, Indonesia, Ghana, Gambia, Malawi, Nigeria, Uganda, Ethiopia, Sudan, Kenya, and Tanzania. The report linked the high levels of contamination to

climatic conditions, agricultural practices, cultivars grown, mechanical and pest damages on the cereals. The countries are in the tropical and sub-tropical regions, where aflatoxin prevalence rate in foods and feeds is higher than other global regions. The other contributing factor was associated with low knowledge levels at the grass-root, particularly relating to harmful effects of food-borne toxins (Cotty & Garcia., 2007; Wu *et al*., 2011). Studies carried out in Kenya since 1960s have reported high aflatoxins contamination levels, some exceeded 5,000 ng/g, which are more than 500 times the WHO limits for safe human consumption that is pegged at 10 ng/g total aflatoxin (Leslie *et al*., 2008). The infested maize exposes human directly and indirectly to mycotoxins (Mutegi *et al.*, 2018). Kenya lost millions of dollars due to aflatoxicosis outbreak, creating barriers for agricultural trade on maize from some regions (Leslie *et al.,* 2008). Due to high risks associated with aflatoxin and associated diseases, the World Health Organization (WHO) has been at the forefront in raising awareness to control these mycotoxins (Mutegi *et al*., 2018).

1.1 Statement of the Problem

Aflatoxins are associated with adverse health effects to human and livestock and cause serious economic losses to farmers. This has led to increased research activities and policies geared towards alleviating the impact of mycotoxin infestations in grain yields in the field and during storage. However, most of the farmers in developing countries still experience high incidences of aflatoxin contamination due to poor storage conditions and lack of knowledge on good agricultural practices. In addition, most aflatoxin decontamination technologies employ extreme temperatures and pressure conditions that cannot be easily adapted by small scale farmers. Furthermore, the intermediate metabolites generated during decontamination processes have not been adequately characterised and their safety is not well known. This study aimed at understanding the effect different concentrations of ferulic acid catalysed by hydrogen peroxide on degradation of aflatoxin in maize.

1.2 LITERATURE REVIEW

Different studies focusing on biological, physical and chemical methods to detoxify and decontaminate mycotoxins contaminated foods and feeds have been done (Karlovsky *et al.,* 2016). The target of most studies has been to break down the toxic mycotoxin molecules into safer metabolites. The main limitation in most of the studies has been the extreme conditions of pressure and temperature, reduced sensory, nutritional or functional properties of the food materials, and toxic residues in the products (Hojnik *et al.,* 2017). Aflatoxin molecule contains four chemically active sites that leads to its toxicity when ingested- the oxygen- carbon bond of the lactone in coumarin ring, the double furan ring, the methoxy connection and the cyclopentanone bridge (Figure1). Hence treatment aflatoxins in foods and feeds requires innovative, efficient, cost-effective and environment-friendly technologies. Many of these targets to dislocate the structure of the terminal furan ring which is directly involved in the toxicity or the coumarin group as well as to disrupt or remove lactone that leads to drastic reduction or loss of toxicity (Benkerroum, 2020; Kim *et al.,*2017). These rings opened under extreme pH values, and modified by

decarboxylation reaction (Bond *et al.,* 2016). The lactone can also be disrupted by different enzymes such as laccases, peroxidases, reductases, and oxidases (Kim *et al.,* 2017).
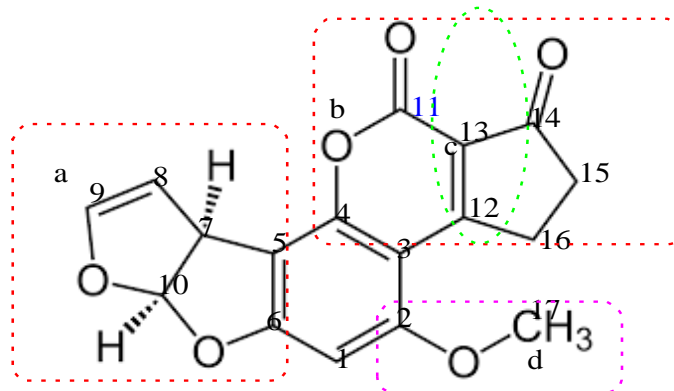

Figure 1. **Active parts of aflatoxin molecule**

Key; a – Furan ring, b- lactone ring, c- cyclopentanone bridge and d- Methyoxyl link.

Ozone is highly oxidative, in acidic environments, it reacts with the furan ring's double bond on carbon number 8 and 9 of the aflatoxin molecules. The mycotoxin is inactivated and its action limited in all aflatoxin molecules B1 and G1 (Agriopoulou *et al.,*2016). Reaction initiates ozonation and rearrangement of the formed molecules into less toxic aldehydes, ketones and organic acids derivatives (Mallakian *et al.,* 2017). Experiments done using ozone gas on different farm produce including maize reduced the aflatoxin load by more than 99% of the initial level of contamination (Udomkun *et al*., 2017). The treated products retained their nutritional value and chemical composition with no safety concerns (Chen *et al.,* 2014; Luo *et al.,* 2014). When ozone was used in storage facilities, it reduced mould counts and aflatoxigenic species A. flavus and A. parasiticus significantly (Porto *et al.,* 2019).

