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The Impacts of Mycotoxins on the Proximate Composition and Functional Properties of Grains

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Abstract

The research focused on the evaluation of the levels of some mycotoxins and their impacts on the proximate composition and functional properties of grains. Cowpea, sorghum, maize, groundnut, rice, millet, and acha were obtained from all the LGAs in Owerri and milled into flour. The proximate composition, functional properties, and mycotoxin levels in the grains were evaluated. The effects of the mycotoxins on the proximate composition and functional properties of the grains were also assessed. Aflatoxin was detected in all the grains, with values ranging from 0.12 to 58.65 µg/kg. The levels of Ochratoxin in the grain flours ranged from 0.09 to 54.41 µg/kg but was not detected in some rice samples. The Patulin concentration in the grains ranged from 0.11 to 13.19 µg/kg. Unlike total aflatoxin and ochratoxin, most patulin and penicillic acid levels in these grains were

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within WHO limits; although a few were beyond the limit for infant foods. Results of proximate composition of the grain samples showed that acha and millet had the highest (10.59 %) and lowest (7.30%) mean moisture contents, respectively. Rice had the highest mean carbohydrate content. Groundnut and Cowpea had a high contents of protein than other grains. Groundnut had the highest mean fat content of 41.84 %. The ash content of the grains ranged from 0.73 to 3.61%. Cowpea and groundnut had relatively highest crude fiber contents. The presence of the mycotoxins had significant impact on carbohydrates (93.8%), proteins (78.3%), and fat (94.9%) contents of the grains. Their presence had moderate impacts on crude fibre (47.2%), ash (54.9%), and functional properties including water absorption capacity (WAC) (47.0%), Bulk density (63.5%), Swelling index (24.0%), Emulsion capacity (25.2%), Foaming capacity (26.2%), oil absorption capacity (OAC) (33.6%). There was a negligible effect on the moisture content (16.3%) of the grains.

Key words: Mycotoxins; Total Aflatoxins; Total Ochratoxins; Patulin; Penicillic Acids; Proximate Compositions; Functional Properties

1. INTRODUCTION

Grains are hard, dry seeds, with or without fixed hulls or fruit layers, harvested for human or animal consumption (Babcock, 1976). Agronomists call the plants making such seeds "grain crops." The two key types of commercial grain crops are cereals, such as rice, wheat and rye, and legumes such as cowpeas and soybeans. Grains are naturally endowed with food nutrients, including antioxidants.

Mold is a fungus which develops in the form of multicellular filaments known as hyphae (Moore *et al.*, 2011). Molds are large and taxonomically various number of fungi where the development of hyphae leads to change in coloration and an unclear appearance, especially on food. The network of these tubular branched hyphae, known as a mycelium, is considered a single organism (Madigan and Martinko, 2005). Mycotoxins are toxic secondary metabolites produced by microorganisms of the fungus kingdom, commonly known as molds. The word 'mycotoxin' is commonly set aside for the toxic products produced by some fungi that readily inhabit crops (Turner *et al.,* 2009). A mold species can yield many different mycotoxins, and numerous species could produce similar mycotoxin (Robbins *et al.,* 2000).

Many fungi require oxygen and are seen almost in all places in little quantities owing to the tiny spore size. They ingest organic material anywhere temperature and humidity are appropriate to support their activity. Wherever environments are suitable, fungi form clusters, and mycotoxins are high. The main motive for the making of mycotoxins is unknown; they are not compulsory for the growth or the system development of the fungi (Fox and Howlett, 2008). The production of these toxins rest on the neighboring internal and external environments, and these materials differ significantly in their potent toxicity, reliant on the organism infested and its predisposition, metabolism, and defense machineries (Hussein and Brasel, 2001).

Aflatoxins are type of mycotoxin produced by *Aspergillus* species of fungi, such as *Aspergillus flavus* and *Aspergillus parasiticus* (Martins *et al.,* 2001). The word "aflatoxin" denotes to four different types of mycotoxins produced, which are B_1, B_2, G_1, and G_2 (Yin *et al.,* 2008). Aflatoxin B_1, the utmost toxic, is a strong cancer-causing agent and has been directly linked to liver cancer in numerous animal species (Martins *et al.,* 2001). Aflatoxins are largely connected with Agric produces produced in the tropics and subtropics, such as peanuts, spices, and maize, among others (*Jiang et al.,* 2008).

Ochratoxin is a type of mycotoxin which comes in three key secondary metabolite forms; A, B, and C. All are made by species of *Penicillium* and *Aspergillus*. The three forms vary given that Ochratoxin B is a non-chlorinated form of Ochratoxin A & that Ochratoxin C is an ethyl ester form of Ochratoxin A (*Bayman and Baker, 2006).* Ochratoxin A has been considered as a cancer-causing agent and a nephrotoxin and was linked to malignant tumors in the human urinary tract, although research in humans is restricted by perplexing factors (*Mateo et al.,* 2007).

Patulin is a mycotoxin produced by *Penicillium expansum*, *Penicillium*, *Aspergillus,* and *Paecilomyces* fungal species. *P. expansum* is mainly associated with a range of rotten fruits & vegetables, particularly, rotting apples & figs (Moss, 2008: Trucksess and Scott, 2008). Although patulin has not shown carcinogenicity, it has been told to impair the immune system in some animal species (Chinaza *et al*., 2019b; Moss, 2008). In 2004, the European Community set restrictions on the concentrations of patulin in food products. They presently stand at 50 µg/kg in all fruit juice concentrates, at 25 µg/kg in solid apple foods used for straight consumption, and at 10 µg/kg for children's apple foods, including apple juice (Moss, 2008: Trucksess and Scott, 2008).

Penicillic acid is a mycotoxin with both antibiotic and carcinogenic activities made by many strains of *Penicillium* and *Aspergillus* species. It has been seen in tobacco, sausages, and corn. Penicillic acid is a known strain of *P. roqueforti.*

The functional properties of grains are determined by their physical, chemical, and organoleptic characteristics (Adeleke and Odedeji, 2010). Examples of functional properties in grains may include solubility, absorption, water retention, frothing ability, elasticity, and absorptive capacity for fats and foreign particles. While functional properties describe how ingredients behave during preparation and cooking, as well as how they affect the finished product in terms of appearance, tastes, feels, etc. (IFST, 2017), the nutritional properties of food define what a food is made of, as regards the composite nutrients, and its' impact on the body.

The potency, carcinogenicity, toxicity, and lethality of some mycotoxins, when ingested by animals and humans, demand that proper attention is given to evaluating the levels contained in various grains commonly consumed in Nigeria. It is recognized that mycotoxins constitute one of the main physiologically active components of food of plant origin, and some of them may have an effect on the nutritional and functional properties of foods of plant origin. The consumption of foods containing them can have serious consequences on the health of consumers. The toxic effect of mycotoxins has been observed in previous works. However, many have been victims of mycotoxins toxicity due to consumption of improperly prepared foods high in mycotoxins. Many of the mycotoxins found in grains have been implicated in various health problems, which call for a proper study to ascertain their levels as well as effects on the functional and nutritional properties of grains commonly consumed in Nigeria metropolises, especially Owerri, Imo State.

This research will give concise information on the effects of mycotoxins on nutritional and functional properties of grains sold in Owerri, as well as quantify the levels of mycotoxins present in these grains, and their proximate and functional properties. Furthermore, information generated will be significant in advising food processors, consumers, vendors, and other food handlers on the level (quantity) of these mycotoxins in grains sold in Owerri, Imo State. The result of the study is useful to grain importers/exporters and policy formulators on designing the bench levels for monitoring purpose and thereby improve the acceptance of these grains in the international market.

2. MATERIALS AND METHODS

This chapter reports the materials and methods used for analyses and survey during the course of this research. The research analyses were done at NAFDAC Office, Independent Layout, Enugu; Root Crop Research Institute, Umudike, Umuahia, Abia State; and Food Science and Technology Food Chemistry Laboratory, Federal University of Technology Owerri, Imo State.

2.1. Sources of Raw Materials:

The raw materials for this study, cowpea, maize, acha, rice, groundnut, sorghum, and millet were obtained from markets in all the three Local Government Areas in Owerri (Owerri North, Owerri West, Owerri Municipal): Orie Uratta, Orie Umuorii, and Ekeobi markets (Owerri North): Nkwo Ukwo (Ihiagwa), Obinze, and Umuerim markets (Owerri West): Ekeonunwa, Ama-Hausa markets, and Relief, (Owerri Municipal).

2.2. Sample Selection and Preparation

Three sample representatives were randomly collected for each grain from different markets in every local government area in Owerri around November 2016 and July 2017. Samples were selected during harmattan and rainy seasons. The samples were examined physically and milled into flour of ≤118 µm particle size using Art's-Way's portable roller mill (PRM30: USA) and then labeled accordingly as directed by AOAC (2000).

2.3. Determination of mycotoxin levels

2.3.1. Determination of Penicillic Acid

The Ehnert *et al.* (1981) spectrometric method was used for the quantitative evaluation of Penicillic acid in foods. A 10g of the test food sample was extracted with 1:1 mixed solvent containing dichloromethane and methanol. Subsequently, the extracts were concentrated by evaporation and the Penicillic acid was separated by thin layer chromatography. The Penicillic acid was then treated using Diphenyl boric acid-2 amino ethyl ether to transform it, in situ, to a strongly fluorescent derivative with absorption max at 440 – 450nm, the luminous intensity was proportionate to the concentration of Penicillic acid. The absorption was measured in a spectrophotometer at 445 nm. The measurement was made against a standard and the formula below was used to calculate the quantity of Penicillic acid in the food sample.

$$PA\ (\mu g/kg) = \frac{1000}{w} \times \frac{au}{as} \times c \times D$$

Where; w = weight of sample analyzed, au = absorbance of the sample extracted, as = absorbance of standard penicillic acid, c = concentration of the standard, D = Dilution factor where applicable.

2.3.2. Quantitative Determination of Total Aflatoxin and Ochratoxin

For the evaluation of total aflatoxins and total ochratoxins, the ELISA (kit) method was used. The flour samples (10g each) were extracted with 100ml of

80 percent acetonitrile (in water) for total aflatoxins, whereas a mixture of dichloromethane and ethyl acetate (1:3) was used for extraction of total ochratoxins. In each case, the extract was diluted with phosphate Buffer solution (PBS), pH 7 – 7.5. The extract diluent was used for the analysis.

First, the kit reagents were allowed to equilibrate to room temperature before being used. The titer wells for use were first coated with antibody. Then 0.2ml of the extract diluent was put in marked antibody coated well, while the toxin standard was put in its marked coated well. They were allowed to stand for 30 minutes at room temperature and the wells washed three times with the phosphate buffer solution and then turned and tapped to dry. Following this, 0.1ml of the toxin conjugate was added to each of the wells and allowed to stand for 10mins. The reaction stop reagent was added and the absorbance was read at 450nm. The formula was used for calculation.

$$\text{AT or OT } (\mu g/kg) = \frac{1000}{w} \text{ x} \frac{au}{as} \text{ x c x} \frac{Vf}{Va} \text{ x D}$$

Vf= total volume of extract diluent, Va= volume of extract diluent analyzed. au, as, c and D are the same as in penicillic acid determination.