Ammonia reacted with the lactone oxygen in the lactone ring to decreased aflatoxin toxicity by inactivating the lactone of the coumarin ring. Ammonia, however does not attack the furan ring double bond thus making it suitable to a wider range of aflatoxin strains with a high efficiency. The reaction processes are decarboxylation and hydrolysis which convert aflatoxin molecule to non-toxic products (Rushing & Selim., 2019; Porto *et al.,* 2019; Chen *et al.,* 2014; Luo *et al.,* 2014). Ammonia decontaminate aflatoxin contaminated feeds with more than 99% efficacy at high pressure and temperature conditions and moisture range between 13 to 16% for the feed being treated, with humidification on the feeds is done before treatment through drying. The nutritional content of the feeds is, however, decreased and also it increases the cost (Jard *et al.,* 2011).

The use of 0.0088M sodium hypochlorite under acidic conditions decomposes aflatoxin molecules completely in foods in two-hour time. A 0.5% hydrogen peroxide decomposes aflatoxin contamination completely at pH = 4 and when the concentration increased to 6% at pH=9.5. Sodium hydrogen sulfite at 0.5 – 1 M, deactivates aflatoxin molecules through formation of

aflatoxin sodium sulphate by breaking the C15-C16 bond. For this reason, sodium hydrogen sulfite is an industrial food additive.

Data on the conditions required for decontamination of aflatoxin contaminated maize using ferulic acid catalysed by hydrogen peroxide are not available. Hence this study aimed at evaluating the effect of concentration of ferulic acid on rate of degradation of aflatoxin in naturally contaminated maize. The effect of dehulling and grinding of maize on degradation of aflatoxin was also investigated.

2.0 METHODOLOGY

2.1 Preparation of Decontamination media

0.1 M solution of ferulic acid was prepared by dissolving 200 g of the acid in 1000 mL dimethyl formamide (DMF). The solution was serially diluted from 1.0 M to 0.0005 M (1.0, 0.5, 0. 05, 0.005, 0.0005) with DMF. In addition, 20 mL of 0.05M of hydrogen peroxide was prepared to catalyze the reaction.

2.2 Decontamination Experiments

Naturally aflatoxin contaminated maize samples were collected from Makueni county and analysed to determine the total aflatoxin concentration before treatment. The most affected maize grains were processed into three sets consisting of whole maize, dehulled maize and ground maize flour. For each set, 5 subsets of 50 grams each were drawn in triplicates and placed into different 250 mL conical flasks. The flasks were treated with 100 mL five different levels of ferulic acid at concentrations of 1, 0.5, 0.05, 0.005 and 0.0005 M, heated to 80 ºC for x minutes and cooled to 25 ºC for analysis. The experiment was repeated with 20 mL of hydrogen peroxide catalyst and ferulic acid added into into each of the flasks. At regular intervals aliquot samples were drawn and homogenized for extraction of aflatoxin. 5 g of the homogenate was placed into a tube containing acetonitrile/methanol in the ratio of 4:6 v/v and centrifuged at 3000 rpm for 5 min. 1 g of sodium chloride and 4 g of anhydrous magnesium sulfate were added to the mixture and shaken to induce phase separation and partitioning of aflatoxin. 1 mL of the supernatant layer was evaporated until near dry under nitrogen gas. The residue was reconstituted in 900 µl of 10% acetonitrile followed by 100 µl of trifluoroacetic acid and incubated at 50 °C for 15 min. The derivatized solution was centrifuged at 1000 rpm for 10 min and injected into HPLC-FLD for analysis.

2.3 Sample preparation for Aflatoxin enzyme-linked immunosorbent assay (ELISA) Kit techniques

Maize samples for ELISA analysis were prepared according to modified Joanna et al. (2001) methods. 2.00 g sample was weighed into a conical flask, 10 mL of extraction mixture of methanol and water (7:3) was added and homogenized for 10 minutes at room temperature. The resultant mixture was centrifuged for 10 minutes at 4000 rpm and allowed to evaporate. Using a micropipette, 100 µl of the supernatant was drawn and diluted with 600 µl of phosphate buffer saline at pH of 7.2. 50 µl aliquots of the resultant solution, 50 µl of the aflatoxin-peroxidase conjugate and 50 µl of the mouse antibody solution against aflatoxin were added to each well of polystyrene microtiter plates. Filtrate was passed through an immunoaffinity column (Afla B

G.1003, VICAM), the aflatoxin residues were eluted with 1.25 mL of methanol at room temperature and in the darkness. Test samples and blanks were incubated for 30 min following the same procedure. After the incubation, the plate was emptied and washed five times with 0.15 M phosphate buffer saline at pH of 7.2.

Tetra-methyl benzidine and urea peroxide of 50 µl each were added to the same wells and incubated again for 30 min in darkness. After the second incubation, 100 µl of reaction termination reagent was added and absorbance measured using the ELISA reader at 450 nm. Immunochemical techniques rely on the specificity of binding between antibodies and antigens. The high affinity and specificity of antibodies for antigens have been used in the development of the various immunochemical methods.