2.3.3. Patulin Determination

The 2000 method of AOAC was used as described here.

Extraction

Fifty grams of grain flour sample was added to three 50 ml rations of ethyl acetate in a 250 ml separator and extracts recovered. Extracts were dried (upper phase technique) for 30 minutes over anhydrous sodium sulfate, and emptied into 250 ml graduated beaker. Sodium sulfate was washed using two 25 ml rations of ethyl acetate and then added to extract. Steady evaporation to <25 ml on the steam bath in a gentle stream of nitrogen was done (did not evaporate to dryness). It was allowed to cool to room temperature; adjusted to 25 ml mark with ethyl acetate and diluted to 100 ml with benzene.

Thin-layer Chromatography (TLC)

Plates' preparation: 30 g Silica gel was weighed into a 300 ml glass stoppered flask; water was added to the flask. The solution was vigorously shaken and emptied into the applicator. Instantly, five plates of 20x20 cm with 0.25 mm thickness were coated. Plates were rested until gel was formed. Coated plates were dried at 110^{o}C for 1 hour and stored in dessicating cabinet.

The preliminary Thin Layer Chromatography – By use of a 10 µl syringe, two spots of 5 µl and one spot of 10 µl of the test solution from the above and 1, 3, 5, 7, & 10 µL of the working standard on imaginary line of 4 cm from the bottom edge of the plates, were spotted, which were followed by spotting of 5 µl of standard solution on the top of the 5 µl spot of the test solution.

Plate with toluene-ethyl acetate – 90 percent formic acid (5+4+1) was developed and contained in a V-shaped metal trough in the unlined equilibrated tank with the Silica gel facing highest tank volume. When the solvent front extended to 4 cm from the plate top, the plate was brought out and dried under air, in hood Spray plate with 5 percent MBTH solution until the layer appears wet, and then heated for 15 minutes in 130°C oven. The plate was inspected in reflected and transmitted long wave Ultra Violet radiation.

NB: Patulin appeared as a yellow spot in visible light when the concentration was higher than 0.05 µg.

NB: Patulin was also seen as yellow-brown fluorescent spot at Rf of 0.5 – 1 µL standard solution. TLC patterns were examined.

Calculation

$$\mu g\ Patulin = \frac{S \times Y \times V}{50 \times X}$$

Where, Y = Concentration of standard in µg/ml, X = µL test solution spotted offering fluorescent intensity equal to standard (S) Sprayed, S = uL standard equal to test solution, V = Volume of test solution.

TLC plate slowly turned to blue on standing in air for few hours unless covered by a second glass plate.

2.4. Proximate Analysis

2.4.1. Moisture Content Determination

As recommended by Association of Official Analytical Chemist (AOAC, 2005), 5g each of the flour samples were weighed up into a petri dish of a known weight and then dried up in the oven at 105.5 ± 1.5°C for about four (4) hours. The samples were allowed to cool in a desiccator and weighed. The percent moisture composition of the grains was calculated as follows

$$\%\ moisture\ content = \frac{change\ in\ weight}{Initial\ weight\ of\ sample\ before\ drying} \times 100$$

2.4.2. Total Ash Determination

Ash content was assessed using the method of AOAC, 2005. About 5g of each sample was weighed into crucibles in duplicate, and then the grain samples were incinerated in an MF-4A 673in^3 (11L) Muffle Furnace w/Digital Controller at temperature of 552^{o}C till a light gray ash was detected and a constant weight maintained. The grain samples were allowed to cool in desiccators to avoid moisture absorption and weighed to find percent ash content.

Percentage ash was calculated using the formula: $\frac{W2-W1}{W} \times 100$

Where, W= Dry weight of food sample, W_1= weight of crucible, W_2 = weight of crucible and ash

2.4.3. Crude Protein Determination

The protein content was analyzed using the micro-Kjeldahl analytical method as recommended by the AOAC (2005); wet digestion, distillation, and titration. The protein content was assessed by weighing 0.5g of each flour sample into a boiling tube containing about 25ml concentrated Sulfuric acid and one catalyst tablet containing 5g H_2SO_4, 0.15g titanium dioxide (TiO_2). The tubes were heated at a very low temperature for thorough digestion to take place. The digest was diluted with 10ml of 40 percent sodium hydroxide (NaOH), 100ml distilled water (H_2O), and 5ml sodium thiosulfate ($Na_2S_2O_3$). The anti-bumping agent was included, followed by diluting the sample with 10ml of Boric acid (H_3BO_3). The Ammonium, NH_4, content in the distillate was obtained by titrating with 0.1N standard Hydrochloric acid, HCl, using a 255ml burette. Preparation of a blank was made without the sample. The protein value was calculated using a conversion factor (6.25), and the result expressed as the amount of crude protein.

$$\% \text{ crude protein} = = \ \%N2\frac{100 \text{ x N } 14 \text{ x Vf T}}{W \text{ x } 1000 \text{ x Va}} \text{ x } 6.25$$

Where W = weight of sample analyzed, N = Concentration of H_2SO_4 titrant, V_f = Total volume of digest, V_a = Volume of digest distilled, T = titer value- blank

2.4.4. Fat Content determination

Fat content was obtained using the 2005 method of Association of Official Analytical Chemists. Ten grams (10g) each of the flour sample was weighed using a sensitive weighing balance and enveloped in a filter paper. It was then positioned in an extraction thimble, cleaned and dehydrated in a laboratory oven, and cooled in desiccators prior to weighing. Then, petroleum ether solvent (about 25ml) was poured into the flask and the crude fat extracted. Following extraction, the organic solvent was evaporated by dehydrating in the laboratory oven. The flask and its constituents were cooled

in desiccators and the weight noted. The percent fat content was obtained as follows:

$$\frac{\text{Weight of the extracted}}{\text{Weight of sample}} \times 100$$

2.4.5. Crude Fibre Determination

The 2005 experimental method of Association of Official Analytical Chemists (AOAC) was used to determine the fiber content by defatting (during fat analysis) five grams (5.0g) of each grain sample. The fat-free samples were boiled in 200ml 0f 1.25% H_2SO_4 solution under reflux for 30 minutes. Subsequently, the samples were washed using numerous portions of hot (boiling) water with a two-fold muslin cloth for trapping the sample particles. The washed samples were cautiously conveyed quantitatively back to the holding flask and about 200ml of 1.25 percent of sodium hydroxide, NaOH, solution was added to each of the samples in the flask. For a second time, the grain samples were boiled for about 30 minutes and washed as done earlier, with hot water, and then they were cautiously moved to a weighed porcelain crucibles of known weight, followed by drying in the oven at about 105.5^0C for approximately 3 hrs. Following cooling in desiccators, the grain samples were weighed again, W2, and then positioned in a muffle furnace and heated to the temperature of about 552^0C for approximately 2 hours till they turned to ash. Once again they were allowed to cool in desiccators and weighed thereafter. The percent crude fiber content of each grain sample was estimated gravimetrically as;

$$\% \text{ crude fibre } = \frac{W2 - W3}{W1} \text{ x } 100$$

Where, W1 = weight of sample, W2 = weight of crucible + sample after washing and drying in oven, W3 = weight of crucible + sample ash

2.4.6. Carbohydrate Determination

The carbohydrate content was obtained using the difference method as the nitrogen-free extract, NFE, a method distinctly described by AOAC (2005). The nitrogen-free extract was calculated as

$$\% \text{ NFE } = 100 - \% (a + b + c + d + e)$$

Where; a = protein, b = fat, c = fibre, d = ash, and e = moisture

2.5. Determination of Functional Properties

The methods described by Onwuka (2005) were used to evaluate the functional properties (except foaming capacity) of the grain samples. The functional properties determined include water and oil absorption capacities, bulk density, swelling index, foaming capacity, and emulsion capacity.

2.5.1. Water and Oil Absorption Capacity

A gram each of the grain samples was weighed into a clean graduated (calibrated) conical centrifuge tube and mixed exhaustively with about 10 ml distilled water/oil using a clean warring mixer for almost 30 seconds. The grain samples were kept standing for about 30 minutes at ambient temperature, afterward centrifuged for 30 minutes at about 5000 rpm. After centrifugation, the volume of the supernatants (free water or oil) were read straight from the graduated (calibrated) centrifuge tube. The absorbed oil/water was calculated in terms of weight in grams by multiplication with the density of oil, 0.894 gram per milliliter, and water, 1 gram per milliliter, for oil and water respectively. The water and oil absorption capacities were stated in milliliters of water/oil absorbed per gram of each flour sample.

Absorbed water, ml/g = total water – free water

2.5.2. Bulk Density

The gravimetric method of Onwuka (2005) was used. A weighed grain sample of 10 g was placed in a graduated 10 ml measuring cylinder. Then the end of the graduated cylinder was tapped repetitively on a fixed cushion on a laboratory workbench till a steady volume was obtained. The steady volume was noted. The bulk density was obtained as a ratio of the grain sample weight to the volume filled by the grain sample after tapping, and expressed in gram per ml.

Bulk density, g/ml = weight of sample (g) / volume of sample (ml)

2.5.3. Swelling Index

These swelling indices of the flour samples were evaluated as per the ratio of the enlarged volume to the ordinary volume per unit weight of the samples. A gram of the flour sample was poured into a very clean dry measuring cylinder. The volume filled by the flour sample was noted before about 5 ml of distilled water was weighed into the sample. The solution stood undisturbed for approximately an hour; the new volume was noted again. The swelling index of the flour sample was obtained by using the formula:

$$\text{Swelling index} = \frac{\text{volume occupied by sample after swelling}}{\text{volume occupied by sample before swelling}}$$

2.5.4. Emulsion Capacity

The analytical method described by Onwuka (2005) was used to determine the emulsion capacities of the flour samples. Two grams (2g) each of the flour samples were mixed with 25 ml of distilled water at ambient temperature for roughly 30 seconds using a Kenwood blender (BL 330 series). Following thorough dispersal, 25ml of vegetable oil was slowly but steadily added and mixed for additional 30 seconds. At this moment, 15 ml of the sample was

conveyed into a centrifuge and spun for about 5 minutes at 1600 rpm. The volume of the vegetable oil alienated from the flour sample after centrifuge was taken straight from the table. The emulsion capacity was calculated as the volume of the vegetable oil emulsified and retained per gram of oil emulsified and retained per gram of flour sample.

$$\text{Emulsion capacity} = \frac{X}{Y} \text{ x } 100$$

Where, X = height of emulsified layer, and Y= height of the whole solution in the centrifuge tube.

2.5.5. Foaming Capacity (FC)

The analytical method defined by Coffmann and Garcia (1977) was used to evaluate the FC of the flour sample. About 2g of the flour sample was mixed with approximately 100 ml distilled water using a Kenwood blender. The suspension was homogenized for 5 minutes using ace homogenizer (NSEIAM-6) at 1600 rpm. The blended mixture was transferred to 250 ml calibrated measuring cylinder. The volume was noted after 30 seconds. The foaming capacity was calculated as % increase in volume using:

$$\%\text{Foam capacity} = \frac{\text{volume after whipping} - \text{volume before whipping}}{\text{Volume before whipping}} \text{ X } 100$$

3. RESULTS AND DISCUSSIONS

The results and discussions of the survey of mycotoxins and their impacts on the proximate compositions and functional properties of the grain samples are reported in this chapter and a detailed discussion followed.

3.1. Mean Levels of Mycotoxins in the Grain Samples

The results of the mycotoxins (aflatoxins, ochratoxins, patulin, penicillic acid) are shown in Table 1. The levels of the mycotoxins ranged from 0.65 ± 0.39 to 31.35 ± 21.06, 0.41 ± 0.52 to 31.24 ± 17.54, 0.66 ± 0.47 to 11.28 ± 1.53, and 0.00 ± 0.00 to 5.43 ± 0.59 for total aflatoxins, ochratoxins, patulin, and penicillic acid, respectively.

Table 1: Mean values of Total Aflatoxin concentrations in the grain samples

Grains	Total Aflatoxin (µg/kg)	Total Ochratoxin (µg/kg)	Patulin (µg/kg)	Penicillic Acid (µg/kg)
Rice	$0.65^{e} \pm 0.39$	$0.41^{e} \pm 0.52$	$0.79^{e} \pm 0.22$	$0.00^{e} \pm 0.00$
Cowpea	$31.35^{a} \pm 21.06$	$4.22^{c} \pm 5.51$	$2.44^{c} \pm 0.58$	$1.18^{c} \pm 0.40$
Acha	$0.92^{e} \pm 0.72$	$1.63^{d} \pm 0.88$	$0.71^{e} \pm 0.19$	$0.59^{d} \pm 0.30$
Maize	$3.14^{d} \pm 3.01$	$7.91^{b} \pm 5.29$	$2.78^{b} \pm 0.89$	$1.18^{c} \pm 0.30$
Millet	$3.37^{d} \pm 3.07$	$1.90^{d} \pm 1.63$	$1.84^{d} \pm 0.59$	$1.50^{b} \pm 1.18$
Sorghum	$20.16^{c} \pm 18.09$	$1.97^{d} \pm 2.23$	$0.66^{e} \pm 0.47$	$0.62^{d} \pm 0.39$
Groundnut	$26.30^{b} \pm 11.47$	$31.24^{a} \pm 17.54$	$11.28^{a} \pm 1.53$	$5.43^{a} \pm 0.59$
LSD	2.18	1.04	0.33	0.27
Grand Mean	12.27 ± 16.47	7.04 ± 12.36	2.93 ± 3.60	1.50 ± 1.77

WHO/EU/FAO/CODEX Limits for total Aflatoxins = 4.00 µg/Kg (Ready-to-eat), and 10.00 – 15.00 µg/Kg (Unprocessed)
WHO/EU/FAO/CODEX Limits for total Ochratoxins = Tolerable Weekly Intake of 1.20 µg/Kg Body Weight; 5.00 µg/Kg (raw or unprocessed).
WHO/EU/FAO/CODEX Limits for Patulin = 10.00 µg/Kg in infant food; Daily Intake of 0.40 Body Weight; 50.00 µg/Kg in adult food
WHO/EU/FAO/CODEX Limits Penicillic acid = 20.00 µg/Kg
Means with different alphabets along the column differed significantly at $p<0.05$ *P= 0.05 *

Aflatoxins: Results in Table 1 showed that aflatoxin was present in the grains at different levels. The presence of aflatoxin in all the grains was not a surprise, as grains are known to contain aflatoxin in various amounts. There was significant difference ($p=0.05$) in the levels of Aflatoxins in the grain samples, as shown by ANOVA and LSD results. Aflatoxins are toxic cancer-causing agents that are made by certain molds, *Aspergillus flavus* and *Aspergillus parasiticus*, which propagate in soil, decaying vegetation, dry environs, hay, and grains (Fratamico *et al.*, 2008). The most frequently occurring limit for total aflatoxin in food is at 4 µg/kg for ready-to-eat foods, and 10 to 15 µg/kg for foods requiring further processing; a limit found in the harmonized regulations in the European Union (EU/USDA, 2010). The highest average Aflatoxin level was detected in cowpea flour, 58.65 ± 2.24 µg/kg, while the lowest was detected in acha, 0.12 ± 0.02 µg/kg. This shows that grains sold in Owerri markets are high in aflatoxins, and may expose consumers to risk of aflatoxicosis. Groundnut and sorghum also contain high levels of Aflatoxin – 26.30 ± 11.47 and 20.16 ± 18.09 respectively – while rice, millet, and maize contain relatively lower levels of aflatoxins.

Aflatoxins are secondary metabolites, difuranocoumarins, produced by *Aspergillus flavus* and *A. parasiticus,* commonly found in food and feeds causing various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world (Ashiq, 2015), including Imo State. Among the mycotoxins, aflatoxins have been implicated in human diseases including liver cancer, Reye's syndrome, Indian childhood cirrhosis, chronic gastritis, kwashiorkor and certain occupational respiratory diseases in various parts of the world, particularly in African and Asian countries (Ashiq, 2015). Aflatoxin B_1, the utmost toxic, is a strong cancer-causing agent and has been directly connected to many adverse health effects, such as liver cancer, in many human and animal species (Ashiq, 2015). In high dosage, aflatoxins can lead to serious illness and even death in both humans and animals. They can cause acute liver cirrhosis and are strongly linked to an increased risk of liver cancer. It is estimated that aflatoxins cause between 5% and 30% of all liver cancer in the world, with the highest incidence of 40% occurring in Africa (Partnership for Aflatoxins Control in Africa, 2018). Limited recent studies show an association between aflatoxins exposure and stunted growth in

children under five years old (Partnership for Aflatoxins Control in Africa, 2018).

Ochratoxin: The average level of total Ochratoxin in the grain flours – 7.04 ± 12.36 µg/kg – was beyond the WHO/EU limit of 5.0 µg/kg for unprocessed grains. When inadequately processed, consumption of these grains may expose consumers to urinary tract cancer, kidney damage, etc. There is a significant difference (p=0.05) in the levels of ochratoxins in the grain samples from A, B, and C. However, the toxin was not detected in some rice samples. Ochratoxins are group of mycotoxins made by some Aspergillus species (mainly *A. ochaceus*; also by 33% of *A. niger* industrial strains) and some *Penicillium* species, especially *P. verrucosum* and *P. carbonarius* (Fratamico *et al.*, 2008). Ochratoxin is a type of mycotoxin which comes in three main secondary metabolic forms; A, B, and C. They are made by *Penicillium* & *Aspergillus* species. The three vary as Ochratoxin B (OTB) is a nonchlorinated arrangement of Ochratoxin A (OTA), and Ochratoxin C (OTC) is ethyl ester arrangement of Ochratoxin A (Ashiq, 2015; Bayman and Baker, 2006; Jeswal and Kumar, 2015). Due to the toxicity of mycotoxins, their presence in foods is regulated. The European Food Safety Authority (EFSA) has, based on a request from the European Commission, adopted an updated scientific opinion relating to ochratoxin A in Food on 4 April 2006, bearing in mind some new scientific information and developed a tolerable weekly intake (TWI) of 120ng/kg (1.2 µg/kg) body weight (European Food Safety Authority, 2000). Ochratoxin is a probable human carcinogen which has been reported to cause urinary tract cancer and kidney damage in people from Eastern Europe. Exposure to ochratoxin seems to be the biggest hazard correlated to microscopic fungi for the European consumers of cereals (Ashiq, 2015; Richard, 2007).

Patulin: The concentration of Patulin in the grains ranged from 0.11 to 13.19 µg/kg (Refer to Appendix VII). These levels were within the WHO/EU limits of 50 µg/kg for adult foods, but some were above the WHO/EU limits of 10 µg/kg for infant foods. The highest and lowest average Patulin concentrations – 11.28 ± 1.53 and 0.66 ± 0.47 µg/kg – were seen in groundnut and sorghum flour, respectively. Groundnuts are known to contain high levels of mycotoxins. The WHO and the European Union recommended a concentration of not more than 50 µg/kg (micrograms per kilogram) of Patulin in adult foods & 10 µg/kg for infants and young children products (Moss, 2008). The Scientific Committee on Foods (SCF) validated in its summit on 8th March 2000 the interim maximum tolerable daily intake of 0.4 µg/kg body weight for Patulin (European Food Safety Authority, 2000). Patulin is damaged and destroyed by the rigours of fermentation. Even though Patulin has not been revealed to be a cancer-causing agent, it has been detailed to harm animals' immune system (Moss, 2008).

Penicillic acid: Penicillic acid was not detected in all the rice flour, as well as some millet flour (Table 1). Its presence in other grain samples ranged from 0.59 to 5.43 µg/kg. Groundnut flour had the highest – 5.43 ± 0.59 µg/kg – while acha flour has the lowest concentration of 0.59 ± 0.30 µg/kg. Penicillic acid and ochratoxin A are synergistic toxic fungal metabolites. The effect of Penicillic acid on the pancreatic enzyme carboxypeptidase A in vitro was decreased conversion of parent ochratoxin A to alpha-ochratoxin, a non-toxic metabolite (Parker *et al.,* 1982). In vivo, Penicillic acid inhibits mouse and chicken pancreatic carboxypeptidase A after multiple oral exposures. The mode of toxic interaction of the 2 mycotoxins may have been due to impaired detoxification of ochratoxin A through Penicillic acid depletion of carboxypeptidase A activity (Parker *et al.,* 1982).

3.2. Proximate Composition of Grain Samples

Results in Table 2 show the proximate composition of the grain samples. The order of increasing average carbohydrate content was groundnut, cowpea, sorghum, millet, maize, acha, and rice, respectively. Acha had the highest average moisture content, while millet had the lowest. The mean values of other proximate components are as shown in the Table 2.