2.4 High Performance Liquid Chromatography (HPLC) system

The Shimadzu High Performance Liquid Chromatography (HPLC) system (Tokyo, Japan made) was used for separation, detection and quantification of all aflatoxin strains using a reverse-phase HPLC and fluorescence detector. The HPLC method included the use of an LC-20AT pump, a reagent organizer, a column oven- CT 10AS- VP thermo-controller, a RF–20A fluorescence detector and a Genesis RP C18 analytical column (250 x 4.6 mm x4 mm). The fluorescence detector was operated at 360 and 450 nm wavelengths for excitation and emission, respectively. UV–Visible spectra of aflatoxins stock solution was determined using UV–Visible spectrometer equipped with a standard cell of 10 mm path length.

2.5 Maize Sample Preparation

100 g of maize sample was weighed and grounded using a laboratory hammer mill. The ground sample was divided into 2 portions of 50 g each. 20 g was drawn from each sample and treated with 100 mL of methanol/deionized water in ratio of 8:2, 50 mL of Acetonitrile, 1 g NaCl and 4 g of anhydrous magnesium sulphate, and blended for 30 min at 120 RPM for extraction of aflatoxin. The extract was filtered through a glass fiber micro filter. 20 mL of the filtrate was diluted 4:1 with Phosphate Buffer Solution (PBS) and pH of 7. 2. The diluted solution was centrifuged at 3,400 RPM for 15 min, and filtered using a nylon membrane filter (pore size, 0.45µm). About 10 mL of the filtrate was applied onto the Immunoaffinity column and eluted with 2 mL of methanol at a flow rate of 1 mL/minute by gravity. The eluate was incubated at 50 °C and the solvent evaporated using Nitrogen and reconstituted with 200 µl trifluoroacetic acid and 800 µl methanol- water. 20 µl of the sample solution was injected into the HPLC for quantitative determination of Aflatoxin B1, B2, G1 and G2.

2.6 Sample analysis

Aflatoxin standards and samples were analysed using HPLC (Shimadzu, Kyoto, Japan) with fluorescent detection. The analytical column used was a Symmetry® C-18 3.9x150 mm with 5 µm particle size from Waters (Massachusetts) with a guard column Silfilter STD C-18 3.0 x10 mm. The fluorescence detection (FLD) was set at an excitation wavelength 360 nm and 440 nm for emission. The column was maintained at 40 °C temperature. Analysis was run at a flow rate of

1 ml/min by an isocratic mobile phase using a mixture of acetonitrile/methanol/water (15/30/70 v/v/v). An aliquot of a 10 µl sample extract was injected into the chromatographic system.

2.7 Calibration Curve

An external standard curve was constructed using aflatoxin standards to quantify the aflatoxins concentrations in all the samples. A stock solution was prepared containing 100 ng/mL, and serially diluted into seven different concentrations using methanol/water (4:6, v/v), according to AOAC reference method 994.08. The method was validated according to SANCO/12571/2013 which demonstrates the conformity of the analytical performances with criteria established in the European Commission (EC) regulation no. 178/2010 (EC, 2010), this provides guidelines for validation procedure for linearity, specificity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision. The linearity was tested by external standardization using matrix calibration curves constructed from AFB1, AFB2, AFG1 and AFG2, standard solutions of six different concentrations within the range of 5-100 ng/mL (1,5, 10, 30, 50, and 100 ng/g). Analytical curves were established by plotting the peak areas which were used as the analytical signal response (y) versus the concentration of the Aflatoxin (x). The specificity of the method was evaluated by comparing the retention times in the blank sample matrices and the samples were spiked with 100 ng/g of AFB1 to ensure there was no interference to the target analyte. The sensitivity of the method was considered according to the LOD and LOQ. The LOD was calculated as the lowest concentration of the aflatoxins giving a signal response 3 times greater than the average of the baseline noise obtained from 10 independent blank samples. The LOQ was defined as an aflatoxin signal response 10 times greater than the average of the baseline noise obtained from 10 independent blank samples. The accuracy was tested through recovery studies by spiked aflatoxin standard solution at 3 different concentration levels (equivalent to 20, 40, and 100 ng/g) into blank sample matrices. Six replicates of each concentration were prepared for each matrix. The level of precision, which is expressed as relative standard deviations (RSDs), was estimated by performing daily repeatability, expressed as the confidence interval of the mean value. In addition, 6 replicates of each concentration were prepared for each matrix and determined within 1 day and in 3 consecutive days.

2.8 Calculations

The peak area of the aflatoxin standards was plotted against concentration. From the plot curve the slope (S) was determined as well as the Y-intercept (a). The level of aflatoxins in the sample was calculated using the formula (Trucksess et al., 2009) shown:

Aflatoxin, $\mu g/kg = \{(L-a)/S) \times V/W\} \times F.$ (1)

Where L represent the test solution peak area, V the final volume (mL) of the injected test solution, F the dilution factor and W amount in grams of test sample passed through the immune-affinity column. The total aflatoxin was calculated as the sum of the aflatoxin G2, G1, B2, and B1 based on AOAC method 994.08.