Table 2: Mean Values of Proximate Composition of the Grain Samples

Grain	% Crude Protein	% Crude Fat	% Crude Fibre	% Ash	% Moisture	% Carbohydrate
Rice	$8.38^{de} \pm 0.69$	$0.83^{f} \pm 0.06$	$0.93^{f} \pm 0.05$	$0.86^{g} \pm 0.10$	$8.04^{d} \pm 0.42$	$80.95^{a} \pm 0.68$
Cowpea	$20.98^{b} \pm 1.22$	$2.12^{e} \pm 0.07$	$4.56^{a} \pm 0.29$	$3.41^{a} \pm 0.16$	$9.80^{b} \pm 0.66$	$59.14^{d} \pm 1.19$
Acha	$7.71^{de} \pm 0.49$	$0.99^{f} \pm 0.13$	$1.83^{e} \pm 0.12$	$1.75^{e} \pm 0.18$	$10.59^{a} \pm 0.93$	$77.12^{b} \pm 1.48$
Maize	$8.57^{de} \pm 0.31$	$4.28^{c} \pm 0.18$	$2.63^{c} \pm 0.32$	$1.40^{f} \pm 0.30$	$9.78^{b} \pm 0.62$	$73.34^{c} \pm 0.90$
Millet	$10.40^{c} \pm 0.37$	$5.41^{b} \pm 0.25$	$2.24^{d} \pm 0.12$	$1.95^{c} \pm 0.12$	$7.30^{e} \pm 0.80$	$72.70^{c} \pm 0.78$
Sorghum	$9.52^{cd} \pm 0.47$	$3.31^{d} \pm 0.19$	$2.27^{d} \pm 0.08$	$1.78^{d} \pm 0.09$	$10.38^{a} \pm 0.56$	$72.58^{c} \pm 0.74$
Groundnut	$23.86^{a} \pm 1.11$	$41.84^{a} \pm 0.73$	$3.48^{b} \pm 0.29$	$3.03^{b} \pm 0.11$	$8.70^{c} \pm 0.50$	$19.09^{e} \pm 1.19$
LSD	1.54	0.38	0.27	0.02	0.33	2.06
Grand mean	12.83 ± 6.33	8.40 ± 13.86	2.56 ± 1.11	2.03 ± 0.85	9.23 ± 1.32	64.99 ± 19.93

The Standard Deviation (± SD) showed how spread out the values of proximate compositions of the samples are around the mean. *P = 0.05

The mean values of proximate composition of the grains are as shown in Table 2. There were significant differences in proximate composition (carbohydrates, protein, fat, fibre, ash, and moisture) of the grains (p = 0.05). These agreed with the work of Iwe *et al.* (2016) who reported significant differences in the proximate compositions of FARO 44 Rice, African yam bean, and brown cowpea seeds composite flour. However, there was no significant difference in carbohydrate contents of the grain samples from A, B, and C, except in millet and acha. The results of the ANOVA show that the source of variation in the functional properties was from either the proximate

composition or from the interaction between the proximate composition and the mycotoxin levels (p=0.05 in all cases). This implied that there were significant differences (p = 0.05) in the levels of the proximate composition of the grains.

Moisture: The difference in the moisture content of the grain samples from the three Local Government Areas was significant at p = 0.05. The moisture content of the grain samples ranged from 6.65 to 11.72%, with acha having the highest average moisture content, 11.69 ± 0.03 %, while millet had the lowest, 6.69 ± 0.04%. Acha from Owerri municipal and Millet from Owerri North had the highest (11.72%) and lowest (6.65%) moisture content, respectively. The relative low moisture content of the grains is an indication of storage and shelf stability. This result agrees with the values reported by Iwe *et al*. (2016) – 8 to 14.00% – for the Proximate, functional and pasting properties of FARO 44 rice, African yam bean and brown cowpea seeds composite flour, as well as that of Adebayo-Oyetoro *et al.* (2011) – 14% average – for proximate and functional properties of Ofada rice. The American Association of Cereal Chemists permitted methods for determining several properties of grain flour stipulate that the greater the moisture content, the lesser the quantity of dry solids in the grain flour (Hannington *et al.,* 2020; Chinaza, 2019). Flour stipulations generally limit the moisture content to 14 percent or less. Flours with percent moisture content beyond 14% are not stable at ambient temperature and as such microorganisms present in them start to grow, thereby generating off odours and flavours (Iwe *et al*., 2016; Hannington *et al.,* 2020).

Carbohydrates: There was significant difference (p = 0.05) in carbohydrates content of the millet and acha from different Local Government Areas. There was no significant difference (p >0.05) in carbohydrates content of other grains. It is evident from Table 2 that rice had the highest average carbohydrate content, 80.95 ± 0.68%, whereas groundnut had the lowest, 19.09 ± 1.19%. The Institute of Medicine recommends that adults get between 45 to 65% of dietary energy from whole-grains' carbohydrates (Food and Nutrition Board, 2002/2005). The order of increasing average carbohydrate contents was 19.09, 59.14, 72.58, 72.70, 73.34, 77.12, and 80.95% for groundnut, cowpea, sorghum, millet, maize, acha, and rice respectively. The values of carbohydrates obtained were similar to the values of 52.62 to 72.58% reported by Iwe *et al*. (2016).

Protein: There was significant difference (p = 0.05) in average protein content of the grains. Groundnut and Cowpea have considerable high content of protein than sorghum, rice, acha, millet, and maize. This considerable difference in protein content was expected, as legumes (groundnut, cowpea) were reported to have high protein content than their cereal counterparts (rice, acha, millet, sorghum, and maize). Legumes are rich sources of protein

(Udeogu and Awuchi, 2016). Groundnut had the highest average crude protein content, while acha had the lowest. The mean values of the proteins obtained, 7.71 to 23.86%, were similar to the average values of 12.86 to 28.13% reported by Iwe *et al.* (2016).

Fats: With the exception of groundnut, other grains had relatively low-fat content, ranging from 0.73% in rice to 5.73% in millet especially for samples obtained from Owerri west. Groundnut had a considerable high-fat content with the highest (42.68%) from Owerri North. There's significant difference ($p = 0.05$) in fat content of the grains. The low-fat content in rice, millet, cowpea, acha, sorghum, and maize may be due to the fact that cereals, some legumes, and tubers store energy in the form of starch rather than lipids (Iwe *et al.*, 2016). The little percent fat levels are advantageous as it guarantees longer shelf life for the grain products (Reebe *et al.*, 2000) because all fats and fat-containing foods contain some unsaturated fatty acids and hence are potentially susceptible to rancidity.

Ash: With the exception of groundnut, the difference in ash content of the grains from A, B, and C was significant ($p = 0.05$). The average ash content of the grains ranged from 0.73 ± 0.02 to 3.57 ± 0.06 %, with rice and cowpea having the lowest and highest average % ash content, respectively. The ash content of the grains gives an estimate of the total mineral content of the grains. It gives the total inorganic constituents after organic constituents and moisture have been converted to CO_2 and oxides of nitrogen. Minerals are essential elements (trace elements) needed in minute quantity by organisms (including human) to perform functions necessary for life (Bender *et al.*, 2009). They may serve as cofactors to enzymes for efficient and effective metabolic functions and are also parts of biomolecules such as hemoglobin, myoglobin, ATP, NADP, chlorophyll, nucleic acids, among others (Bender *et al.*, 2009; Hannington *et al.,* 2020).

Fibre: There was significant difference ($p = 0.05$) in average fibre content of the grains, except rice and maize. Cowpea and rice had the highest and lowest average fibre contents of 4.56 and 0.93% respectively. The order of increasing fibre content was rice, acha, millet, sorghum, maize, groundnut, and cowpea, respectively. The crude fibre content of the grains ranged from 0.93 to 4.96%. The relative high fibre content in cowpea and groundnut was not stunning. This may be ascribed to the high percent crude fibre content of the legumes. Crude fibre decelerates the release of glucose into the blood and decreases intercolonic pressure hence reducing the risk of colon cancer, diverticulosis (Gibney, 1989). Although fibre is indigestible, it plays an important role in maintaining the integrity of the gastrointestinal (GI) tract and overall health (Gibney, 1989). It prevents constipation and diverticular disease and enhances the management of body weight, blood glucose and

cholesterol levels. It reduces the risk of obesity, promotes intestinal transit, and reduces the risk of cardiovascular diseases (Gibney, 1989).

3.3. Functional Properties of the Samples

The results of the functional properties of the grain samples are shown in Table 3. Cowpea flour had the highest average foaming capacity, while millet flour had the lowest average foaming capacity. There was slight variation in bulk density. The mean values of Emulsion capacity, Oil Absorption Capacity, Swelling index, and Water Absorption Capacity can be seen in Table 3.

Table 3: Mean Values of the Functional Properties of Grain Samples

Grains	Oil Absorption Capacity	Water Absorption Capacity	Bulk Density	Swelling Index	Emulsion Capacity	Foaming Capacity
	ml/g	ml/g	g/ml		%	%
Rice	$1.27^{g} \pm 0.03$	$3.24^{a} \pm 0.06$	$0.71^{b} \pm 0.01$	$1.47^{a} \pm 0.06$	$46.76^{e} \pm 0.46$	$8.49^{d} \pm 0.06$
Cowpea	$2.20^{b} \pm 0.09$	$1.80^{f} \pm 0.10$	$0.71^{b} \pm 0.01$	$1.09^{g} \pm 0.05$	$66.15^{a} \pm 2.02$	$14.22^{a} \pm 0.09$
Acha	$1.78^{f} \pm 0.03$	$2.51^{d} \pm 0.10$	$0.76^{a} \pm 0.02$	$1.17^{e} \pm 0.04$	$45.77^{f} \pm 0.70$	$8.06^{e} \pm 0.05$
Maize	$2.10^{d} \pm 0.05$	$3.05^{c} \pm 0.04$	$0.63^{e} \pm 0.02$	$1.25^{d} \pm 0.04$	$47.86^{d} \pm 1.24$	$9.70^{b} \pm 0.14$
Millet	$1.87^{e} \pm 0.08$	$2.10^{e} \pm 0.06$	$0.67^{c} \pm 0.01$	$1.30^{c} \pm 0.05$	$58.57^{b} \pm 0.78$	$7.23^{g} \pm 0.11$
Sorghum	$2.15^{c} \pm 0.05$	$3.22^{b} \pm 0.06$	$0.66^{d} \pm 0.02$	$1.14^{f} \pm 0.10$	$43.64^{g} \pm 0.80$	$7.56^{f} \pm 0.05$
Groundnut	$2.22^{a} \pm 0.09$	$1.67^{g} \pm 0.08$	$0.56^{f} \pm 0.01$	$1.32^{b} \pm 0.03$	$53.58^{c} \pm 1.11$	$8.51^{c} \pm 0.16$
LSD	0.01	0.02	0.01	0.01	0.24	0.10
Grand Mean	1.94 ± 0.32	2.51 ± 0.63	0.67 ± 0.06	1.25 ± 0.13	51.76 ± 7.68	9.11 ± 2.23

The Standard Deviation (± SD) showed how spread out the values of functional properties of the samples are around the mean. *P = 0.05

The results of the functional properties of grains were presented in Table 3. There were significant differences (p=0.05) in the functional properties of the grain samples, as shown by ANOVA and LSD results.

Foaming capacity: There was significant difference (p = 0.05) in foaming capacity of the grain flours from the three Local Government Areas, with the exception of millet and cowpea. Foaming capacity of protein refers to the amount of interfacial area that can be created by the protein (Fennama, 1996). Cowpea flour had the highest average foaming capacity, with cowpea from Owerri West (14.26 ± 0.03 %) having the highest, while millet flour had the lowest average foaming capacity. Foam is a colloidal of many gas bubbles trapped in a liquid or solid; the small bubbles are surrounded by thin liquid films (Suresh *et al.,* 2014).

Bulk density: There was a slight variation in bulk density of the samples. Table 4 shows that there was significant difference (p = 0.05) in groundnut and cowpea from A, B, and C, while there was no significant difference (p >0.05) in bulk density of other grain samples. Groundnut had the lowest average bulk density, with the sample from Owerri North (0.55 ± 0.01 g/ml) having the lowest, while acha had the highest average bulk density. The

small variation in the bulk density might be as a result of the difference in starch content (Iwe *et al*., 2016). Iwe and Onuh (1992) and Iwe and Onadipe (2001) stated that higher starch content enlarged bulk density. Bulk density is similarly reliant on factors such as method of measurement, geometry, size, surface properties of the materials, etc., and could be enhanced when the elements are small, compactible, appropriately tapped and vibrated and with a suitable packaging material (Awuchi *et al.,* 2019a; Machuka *et al*., 2000). These may be the reason the average bulk density is relatively higher in acha and rice. Bulk density mirrors the relative capacity of packaging material required (Iwe *et al*., 2016). The greater the bulk density, the more compressed the packing material required. It specifies the porosity of a produce which impacts the package design and can be used in determining the kind of packing material required (Iwe & Onadipe, 2001).

Emulsion capacity: Protein being the surface active agents can form and stabilize the emulsion by creating electrostatic repulsion on oil droplet surface (Kaushal *et al.,* 2012). Results showed that the average values of emulsion capacity of the grains ranged from 43.06 ± 0.04% to 67.68 ± 0.15%. These values are similar to the values of 42.5 to 56.78% reported by Iwe *et al*. (2016). There was significant difference in emulsion capacity of the grain samples. Cowpea flour had the highest average emulsion capacity, while sorghum had the lowest. The high emulsion capacity of cowpea flour may be due to its relative high protein content Kaushal *et al.,* 2012).

Swelling index: There were slight variations in mean values of the swelling indices of the grain flours. The highest, 1.53 ± 0.04, was noticed in rice sample while the lowest, 1.05 ± 0.04, was observed in sorghum sample. There was no significant difference ($p > 0.05$) in mean values of the swelling indices of cowpea and groundnut from the three Local Government Areas, while mean values of other grain samples showed significant difference ($p = 0.05$). Swelling capacity is considered a quality benchmark in some good preparations as bakery products (Iwe *et al*., 2016). It is an indication of non-covalent bonding among molecules in starch granules and also an element of the ratio of α-amylose and amylopectin (Rašper, 1969).

Water Absorption Capacity: With the exception of millet samples, there was significant difference (variations) ($p = 0.05$) in the Water Absorption Capacity (WAC) of the grain samples. The WAC ranged from 1.58 to 3.29 ml/g. The lowest and highest values were observed in groundnut and rice samples, respectively. WAC is a vital functional property essential in food preparations especially those involving dough handling (Lorenz & Collins, 1980). The observed variation in WAC of the grain samples may be credited to diverse protein concentrations, their degree of interface with moisture and their conformational features (McWatters *et al*., 2003). This influence could be a result of the loose link of amylose & amylopectin in the starch granules &

fragile association forces sustaining the granular structure. Water absorption capacity is imperative in bulking and stability of products as well as in baking applications (Awuchi *et al.,* 2019a; Lorenz & Collins, 1980). Thus, the grain sample with the highest water absorption capacity is paramount in baking applications (Iwe *et al.,* 2016). The increase in WAC has always been associated with increase in the amylose leaching and solubility, and loss of crystalline starch structure (Awuchi *et al.,* 2019a; Hannington *et al*., 2020). Rice flour with the highest mean WAC of 3.24 ml/g may have more hydrophilic constituents such as polysaccharides. Proteins are both hydrophilic and hydrophobic, and therefore can interact with water in foods (Suresh *et al.,* 2014). The observed variation in different flours may be due to different protein concentrations, their degree of interaction with water and conformational characteristics (Butt and Batool, 2010).

Oil Absorption Capacity (OAC): Oil absorption capacity is an essential functional property that boosts the mouthfeel while maintaining the flavour of food products (Adebowale & Lawal, 2004). There was significant difference in OAC of the grain samples. Groundnut sample had the highest average OAC, 2.22 ± 0.09 ml/g, while rice sample had the lowest, 1.27 ± 0.03 ml/g. This shows that the level of fat might have influenced the OAC of the grain flours. The major proximate components affecting OAC are protein and fat. Protein is composed of both hydrophilic and hydrophobic parts. Non-polar amino acid side chains can form hydrophobic interaction with hydrocarbon chains of lipids (Jitngarmkusol *et al.,* 2008).

3.4. Impact of the Mycotoxins on the Nutritional (Proximate Composition) and Functional Properties of the Grains

Results in Table 4.6.1 and 4.6.2 show the effect of mycotoxins on nutritional (proximate composition) and functional properties of the grain samples.

Table 4: Correlation results of the mycotoxins and proximate composition

Model	% Crude Protein	% Crude Fat	% Crude Fibre	% Ash	% Moisture	% Carbohydrate
R	.885	.974	.687	.741	.403	.969
R^2	.783	.949	.472	.549	.163	.938

Table 5: Correlation of each mycotoxin and proximate composition

Model	R (Aflatoxin)	R Square (Aflatoxin)	R (Ochratoxin)	R Square (Ochratoxin)	R (Patulin)	R Square (Patulin)	R (Penicillic Aid)	R Square (Penicillic Acid)
% Carb	.963	.927	.925	.856	.940	.884	.938	.880
% Moisture	.426	.182	.410	.168	.398	.158	.403	.163
% Ash	.758	.575	.699	.489	.695	.483	.627	.393
% fibre	.701	.491	.702	.493	.707	.500	.686	.471
% fat	.932	.869	.971	.943	.924	.854	.875	.766
% protein	.877	.769	.897	.805	.891	.794	.819	.671

Carbohydrates: From Table 4, the R value indicates the degree of correlation between the mycotoxins and % carbohydrate content of the grains. An R value of 0.969 indicated a very high degree of correlation. The R square value

indicated how much of the total variation in the % carbohydrates could be explained by the mycotoxins. The production of mycotoxins rest on the neighboring internal and external environments, and these materials differ significantly in their potent toxicity, reliant on the organism infested and its predisposition, metabolism, and defense machineries (Hussein and Brasel, 2001).The R^2 value of 0.938 indicated that the effect of the mycotoxins on the % carbohydrate content of the grains was very high. Mycotoxins such as aflatoxins reduce the nutritional components of grains (PACA, 2019). In other words, 93.8% change in the carbohydrates content of the grains was caused by the mycotoxins' production, while the remaining 6.2% may be due to other factors. The Adjusted R square tells that actually, this explained 93.4% of the variance in the % Carbohydrate. The low P (Sig.) value which was 0.00 (ie <0.005) indicated that there was a significant variation in the % carbohydrates and also the mycotoxins were jointly significant in predicting the % carbohydrate. The symptoms of mycotoxicosis depend largely on the type of mycotoxin, the length of exposure and concentration, as well as health, age, and sex of the individual exposed (Bennett and Klich, 2003). This also meant that the regression model was significantly better in predicting % carbohydrate than using the average %carbohydrate as the best option. The coefficient tables defined the model in a linear equation. The equation for this regression model is % carbohydrate = 82.781 – 0.202 (Aflatoxin) + 0.291 (Ochratoxin) – 4.185 (Patulin) – 3.409 (Penicillic Acid). VIF means Variance Inflation Factor and is used to access multicollinearity problems. Normally, we do not want multicollinearity. A VIF value higher than 10 indicates a multicollinearity problem. <10 is acceptable VIF. All the values were < 10 and that indicated good linearity.

Moisture: From Table 4, the R value indicated the degree of correlation between the mycotoxins and % moisture content of the grains. Some of these mycotoxins in the internal environment are made by *Alternaria*, *Aspergillus*, *Penicillium*, and *Stachybotrys* (Fog, 2003). An R value of 0.403 indicated a low correlation compare to that of % carbohydrates. The R square value indicated how much of the total variation in the % moisture could be explained by the mycotoxins. The R^2 value of 0.163 indicated that the effect of the mycotoxins on the % moisture content of the grains was very low. The Adjusted R square tells us that actually, the mycotoxins explains 10.5% of the variance in the % moisture content of the grains. This shows that the presence of the mycotoxins had insignificant influence on the moisture content of the grains. Flour stipulations generally limit the moisture content to 14 percent or less (Iwe *et al*., 2016). The P (Sig.) value of 0.03 indicated that there was an insignificant variation in the % moisture and the mycotoxins were jointly insignificant in predicting the % moisture (p = 0.05). It is not a good fit for the data. Flours with percent moisture content beyond 14% are

not stable at ambient temperature and as such microorganisms present in them start to grow, thereby generating off odours and flavours (Iwe *et al*., 2016). The coefficient tables help us to describe this model in a linear equation. The equation for the regression model is % moisture = 9.334 – 0.026 (Aflatoxin) + 0.015 (Ochratoxin) – 0.016 (Patulin) – 0.326 (Penicillic Acid). VIF means Variance Inflation Factor. A VIF value higher than 10 indicates a multicollinearity problem. <10 is acceptable VIF. All the values were < 10 and that indicated good linearity. Molds require moisture for development and growth and some may live in aquatic environments (Wareing, 2013).

Ash: in Table 4, an R value of 0.741 indicated a slightly high correlation of % ash and mycotoxins. . Minerals are essential elements needed in minute quantity by organisms, including molds, to perform functions necessary for life (Bender *et al.*, 2009). The R^2 value indicated how much of the total variation in the % ash can be explained by the mycotoxins. The R^2 value of 0.549 indicated that the effect of the mycotoxins on the % moisture content of the grains was moderate. It showed that 54.9% variation in % ash was due to presence of mycotoxins, while 45.1 % may be due to other causes. The Adjusted R square showed that actually, the model explains 51.8% of the variance in the % ash content of the grains. This showed that the presence of the mycotoxins had significant influence on the % ash content of the grains. The low P (Sig.) value which was 0.00 (ie <0.005) indicated that there was a significant variation in the % ash and the mycotoxins were jointly significant in predicting the % ash. Mineral elements may serve as cofactors to enzymes for efficient and effective metabolic functions and are also parts of biomolecules such as hemoglobin, chlorophyll, nucleic acids, myoglobin, ATP, NADP, among others (Bender *et al*., 2009). This also means that the regression model was significantly better in predicting % ash than using the average % ash as the best option. i.e. it was a good fit for the data. The coefficient defined the model in a linear equation. The equation for this regression model is % ash = 1.379 – 0.027 (Aflatoxin) – 0.03 (Ochratoxin) + 0.075 (Patulin) + 0.21 (Penicillic Acid). All the VIF (Variance Inflation Factor) values were < 10 and that indicated good linearity.

Crude fibre: The enzymes secreted by molds degrade complex biopolymers such as cellulose and lignin into simpler substances which can be absorbed by the hyphae (Wareing, 2013). Results in Table 4 showed the R value was 0.687, which indicated a slightly high degree of correlation between the mycotoxins and % crude fibre content of the grains. The R^2 value of 0.472 indicated that the effect of the mycotoxins on the % crude fibre content of the grains was slightly low. In other words, 47.2% change in the % crude fibre content of the grains was caused by the mycotoxins' production. Although fibre is indigestible, it plays an important role in maintaining the integrity of the gastrointestinal (GI) tract and overall

health of organisms (Gibney, 1989). The Adjusted R square showed that actually, this model explains 43.5% of the variance in the % crude fibre. The low P (Sig.) value for crude fibre which was 0.00 (ie <0.005) indicated that there's a significant variation in the % crude fibre and the mycotoxins are jointly significant in predicting the % crude fibre. This also meant that the regression model was significantly good enough in predicting % crude fibre than using the average % crude fibre as the best option. In other words, it was a good fit for the data. Crude fibre decelerates the release of glucose into the blood and decreases intercolonic pressure hence reducing the risk of colon cancer, diverticulosis (Gibney, 1989). The coefficient defined the model in a linear equation. The equation for this regression model is % crude fibre = 1.811 + 0.038 (Aflatoxin) – 0.031 (Ochratoxin) + 0.049 (Patulin) + 0.24 (Penicillic Acid). All the VIF (Variance Inflation Factor) values were < 10 and that indicated good linearity.