2.9 Quality Control and Quality Assurance

The chemicals used in the study were purchased from Kobian scientific, the local affiliate for Sigma-Aldrich, Inc., USA and Science lab suppliers limited. Aflatoxin standard solution with purity of 98% was purchased from Sigma (Germany). Standard stock solutions were prepared in acetonitrile according to the modified AOAC method 994.08 (Lupo *et al.*, 2010). Chemicals Sodium Hydrogen Sulfite, Ferulic acid, Ammonium carbonate, Sodium carbonate and Sodium hypochlorite, ammonia solution, hydrogen peroxide, acetic acid and solvents methanol, methylamine, acetonitrile and deionized water used in the study were HPLC grade supplied by Kobian Scientific. The enzymes and aflatest immunoaffinity columns (IAC) and HPLC column (C18) were supplied by Chemo Equip.

2.10 Data Analysis

The data was analyzed using SPSS software IBM PASW Statistics 18, Microsoft excel, Variances (ANOVA) and Fisher's LSD test ($p<0.05$) to compare significant differences. The data was presented using mean and standard deviation, confidence, and bar graphs.

3.0 RESULTS AND DISCUSSION

These tests were carried out to investigate the rate of degration of aflatoxin contaminated maize with different concentrations of ferulic acid and hydrogen peroxide solution. Different samples of whole maize (WM), dehulled maize (DM) and ground maize (GM) were treated with 1, 0.5, 0.05, 0.005 and 0.0005 M ferulic acid. Concentration of aflatoxin was tested in intervals of 30 minutes for five hours. The reactions were carried out at 25 ºC temperature after cooling from 80 ºC. The test findings on the rate of decontamination of aflatoxin contaminated maize, instantaneous concentrations of aflatoxin, different concentration of ferulic acid and the nature of maize are in Table 1 below.

The results show a fast decrease in concentration of aflatoxin in maize for the five different concentrations of ferulic acid within the first two hours, followed by a slower rate thereafter. 1 M ferulic acid reduced the aflatoxin content in whole maize, dehulled maize and ground maize in by 29.99%, 68.99%, 76.0%, 90.0% and 94.0%: 47.0%, 54.9%, 79.0%, 87.7% and 98.3%, and 41.7%, 50.9%, 78.9%, 87.2% and 98.3 % after 1, 2, 3, 4- and 5-hours period respectively. Reduction effectiveness of 1 M ferulic acid recorded an average of 71.79 ± 25.5% for whole maize, 73.4 ± 21.8 % for dehulled maize and 71.4 ± 24.2 % for ground maize. Aflatoxin load reduction in the case of 1 M ferulic acid with 20 ml of hydrogen peroxide for whole maize, dehulled maize and ground maize was 60.00%, 73.00%, 87.00%, 99.1% and 99.1%, 61.8%, 76.3%. 87.5%, 99.2%, and 99.2%, and 62.6 %, 76.8%, 88.9%, 99.2% and 99.3% for 1, 2, 3, 4- and 5-hours period respectively. Therefore, the contamination reduction effectiveness of 1 M ferulic acid with 20 ml of 0.5% hydrogen peroxide on average was 83.6 ± 17.0 % for whole maize, 84.8 ± 16. 0 % for dehulled maize and 85.3 ± 15.7 % for ground maize.

Table 1: Instantaneous Concentrations of Aflatoxin in Maize After Degradation with Ferulic Acid and Hydrogen Peroxide.

Ferulic acid	Without 20ml of 20% Hydrogen peroxide														
	1 1M			0.5 M			0.05 M			0.005 M			0.0005M		
	WM	DM	GM	WM	DM	GM	WM	DM	GM	WM	DM	GM	WM	DM	GM
0	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45
30	148.84	121.25	119.09	142.88	119.27	119.49	121.05	119.67	117.19	127.01	124.63	115.52	134.55	123.63	119.67
60	138.92	105.18	115.7	136.93	99.29	118.3	111.13	98.43	114.43	103.19	98.03	103.67	128.99	96.65	101.58
90	89.3	89.9	97.5	81.36	92.28	95.45	75.41	83.35	81.76	71.44	90.29	90.39	97.24	94.26	93.57
120	61.52	89.5	97.44	60.53	82.56	98.15	54.57	80.77	79.06	58.54	88.55	78.63	73.43	93.07	78.37
150	53.58	53.58	48.22	44.65	41.67	37.13	38.7	51.6	37.57	36.12	35.72	37.25	43.66	81.36	37.49
180	47.63	41.73	41.93	31.75	38.7	34.73	23.81	33.14	31.16	19.85	25.8	25.2	39.29	39.29	33.7
210	33.74	28.78	28.38	29.97	26.79	24.01	20.84	18.06	18.26	17.86	20.04	18	36.71	22.05	24.26
240	19.85	24.49	24.41	13.89	20.24	22.07	7.94	11.71	15.68	5.95	10.72	10.52	17.86	18.87	18.06
270	11.91	11.11	8.93	4.96	10.12	9.78	2.98	9.37	9.37	2.38	8.33	9.84	2.18	9.35	9.83
300	11.91	2.58	3.37	4.96	2.78	3.18	2.98	3.18	2.98	2.38	3.18	2.98	2.18	3.37	3.41
	With 20ml of 20% Hydrogen peroxide														
0	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45	198.45
30	89.3	81.66	79.6	83.35	79.42	77.4	81.36	75.41	75.41	75.41	73.86	74.74	83.35	79.78	77.9
60	79.38	75.81	74.22	75.41	73.03	72.43	67.47	64.3	64.18	63.5	61.76	67.87	69.46	71.24	61.82
90	69.46	64.2	64	65.69	61.98	63.92	63.5	59.97	61.74	59.54	56.36	58.94	64.5	61.77	59.77
120	53.58	47.03	46.11	49.61	44.65	43.89	44.65	40.14	38.2	42.67	35.16	40.09	47.63	48.02	35.37
150	33.74	29.77	30.36	29.77	28.18	28.23	28.97	28.2	24.3	28.18	22.29	28.36	29.97	30.16	24.5
180	25.8	24.81	22.09	23.81	22.07	19.87	22.82	22.03	18.16	20.84	17.86	20.04	23.81	22.05	19.27
210	13.89	12.11	13.3	13.49	12.5	12.11	12.3	12.13	11.55	11.71	11.09	11.87	12.5	12.6	11.89
240	1.79	1.59	1.59	1.79	1.39	1.39	1.79	1.61	1.19	1.94	1.19	1.75	1.98	1.81	1.79
270	1.79	1.57	1.55	1.79	1.35	1.33	1.79	1.59	1.11	1.94	0.91	1.72	1.98	1.79	1.37
300	1.79	1.55	1.49	1.79	1.33	0.99	1.79	1.59	0.79	1.94	0.69	1.67	1.98	1.79	0.91

Key: WM = whole maize, DM = dehulled maize, GM = ground maize

At 0.5 M ferulic acid aflatoxin reduction was 31.0%, 69.5%, 84.0%, 93.0% and 97.5% for whole maize, 50.0%, 58.4%, 84.5%, 89.8% and 98.6% for dehulled maize and 40.4%, 50.4%, 82.5%, 88.9% and 98.4% for ground maize respectively. The results indicate aflatoxin reduction for whole, dehulled and ground maize using 0.5 M ferulic acid alone achieved 75.0 ± 26, 76.25 ± 20.98 %, and 72.14 ± 25.25 %, respectively. When the 0.5 M ferulic acid combine with 20 mL of

0.5 % hydrogen peroxide solution achieved aflatoxin of 62.0%, 75.0%, 88.0%, 99.1% and 99.1% for whole maize, 63.2%, 77.5%, 88.9%, 99.3% and 99.33% for dehulled maize and 63.5%, 77.9%, 90.0%, 99.3% and 99.5% for ground maize respectively. The results showed that 0.5 M ferulic acid combined with 20 mL of 0.5% hydrogen peroxide solution, reduced aflatoxin in whole, dehulled and ground maize by 84.64 ± 16.09 %, 85.64 ± 15.45%, and 86.04 ± 15.39 %, respectively.

0.05 M ferulic acid reduced aflatoxin content in whole, de-hulled and ground maize by 79.8 ± 22.4%, 77.1 ± 21.3% and 75.5 ± 23.5%, respectively. 0.05 M ferulic acid combined with 20 mL of 0.5% hydrogen peroxide solution reduced aflatoxins levels from whole, de-hulled and ground maize by 66.0%, 77.5%, 88.5%, 99.1% and 99.1% for whole maize, 67.6%, 79.8%, 88.9%, 99.1% and 99.2% for dehulled maize and 67.7%, 80.8%, 90.9%, 99.4% and 99.6% for ground maize. The mean percentage reduction for the process was 86.0 ± 14.3 % whole maize, 86.9 ± 13.5% dehulled maize and 87.7 ± 13.6% ground maize.

0.005 M ferulic acid achieved mean reduction of aflatoxin whole maize by 80.9 ± 21.5%, dehulled maize 77.2 ± 22.5 % and ground maize 77.7 ± 22.4%. Combination of 0.005 M ferulic acid with 20 ml of 0.5% hydrogen peroxide reduced by 68.0%, 78.5%, 89.5%, 99.0% and 99.0% for whole maize, 68.9%, 82.3%, 91.0%, 99.4% and 99.7% for dehulled maize and 65.8%, 79.8%, 89.9%, 99.1% and 99.2% for ground maize within 1, 2, 3, 4- and 5-hours, respectively. The percentage mean reduction of aflatoxin in whole maize, dehulled maize and ground maize was $86.8\% \pm 14.2$, 87.6 ± 41.2 %, and 88.8 ± 14.2 %, respectively