Crude fat: From Table 4, the R value indicated the degree of correlation between the mycotoxins and % crude fat content of the grains. Molds can also grow on stored food for animals and humans, making the food unpalatable or toxic and are thus a major source of food losses and illness (Wareing, 2013). An R value of 0.974 indicated a very high degree of correlation. The R square value indicated how much of the total variation in the % crude fat could be explained by the mycotoxins. The R^2 value of 0.949 indicated that the effect of the mycotoxins on the % crude fat content of the grains was very high. In other words, 94.9% change in the crude fat content of the grains was caused by the presence of the mycotoxins, while the remaining 5.1% may be due to other factors. Molds play a major role in causing decomposition of organic material such as starch, fats, proteins, etc. enabling the nutrients recycling throughout ecosystems (Wareing, 2013). The Adjusted R square showed that actually, this model explained 94.6% of the variance in the % crude fat. This showed that the model was good and could explain the results. The low P (Sig.) value which was 0.00 (ie <0.005) indicated that there was a significant variation in the % crude fat and the mycotoxins were jointly significant in predicting this variation. This also meant that the regression model was significantly better in predicting % crude fat than using the average % crude fat as the best option. i.e. it was a good fit for the data. The coefficient Tables described the model in a linear equation. The equation for this regression model is % crude fat = -2.58 – 0.063 (Aflatoxin) – 0.032 (Ochratoxin) + 2.65 (Patulin) + 2.814 (Penicillic Acid). The enzymes secreted by molds degrade complex biopolymers such as starch, protiens, cellulose, fats, and lignin into simpler substances which can be absorbed by the hyphae (Wareing, 2013). Normally we do not want multicollinearity. A Variance Inflation Factor (VIF) value higher than 10

indicates a multicollinearity problem, and <10 is acceptable VIF. All the values were < 10, indicating good linearity.

Crude protein: From the results in Table 4, an R value of 0.885 indicated a very high degree of correlation. Most fungi are aerobic and are found nearly everywhere in extremely small quantities due to the tiny size of their spores; they ingest organic matter anywhere humidity and temperature are sufficient (*Fox and Howlett, 2008).* The R^2 value of 0.783 indicated that the effect of the mycotoxins on the % crude protein content of the grains was considerable. In other words, 78.3% change in the crude protein content of the grains was caused by the presence of the mycotoxins. Like other fungi, molds derive energy not by photosynthesis but from the organic material they live on, utilizing heterotrophy (Wareing, 2013). Typically, molds secrete hydrolytic enzymes, mainly from the hyphal tips. These enzymes degrade complex biopolymers such as starch, protein, cellulose and lignin into simpler substances which can be absorbed by the hyphae (Wareing, 2013). The Adjusted R square showed that actually, this model explained 76.8% of the variance in the % crude protein. This also showed that the model was good and could explain the results. The P (Sig.) value is 0.00 (ie <0.005) indicating that there was a significant variation in the % crude protein and the mycotoxins were jointly significant in predicting the % crude protein. This also meant that the regression model was significantly better in predicting % crude protein than using the average % crude protein as the best option. In other words, it is a good fit for the data. Many molds synthesize mycotoxins and siderophores which, together with lytic enzymes, inhibit the growth of competing microorganisms (Wareing, 2013). The coefficient decribed the model in a linear equation. The equation for this regression model is % crude protein = 7.296 + 0.189 (Aflatoxin) – 0.216 (Ochratoxin) + 1.399 (Patulin) + 0.466 (Penicillic Acid). All the VIF (Variance Inflation Factor) values are < 10 and that indicates good linearity.

3.4.1. Impact of aflatoxins on the nutritional (proximate) composition of the grain samples

Carbohydrates: Typically, molds secrete hydrolytic enzymes, mainly from the hyphal tips. These enzymes degrade complex biopolymers such as complex carbohydrates and lignin into simpler substances which can be absorbed by the hyphae (Wareing, 2013). From Table 5, the R value indicates the degree of correlation between the aflatoxins and % carbohydrate content of the grains. An R value of 0.963 indicated a very high degree of correlation. The R square value indicated how much of the total variation in the % carbohydrates could be explained by the aflatoxins. The R^2 value of 0.927 indicated that the effect of the aflatoxins on the % carbohydrate content of the grains was very high. In other words, 92.7% change in the carbohydrates content of the grains is

caused by the aflatoxins production, while the remaining 7.3% may be due to other factors. In Kenya, over a hundred and twenty-five individuals died, and almost two hundred others were treated after consuming aflatoxin-contaminated corn in 2004 (Lewis *et al.,* 2005).

Moisture: From Table 5, the R value indicated the degree of correlation between the aflatoxins and % moisture content of the grains. An R value of 0.426 indicated a low correlation compare to that of % carbohydrates. Molds need moisture for growth and development (Wareing, 2013). The R square value indicated how much of the total variation in the % moisture could be explained by the aflatoxins. The R^2 value of 0.182 indicated that the effect of the aflatoxins on the % moisture content of the grains was very low (18.2%). This shows that the presence of the aflatoxins had insignificant influence on the moisture content of the grains.

Ash: From Table 5, an R value of 0.758 indicated a slightly high correlation of % ash and aflatoxins. Aflatoxins are poisonous as well as cancer-causing chemicals that are made by certain molds growing in soil, decaying vegetation, hay, and grains (Fratamico *et al.,* 2008), and require mineral elements for their metabolism. The R^2 value indicated how much of the total variation in the % ash can be explained by the aflatoxins. The R^2 value of 0.575 indicated that the effect of the aflatoxins on the % moisture content of the grains was moderate. It showed that 57.5% variation in % ash was due to presence of aflatoxins, while 42.5 % may be due to other causes. This showed that the presence of the aflatoxins had significant influence on the % ash content of the grains. Production of aflatoxins by molds lead to loss of nutrients in grains (PACA, 2018). Also, animals served aflatoxins contaminated feed can pass its transformation products to eggs, milk produce, and meat (Fratamico *et al.,* 2008).

Crude fibre: Results in Table 5 showed the R value was 0.701, which indicated a slightly high degree of correlation between the aflatoxins and % crude fibre content of the grains. The enzymes secreted by molds degrade complex biopolymers such as cellulose and lignin into simpler substances which can be absorbed by the hyphae (Wareing, 2013). The R^2 value of 0.491 indicated that the effect of the aflatoxins on the % crude fibre content of the grains was slightly low. In other words, 49.1% change in the % crude fibre content of the grains was caused by the aflatoxins production

Crude fat: From Table 5, the R value indicated the degree of correlation between the aflatoxins and % crude fat content of the grains. An R value of 0.932 indicated a very high degree of correlation. Aflatoxins are amongst the most cancer-causing substances known (Hudler, 1998). After arriving in the body, aflatoxins can be metabolized by liver to a reactive intermediate or hydroxylated to turn into the less dangerous aflatoxin M_1. The R square value indicated how much of the total variation in

the % crude fat could be explained by the aflatoxins. The R^2 value of 0.869 indicated that the effect of the aflatoxins on the % crude fat content of the grains was very high. In other words, 86.9% change in the crude fibre content of the grains was caused by the presence of the aflatoxins, while the remaining 13.1% may be due to other factors.

Crude protein: Results in Table 5 shows an R value of 0.877 which indicated a very high degree of correlation. The R^2 value of 0.769 indicated that the effect of the aflatoxins on the % crude protein content of the grains was considerable. In other words, 76.9% change in the crude protein content of the grains was caused by the presence of the aflatoxins. Aflatoxins are most normally ingested, but the most toxic kind of aflatoxin, B_1, may infiltrate through the skin (Boonen *et al.,* 2012).

3.4.2. Influences of Ochratoxins on the nutritional compositions of the grain samples

Ash: From Table 5, an R value of 0.699 indicated a slightly high correlation of % ash and Ochratoxins. The R^2 value indicated how much of the total variation in the % ash can be explained by the Ochratoxins. In previous researches, ochratoxin A has been considered to be a carcinogen and nephrotoxin in human urinary tract tumors, although research in humans has been limited by inexplicable factors (*Mateo et al.,* 2007). The R^2 value of 0.489 indicated that the effect of the Ochratoxins on the % moisture content of the grains was moderate. It showed that 48.9% variation in % ash was due to presence of Ochratoxins, while 51.1 % may be due to other causes. This showed that the presence of the Ochratoxins had significant influence on the % ash content of the grains.

Carbohydrates: From Table 5, an R value of 0.925 indicated a high degree of correlation. The R square value indicated how much of the total variation in the % carbohydrates could be explained by the Ochratoxins. Exposure to ochratoxin seems to be the biggest hazard correlated to microscopic fungi for the European consumers of cereals (Ashiq, 2015; Richard, 2007). The R^2 value of 0.85 indicated that the effect of the Ochratoxins on the % carbohydrate content of the grains was very high. In other words, 85.60% change in the carbohydrates content of the grains is caused by the Ochratoxins production, while the remaining 14.4% may be due to other factors.

Crude fibre: Results in Table 5 showed the R value was 0.702, which indicated a slightly high degree of correlation between the Ochratoxins and % crude fibre content of the grains. The R^2 value of 0.493 indicated that the effect of the Ochratoxins on the % crude fibre content of the grains was slightly low. In other words, 49.3% change in the % crude fibre content of the grains was caused by the Ochratoxins production. Crude fibre decelerates the

release of glucose into the blood and decreases intercolonic pressure hence reducing the risk of colon cancer, diverticulosis (Gibney, 1989), and is an essential nutrient.

Crude protein: In commodities such as cereals, legumes, coffee, dried fruit, as well as wine, ochratoxin A is well known to be festering. Grains like legumes (cowpea, peanut) are rich source of proteins (Udeogu and Awuchi, 2016). Results in Table 5 showed an R value of 0.897 which indicated a very high degree of correlation. The R^2 value of 0.805 indicated that the effect of the Ochratoxins on the % crude protein content of the grains was considerable. About 80.5% change in the crude protein content of the grains was caused by the presence of the Ochratoxins. Molds degrade biopolymers like protein, starch, etc. (Wareing, 2013).

Crude fat: From Table 5, the R value indicated the degree of correlation between the Ochratoxins and % crude fat content of the grains. An R value of 0.971 indicated a very high degree of correlation. As biopolymers, fats can be degraded by fungal activities (Wareing, 2013). The R square value indicated how much of the total variation in the % crude fat could be explained by the Ochratoxins. The R^2 value of 0.943 indicated that the effect of the Ochratoxins on the % crude fat content of the grains was very high. In other words, 94.3% change in the crude fibre content of the grains was caused by the presence of the Ochratoxins, while the remaining 5.7% may be due to other factors. The low-fat content in some grains may be due to the fact that cereals, some legumes, and tubers store energy in the form of starch rather than lipids (Iwe *et al*., 2016). The little fat contents may be beneficial as it guarantees longer shelf stability of the grain products (Reebe, Gonzalez, & Rengifo, 2000)

Moisture: From Table 5, the R value indicated the degree of correlation between the Ochratoxins and % moisture content of the grains. An R value of 0.410 indicated a low correlation compare to that of % carbohydrates. Flours with percent moisture content beyond 14% encourage the growth and activities of microorganisms present in them, generating off odours and flavours (Iwe *et al*., 2016), and reducing their quality. The R square value indicated how much of the total variation in the % moisture could be explained by the Ochratoxins. The R^2 value of 0.168 indicated that the effect of the Ochratoxins on the % moisture content of the grains was very low. The presence of the Ochratoxins had insignificant influence on the moisture content of the grains.