0.0005 M ferulic acid reduced aflatoxin content in the three-maize samples by 35.0%, 63.0%, 63.0%, 80.2% and 98.9 % for whole maize, 51.3%, 53.1%, 53.1%, 80.2% and 98.3% for dehulled maize, and 48.8%, 60.5%, 60.5%, 83.0% and 98.3% for ground maize after 1, 2, 3, 4- and 5-hours, respectively. The results showed that 0.0005 M ferulic acid effectively reduced aflatoxin contamination in whole maize by 68.0 ± 23.7%, dehulled maize by 67.2 ± 21.1 % and ground maize by 70.2 ± 20.0%, respectively. 20 mL of 0.5 % hydrogen peroxide combined with 0.0005 M ferulic acid reduced aflatoxin by 65.0%, 76.0%, 88.0%, 99.0% and 99.0 % for whole maize, 64.1%, 75.8%, 88.9%, 99.1% and 99.1% for dehulled maize, and 68.9%, 82.2%, 90.3%, 99.1% and 99.5% for ground maize, respectively. The mean percentage reduction was 85.4 ± 14.8% whole maize, 85.4 ± 15.3%, dehulled maize and 88.0 ± 12.9% ground maize Figure 2.
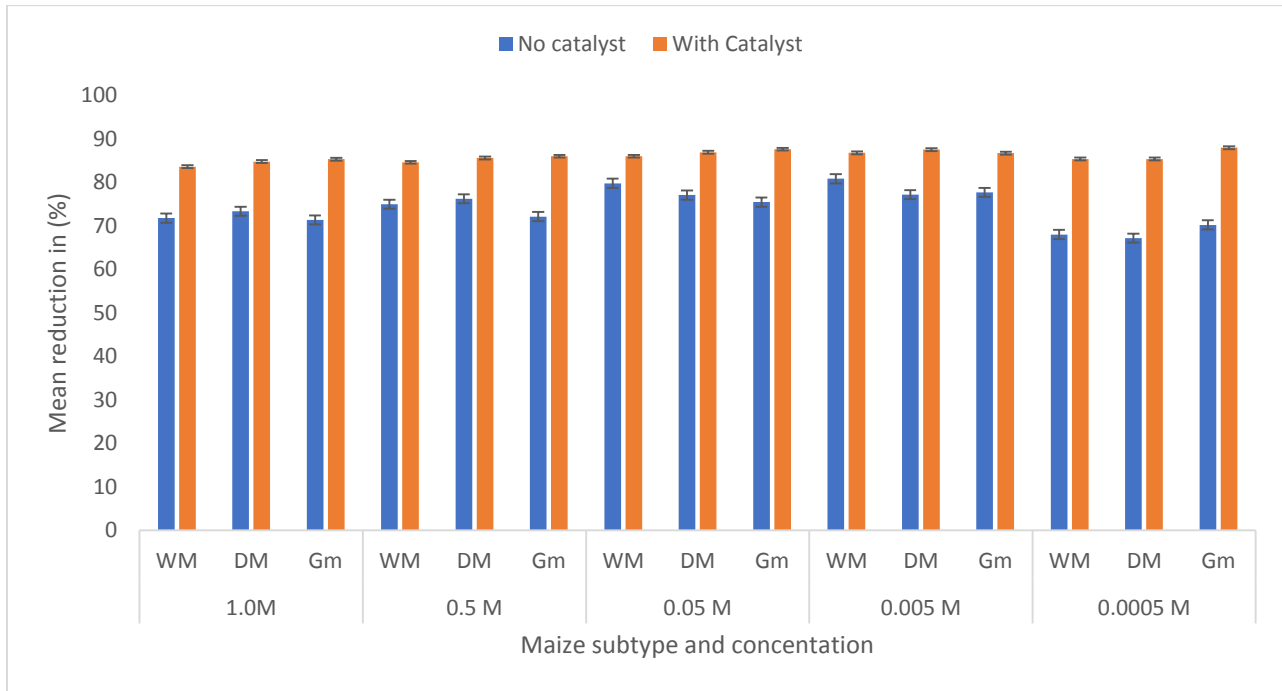
*Figure 2.* **Effect of catalyst on decontamination of contaminated maize.**

This implied that heating, dehulling and grounding of maize, decrease in concentration and use of catalyst had a directly effect on the rate of decontamination of contaminated maize. The effect of changing the concentration of ferulic acid on the rate of decontamination of aflatoxin contaminated in maize, exponentially decreased as shown in Figure 3 A, B, and C.
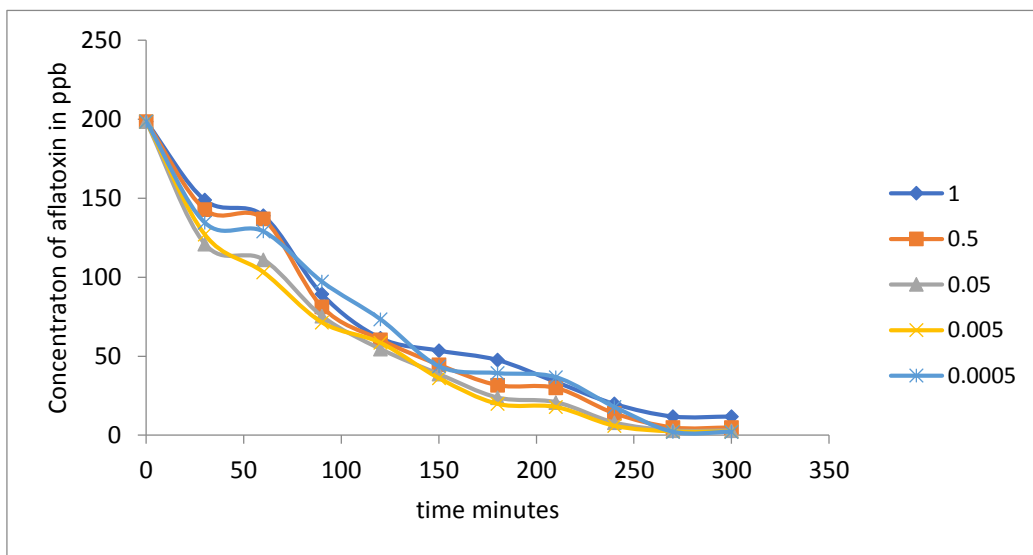
A whole maize
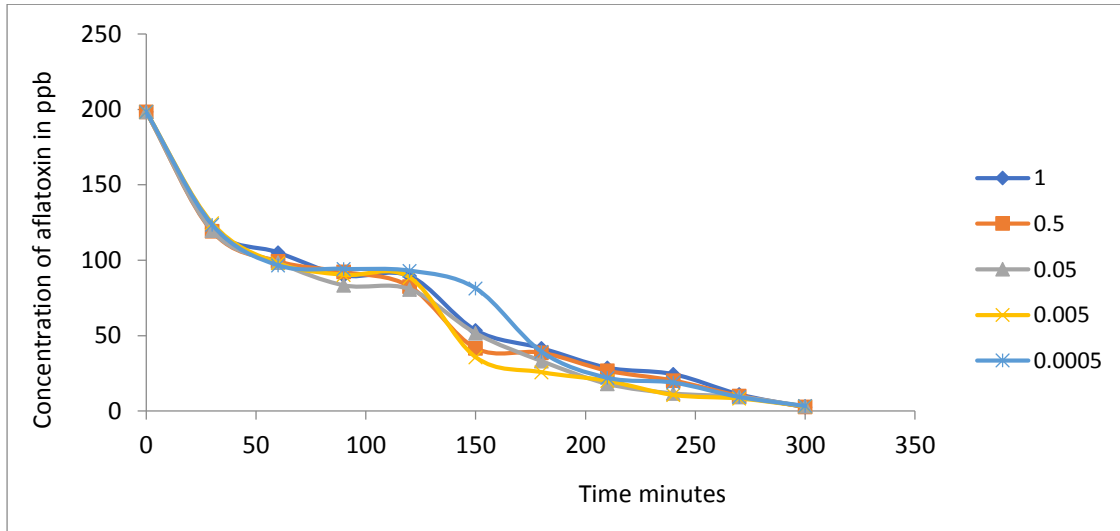
B. dehulled maize
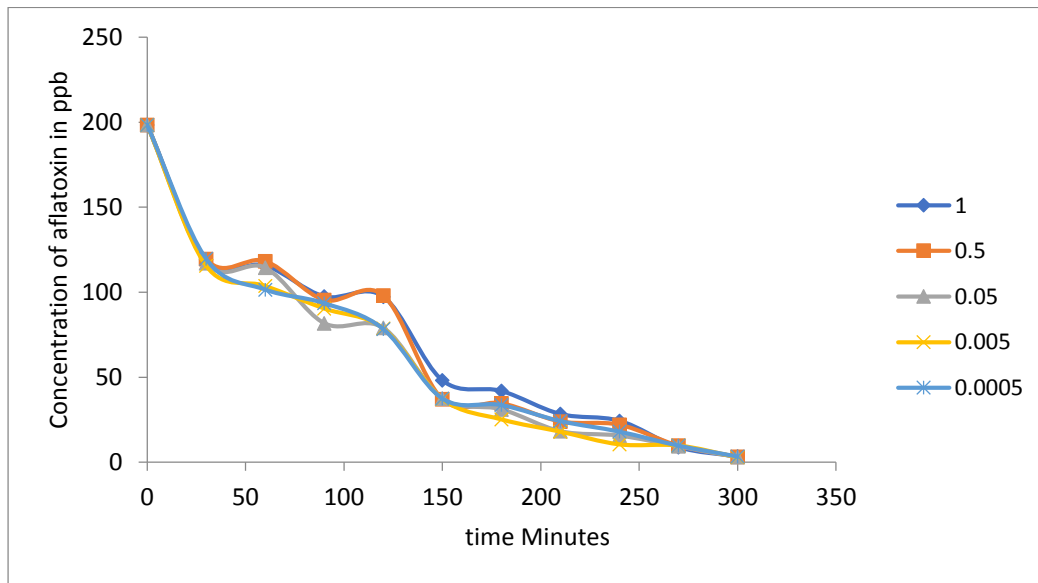
C. Ground maize

Figure 3 A, B and C. **Effect of concentration of ferulic acid with and without hydrogen peroxide on the rate of decontamination of aflatoxin contaminated maize.**

The decontamination results for ferulic acid fitted into a first order kinetic reaction. Ferulic acid reacted with the contaminating aflatoxin molecules to produce afla-ferulate products that were not detected as aflatoxin during the periodic tests for aflatoxin contamination levels.

Aflatoxin + $C_{10}H_{10}O_4$ $\longrightarrow$ Afla-Ferulates products
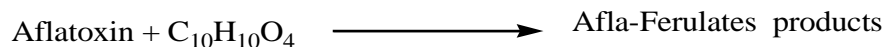
The tests were tried with different concentrations of ferulic acids and their results plotted on semi-log graphs. The linear regression equation y = mx + b, where y- was the natural logarithm of concentration (InCt) of aflatoxin in maize, m- was the gradient or slope (k) of the curve which the reciprocal of k. Decontamination process was represented by x- in valuation of time (t) and b- was the y-intercept value at the start of the decontamination process it was concentration of aflatoxin in the sample (InCo). The k-value in the study represented the rate constant for each decontamination reaction which varied with the concentration of ferulic acid, the nature of maize (whole, dehulled and ground). The magnitude of k, described the rate of decontamination, effect of concentration and the nature of maize at that time. The R^2 value was the coefficient or percentage of determination represented the fitting of experimental results to the regression curve. The decontamination of aflatoxin contaminated maize was effective with ferulic acid only ranged from 82.7 % to 97.5%, but when hydrogen peroxide was used the range increased to 90.49% to 92.95%. The influence of other factors that included variation in temperature accounted for 2.5 – 17.3% without a catalyst but 7.05- 9.51% with a catalyst. These factors were; the variation of ferulic acid concentration, strength of the catalyst, the nature of maize, the particle sizes, the particle orientation and reaction compatibility and the activation energy.