3.4.3. Influence of Patulin on the proximate compositions of the grain samples

Ash: The levels of manganese, nitrogen, and pH, as well as large quantity of necessary enzymes, regulate the biological synthetic pathway of patulin (Puel

et al., 2010). From Table 5, an R value of 0.695 indicated a slightly high correlation of % ash and Ochratoxins. Studies have revealed patulin as genotoxic, leading some to theorize that it may be a cancer-causing agent, even though animal studies have remained indecisive (Yi, 1997). The R^2 value indicated how much of the total variation in the % ash can be explained by the Patulin. The R^2 value of 0.483 indicated that the effect of the Patulin on the % moisture content of the grains was moderate. It showed that 48.3% variation in % ash was due to presence of Patulin, while 51.7 % may be due to other causes. This showed that the presence of the Patulin had significant influence on the % ash content of the grains.

Carbohydrates: From Table 5, an R value of 0.940 indicated a high degree of correlation. The R square value indicated how much of the total variation in the % carbohydrates could be explained by the Patulin. Molds can grow on stored food and feed, making the food unpalatable or toxic and are thus a major source of food losses and illness (Wareing, 2013). The R^2 value of 0.884 indicated that the effect of the Patulin on the % carbohydrate content of the grains was very high. In other words, 88.4% change in the carbohydrates content of the grains is caused by the Patulin production, while the remaining 11.6% may be due to other factors.

Crude fibre: Results in Table 5 showed the R value was 0.707, which indicated a slightly high degree of correlation between the Patulin and % crude fibre content of the grains. Isoepoxydon dehydrogenase is an important enzyme in the biosynthesis of patulin, and the gene is in fungi which can potentially yield the toxin (Puel *et al.,* 2010). It's reactive with sulfur dioxide, consequently antioxidant and antimicrobial agents can be useful to exterminate it (Llewellyn *et al.,* 1998). The R^2 value of 0.500 indicated that the effect of the Patulin on the % crude fibre content of the grains was slightly low. In other words, 50.00% change in the % crude fibre content of the grains was caused by the Patulin production.

Crude protein: Results in Table 5 showed an R value of 0.891 which indicated a very high degree of correlation. Kashif Jilani and co-employees stated that patulin encourages suicidal erythrocyte loss under physiological concentrations (Lupescu *et al.,* 2013). Patulin has been identified in grains like wheat, corn, barley, and their processed products and in shellfish (Llewllyn *et al.,* 1998), where it, like aflatoxins, contributes to nutrient loss (PACA, 2018).The R^2 value of 0.794 indicated that the effect of the Patulin on the % crude protein content of the grains was considerable. About 79.4% change in the crude protein content of the grains was caused by the presence of the Patulin.

Crude fat: From Table 5, the R value indicated the degree of correlation between the Patulin and % crude fat content of the grains. An R value of 0.924 indicated a very high degree of correlation. The R square value

indicated how much of the total variation in the % crude fat could be explained by the Patulin. Food intake of patulin from apple juice is projected at between 0.03 and 0.26 µg/kg bw/day in several age groups and populaces (World Health Organization, 2007). The R^2 value of 0.854 indicated that the effect of the Patulin on the % crude fat content of the grains was very high. In other words, 85.4% change in the crude fibre content of the grains was caused by the presence of the Patulin, while the remaining 5.7% may be due to other factors.

Moisture: From Table 5, the R value indicated the degree of correlation between the Patulin and % moisture content of the grains. An R value of 0.398 indicated a low correlation compare to that of % carbohydrates. Patulin is toxic mainly through affinity to sulfhydryl groups, which leads to inhibiting enzymes (Puel *et al.,* 2010). Oral LD_{50} in rat models have ranged between 20 and 100 mg/kg (Puel *et al.,* 2010). The R square value indicated how much of the total variation in the % moisture could be explained by the Patulin. The R^2 value of 0.158 indicated that the effect of the Patulin on the % moisture content of the grains was very low. The presence of the Patulin had insignificant influence on the moisture content of the grains. Patulin cut sperm count and also changed sperm morphology in rat studies (Selmanoglu, 2006).

3.4.4. Impact of penicillic acids on the nutritional composition of the grain samples

Carbohydrates: From Table 5, the R value indicates the degree of correlation between the penicillic acids and % carbohydrate content of the grains. An R value of 0.938 indicated a very high degree of correlation. The R square value indicated how much of the total variation in the % carbohydrates could be explained by the penicillic acids. Penicillic acid is produced by the actions of *Aspergillus flavus* and *Penicillium roqueforti* (El-Hawary *et al*., 2017). The R^2 value of 0.880 indicated that the effect of the penicillic acids on the % carbohydrate content of the grains was very high. In other words, 88.0% change in the carbohydrates content of the grains is caused by the penicillic acids production, while the remaining 12.0% may be due to other factors.

Moisture: From Table 5, the R value indicated the degree of correlation between the penicillic acids and % moisture content of the grains. An R value of 0.403 indicated a low correlation compare to that of % carbohydrates. The R square value indicated how much of the total variation in the % moisture could be explained by the penicillic acids. The R^2 value of 0.163 indicated that the effect of the penicillic acids on the % moisture content of the grains was very low. This shows that the presence of the penicillic acids had insignificant influence on the moisture content of the grains. Penicillic

acid and ochratoxin A are synergistic toxic fungal metabolites (Parker *et al.,* 1982).

Ash: From Table 5, an R value of 0.627 indicated a slightly high correlation of % ash and penicillic acids. The effect of penicillic acid on the pancreatic enzyme carboxypeptidase A in vitro was decreased conversion of parent ochratoxin A to alpha-ochratoxin, a non-toxic metabolite (Parker *et al.,* 1982). The R^2 value indicated how much of the total variation in the % ash can be explained by the penicillic acids. The R^2 value of 0.393 indicated that the effect of the penicillic acids on the % moisture content of the grains was moderate. It showed that 39.3% variation in % ash was due to presence of penicillic acids, while 60.7 % may be due to other causes. This showed that the presence of the penicillic acids had moderate significant influence on the % ash content of the grains.

Crude fibre: Results in Table 5 showed the R value was 0.686, which indicated a slightly high degree of correlation between the penicillic acids and % crude fibre content of the grains. In vivo, penicillic acid inhibits mouse and chicken pancreatic carboxypeptidase A after multiple oral exposures (Parker *et al.,* 1982). The R^2 value of 0.471 indicated that the effect of the penicillic acids on the % crude fibre content of the grains was slightly low. In other words, 47.1% change in the % crude fibre content of the grains was caused by the penicillic acids production.

Crude fat: From Table 5, the R value indicated the degree of correlation between the penicillic acids and % crude fat content of the grains. An R value of 0.875 indicated a very high degree of correlation. The mode of toxic interaction of penicillic acids and ochratoxins may have been due to impaired detoxification of ochratoxin A through penicillic acid depletion of carboxypeptidase A activity (Parker *et al.,* 1982). The R square value indicated how much of the total variation in the % crude fat could be explained by the penicillic acids. The R^2 value of 0.766 indicated that the effect of the penicillic acids on the % crude fat content of the grains was very high. In other words, 76.6% change in the crude fibre content of the grains was caused by the presence of the penicillic acids, while the remaining 23.4% may be due to other factors.

Crude protein: Results in Table 5 showed an R value of 0.819 which indicated a very high degree of correlation. Molds that produce penicillic acids in grains degrade the biomelecules, like protiens, starch, cellulose, etc. in these grains (Wareing, 2013). The R^2 value of 0.671 indicated that the effect of the penicillic acids on the % crude protein content of the grains was considerable. In other words, 67.1% change in the crude protein content of the grains was caused by the presence of the penicillic acids.

3.5. Impact of the mycotoxins on the functional properties of the grain samples

Table 6: Correlation results of the mycotoxins and functional properties

Model	Water Absorption Capacity	Bulk Density	Swelling index	Emulsion capacity	Foaming capacity	Oil Absorption Capacity
R	.686	.797	.490	.502	.511	.579
R^2	.470	.635	.240	.252	.262	.336

Table 7: Correlation results of each mycotoxin and functional properties

Model	R (Aflatoxin)	R Square (Aflatoxin)	R (Ochratoxin)	R Square (Ochratoxin)	R (Patulin)	R Square (Patulin)	R (Penicillic Aid)	R Square (Penicillic Acid)
WAC	.653	.426	.682	.465	.611	.373	.682	.465
Bulk density	.764	.584	.738	.545	.726	.527	.646	.417
Swelling index	.487	.237	.501	.251	.507	.257	.487	.237
Emulsion capacity	.491	.241	.498	.248	.483	.233	.514	.264
Foaming capacity	.511	.262	.507	.257	.458	.210	.509	.259
OAC	.579	.336	.604	.365	.601	.361	.551	.304

Water Absorption Capacity (WAC): From Table 6, the R value indicated the degree of correlation between the mycotoxins and water absorption capacity (WAC) of the grain flours. The observed variation in WAC of the grain samples may be credited to diverse protein concentrations, their degree of interface with moisture and their conformational features (McWatters et al., 2003). An R value of 0.686 indicated a slightly high degree of correlation. The R^2 value indicated how much of the total variation in the WAC of the flours was explained by the mycotoxins. The R^2 value of 0.470 indicated that the effect of the mycotoxins on the WAC of the grains was slightly or moderately high. In other words, a 47.0% change in the WAC of the grains was caused by the mycotoxins' production. Some mycotoxins like aflatoxins are principally related with commodities produced in the tropics and subtropics, like cotton, peanuts, spices, pistachios, and maize (*Jiang et al.,* 2008). The Adjusted R square showed that actually, this model explained 43.4% of the variance in the WAC. The low P (Sig.) value which is 0.00 indicates that there is a significant variation in the WAC and also tells us that the mycotoxins are jointly significant in predicting the WAC of the flours. This also means that the regression model is significantly better in predicting WAC than using the average WAC as the best option. In other words, it's a good fit for the data. The equation for this regression model is WAC = 2.947 – 0.008 (Aflatoxin) + 0.021 (Ochratoxin) – 0.055 (Patulin) – 0.217 (Penicillic Acid).

Bulk density: Table 6 indicated a high degree of correlation, with R value, 0.797. The R^2 value of 0.635 indicated that the effect of the mycotoxins on the bulk density of the grains was slightly high. The small variation in the bulk density might be as a result of the difference in starch content (Iwe *et al*., 2016), which was also reduced by the molds producing these mycotoxins. The Adjusted R square showed that actually, this model explains 61.0% of the

variance in the bulk density. The low P (Sig.) value, 0.00, indicated a significant variation in the bulk densities of the grains and the mycotoxins were jointly significant in predicting the bulk densities of the grains. It's a good fit for the data. The equation for this regression model is bulk density = 0.711 + 0.000 (Aflatoxin) + 0.000 (Ochratoxin) – 0.011 (Patulin) – 0.008 (Penicillic Acid). Bulk density specifies the porosity of a produce which impacts the package design and can be used in determining the kind of packing material required (Iwe & Onadipe, 2001).