Half-life ($T_{1/2}$) was the time required to reduced half of the initial concentration aflatoxin contaminant in maize. Short time implied that the rate to convert the aflatoxin molecule from maize to afla-ferulate products was fast. The rate of decontamination with ferulic acid, was fast with 0.5 M concentration on whole maize at 45.3 and 36.87 minutes for hydrogen peroxide with 0.05 M concentration on ground maize. The slowest with 0.005 M concentration of ferulic acid alone on ground maize was 73.74 minutes but when the catalyst was used it improved 56.18 minutes on 0.5 M concentration ferulic acid on ground maize. This implied that the nature of maize had little effect on the decontamination process shown in table 2.

Table 2: **Summary of regression equation, slope, coefficient of determination, reaction half-life and y-intercept for each degradation reaction with different concentrations of ferulic acid and hydrogen peroxide.**

Dilution (M)	Maize	Relation Equation	Slope k	R²	Half-life T1/2	y-intercept
Without 20mL of 20% Hydrogen peroxide						
1	WM	y = -0.0098x + 5.3901	-102.04	0.971	70.73	5.39
	DM	y = -0.0125x + 5.5329	-80	0.953	55.45	5.533
	GM	y = -0.0143x + 5.5278	-69.93	0.956	48.47	5.528
0.5	WM	y = -0.0153x + 5.573	-65.36	0.958	45.3	5.573
	DM	y = -0.0144x + 5.7445	-69.44	0.855	48.14	5.744
	GM	y = -0.0104x + 5.233	-96.15	0.969	66.65	5.233
0.05	WM	-0.0121x + 5.3163	-82.64	0.946	57.28	5.316
	DM	y = -0.0118x + 5.2151	-84.75	0.975	58.74	5.215
	GM	y = -0.0134x + 5.3405	-74.63	0.97	51.73	5.34
0.005	WM	y = -0.0108x + 5.2351	-92.59	0.964	64.18	5.235
	DM	y = -0.0102x + 5.4992	-98.04	0.827	67.96	5.499
	GM	y = -0.0094x + 5.4161	-106.38	0.876	73.74	5.416
0.0005	WM	y = -0.0102x + 5.4483	-98.04	0.876	67.96	5.448
	DM	y = -0.0112x + 5.5058	-89.29	0.891	61.89	5.506
	GM	y = -0.0108x + 5.5231	-92.59	0.881	64.18	5.523
Without 20mL of 20% Hydrogen peroxide						
1	WM	y = -0.0165x + 5.526	-60.6061	0.9049	42.00892	5.526
	DM	y = -0.0169x + 5.4827	-59.1716	0.9084	41.01463	5.4827
	GM	y = -0.0169x + 5.4706	-59.1716	0.9084	41.01463	5.4706
0.5	WM	y = -0.0164x + 5.4598	-60.9756	0.911	42.26507	5.4598
	DM	y = -0.0173x + 5.4834	-57.8035	0.9066	40.06631	5.4834
	GM	y = -0.0178x + 5.5141	-38.9409	0.9136	56.17978	5.5141
0.05	WM	y = -0.0163x + 5.4031	-61.3497	0.9165	42.52437	5.4031
	DM	y = -0.0165x + 5.3737	-60.6061	0.9121	42.00892	5.3737
	GM	y = -0.0183x + 5.4904	-53.1915	0.9146	36.86953	5.4904
0.005	WM	y = -0.0158x + 5.3207	-63.2911	0.9237	43.87007	5.3207
	DM	y = -0.0163x + 5.3589	-61.3497	0.9222	42.52437	5.3589
	GM	y = -0.0187x + 5.4771	-53.4759	0.9169	37.06669	5.4771
0.0005	WM	y = -0.0159x + 5.4041	-62.8931	0.9185	43.59416	5.4041
	DM	y = -0.0163x + 5.4164	-59.5238	0.9155	41.25876	5.4164

	GM	y = -0.0174x + 5.4215	-57.4713	0.9295	39.83604	5.4215

Key: WM =whole maize, DM= dehulled maize, GM= ground maize

4.0 CONCLUSION AND RECOMMENDATION

4.1 Conclusion

This study showed that ferulic acid degraded aflatoxin in maize. Degradation rate depended on the nature of maize, concentration of ferulic acid, and the use of catalyst.

4.2. Recommendations

This study recommends more studies on the rate of decontamination of aflatoxin infested maize through the use of LC-MS to follow up the decontamination pathways. To determine utilize LC-MS chemical library to identify the actual reaction sites, the toxicity and the products of decontamination process.

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