Swelling index: Result in Table 6 showed an R value of 0.490, which indicated a moderate correlation of swelling index (SI) and the mycotoxins. The R^2 value of 0.240 indicated that the effect of the mycotoxins on the SI of the grains flours was low. It showed that 24.0% variation in the SI of the flour was due to presence of the mycotoxins, while the remaining % may be due to other causes. Swelling capacity is a quality benchmark in some bakery products (Iwe *et al.*, 2016). The Adjusted R square showed that actually, the model explains 18.8% of the variance in the SI of the grains. This showed that the presence of the mycotoxins had low influence on the SI of the grains. P (Sig.) value 0f 0.003 indicated a slightly insignificant variation in the SI of the flours. This meant that the presence of the mycotoxins had low influence on the SI of the flour. The coefficients defined the model in a linear equation. The equation for this regression model is SI = 1.267 – 0.004 (Aflatoxin) + 0.003 (Ochratoxin) + 0.013 (Patulin) – 0.021 (Penicillic Acid). Swelling index indicates non-covalent bonding of molecules in starch granules and also the ratio of α-amylose and amylopectin (Rašper, 1969).

Emulsion capacity: Table 6 showed the degree of correlation between the mycotoxins and emulsion capacity (EC) of the grain flours. Protein In the grain samples being surface active agent can form and stabilize the emulsion by creating electrostatic repulsion on oil droplet surface (Kaushal *et al.,* 2012). An R value of 0.502 indicated a moderate degree of correlation, while the R^2 value of 0.252 indicated that the effect of the mycotoxins on the EC of the flours was very low. In other words, 25.2% variation in EC of the flours was caused by the presence of the mycotoxins, while the remaining 74.8% may be due to other factors. *Aspergillus ochraceus* is seen as a toxin in an extensive variety of commodities, including beverages (Bayman and Baker, 2006: *Mateo et al.,* 2007). The Adjusted R2 value, 0.200, showed that the mycotoxins accounted little about the variation of SI results. P (Sig.) value of 0.002 indicated that there was slightly insignificant variation in the SI due to presence of mycotoxins. The coefficient tables described the model in a linear equation. The equation for this regression model is SI= 48.160 + 0.200 (Aflatoxin) – 0.376 (Ochratoxin) + 352 (Patulin) + 1.847 (Penicillic Acid).

Foaming capacity: From Table 6, the R value indicated the degree of correlation between the mycotoxins and foaming capacity (FC) of the grain flour. An R value of 0.511 indicated a moderate degree of correlation. Foaming capacity refers to the amount of interfacial area that can be created by susbstances (Fennama, 1996). It is a colloidal of many gas bubbles trapped in a liquid or solid, as the small bubbles are encircled by thin films of liquid (Suresh *et al.,* 2014). The R square value indicated how much of the total variation in the FC could be explained by the mycotoxins. The R^2 value of 0.262 indicated that the effect of the mycotoxins on the FC of the grains was very low. In other words, 26.2% variation in the FC of the grains was caused by the mycotoxins production, while the remaining 73.8% may be due to other factors. The Adjusted R square showed that actually, the model (mycotoxins) explains 21.1% of the variance in the FC. This showed that the presence of mycotoxins was not good enough to account for the variation in the results of the FC of the grain flours. The P (Sig.) value of 0.001 (ie <0.005) indicated that there was very low significant variation and the presence of the mycotoxins were jointly not so significant in predicting the variation in FC of the flours. The equation for this regression model is FC = 8.565 + 0.076 (Aflatoxin) – 0.056 (Ochratoxin) + 0.259 (Patulin) – 0.499 (Penicillic Acid). *Aspergillus carbonarius* is the chief species found in vine fruit, which releases its toxin during the juice making process (Bayman and Baker, 2006: *Mateo et al.,* 2007).

Oil Absorption Capacity (OAC): Result in Table 6 showed an R value of 0.579, which indicated a moderate correlation of oil absorption capacity (OAC) and penicillic acid. The R^2 value of 0.336 indicated that the effect of the mycotoxins on the OAC of the grains flours was slightly low. It showed that 33.6% variation in the OAC of the flour was due to presence of the mycotoxins, while the remaining % may be due to other causes. Oil absorption capacity is an essential functional property that boosts the mouth feel of a food product while maintaining the flavour of that food products (Adebowale & Lawal, 2004). The Adjusted R square showed that actually, this explained 29.0 % of the variance in the OAC of the grains. This shows that the presence of the mycotoxins had slightly low influence on the OAC of the grains. P (Sig.) value 0f 0.000 showed that there's significant difference in the OAC of the flours. This meant that the presence of the mycotoxins had considerable effect on the OAC of the flour. The major proximate components affecting OAC are protein and fat (Jitngarmkusol *et al.,* 2008). The equation for this regression model is OAC = 1.751 + 0.007 (Aflatoxin) – 0.006 (Ochratoxin) – 0.006 (Patulin) + 0.111 (Penicillic Acid).

3.5.1. Impact of total aflatoxins on the functional properties of the grain samples

Water Absorption Capacity (WAC): From Table 7, the R value indicated the degree of correlation between the aflatoxins and water absorption capacity (WAC) of the grain flours. An R value of 0.653 indicated a slightly high degree of correlation. The observed variation in different flours may be due to different protein concentration (as the nitrogen protein may have been affected by the activities of the molds producing the aflatoxins), their degree of interaction with water and conformational characteristics (Butt and Batool, 2010The R^2 value indicated how much of the total variation in the WAC of the flours was explained by the aflatoxins. The R^2 value of 0.426 indicated that the effect of the aflatoxins on the WAC of the grains was slightly or moderately high. In other words, 42.6% change in the WAC of the grains was caused by the aflatoxins production.

Bulk density: Bulk density shows the relative capacity of packaging material required (Iwe *et al*., 2016). Table 7 indicated a high degree of correlation, with R value, 0.764. The R^2 value of 0.584 indicated that the effect of the aflatoxins on the bulk density of the grains was slightly high. Organic crops that are not treated using fungicides can be more vulnerable to contamination with aflatoxins (Halil and Recep, 2013). Children are predominantly affected by aflatoxin contact, leading to liver cancer, stunted growth, liver damage, delayed development (Abbas, 2005).

Swelling index: Result in Table 7 showed an R value of 0.487, which indicated a moderate correlation of swelling index (SI) and the aflatoxins. Aflatoxin makeover products are occasionally found in eggs, dairy products, and meat when animals are given contaminated grains (Fratamico *et al.,* 2008). The R^2 value of 0.237 indicated that the effect of the aflatoxins on the SI of the grains flours was low. It showed that 23.7% variation in the SI of the flour was due to presence of the aflatoxins, while the remaining % may be due to other causes. This showed that the presence of the aflatoxins had low influence on the SI of the grains.

Emulsion capacity: Table 7 showed the degree of correlation between the aflatoxins and emulsion capacity (EC) of the grain flours. Adults have great tolerance for aflatoxin exposure, but children are mainly affected (Abbas, 2005: Williams *et al.,* 2004). An R value of 0.491 indicated a moderate degree of correlation, while the R^2 value of 0.241 indicated that the effect of the aflatoxins on the EC of the flours was very low. In other words, 24.1% variation in EC of the flours was caused by the presence of the aflatoxins, while the remaining 75.9% may be due to other factors.

Foaming capacity: From Table 7, the R value indicated the degree of correlation between the aflatoxins and foaming capacity (FC) of the grain flour. An R value of 0.511 indicated a moderate degree of correlation.

Inadequate availability of food, ecological conditions that favour fungal growth on foodstuffs, and lack of regulatory systems for aflatoxin monitoring and control are among the factors increasing the possibility of aflatoxicosis in humans include (Machida and Gomi, 2010). The R square value indicated how much of the total variation in the FC could be explained by the aflatoxins. The R^2 value of 0.262 indicated that the effect of the aflatoxins on the FC of the grains was very low. In other words, 26.2% variation in the FC of the grains was caused by the aflatoxins production, while the remaining 73.8% may be due to other factors. This showed that the presence of aflatoxins was not good enough to account for the variation in the results of the FC of the grain flours. Molds can also thrive on stored food for humans and animals, thereby making the food unpalatable and toxic (Wareing, 2013).

Oil Absorption Capacity (OAC): Result in Table 7 showed an R value of 0.579, which indicated a moderate correlation of oil absorption capacity (OAC) and the aflatoxins. Small levels of aflatoxin exposure need continuous consumption for some weeks to months for signs of liver dysfunction to appear (Bingham *et al.,* 2003). The R^2 value of 0.336 indicated that the effect of the aflatoxins on the OAC of the grains flours was slightly low. It showed that 33.6% variation in the OAC of the flour was due to presence of the aflatoxins, while the remaining % may be due to other causes. This shows that the presence of the aflatoxins had slightly low influence on the OAC of the grains. Some article has suggested the toxic level of aflatoxins in canine food is 100 to 300 ppb and may require continuous exposure or consumption for weeks to months to lead to aflatoxicosis (Bastianello *et al.,* 1987).

3.5.2. Influences of Total Ochratoxins on the functional properties of the grain samples

Swelling index: Result in Table 7 showed an R value of 0.501, which indicated a moderate correlation of swelling index (SI) and the Ochratoxins. In previous studies, ochratoxin A has been associated with carcinogen and as a nephrotoxin in human urinary tract tumors, although research in humans is limited (*Mateo et al.,* 2007). The R^2 value of 0.251 indicated that the effect of the Ochratoxins on the SI of the grains flours was low. It showed that 25.1% variation in the SI of the flour was due to presence of the Ochratoxins, while the remaining % may be due to other causes.

Oil Absorption Capacity (OAC): Result in Table 7 showed an R value of 0.604, which indicated a moderate correlation of oil absorption capacity (OAC) and the Ochratoxins. Protein and fat affect OAC. The non-polar amino acid side chains can form hydrophobic interaction with hydrocarbon chains of lipids (Jitngarmkusol *et al.,* 2008). The R^2 value of 0.365 indicated that the effect of the Ochratoxins on the OAC of the grains flours was slightly low. It showed that 36.5% variation in the OAC of the flour was due to presence of

the Ochratoxins, while the remaining % may be due to other causes. This shows that the presence of the Ochratoxins had slightly low influence on the Oil Absorption Capacity of the grains.

Foaming capacity (FC): Foam is a colloidal of many gas bubbles trapped in a liquid or solid (Suresh *et al.,* 2014). From Table 7, the R value indicated the degree of correlation between the Ochratoxins and foaming capacity of the grain flour. An R value of 0.507 indicated a moderate degree of correlation. The R square value indicated how much of the total variation in the FC could be explained by the Ochratoxins. The small bubbles created by foaming are surrounded by thin liquid films (Suresh *et al.,* 2014). The R^2 value of 0.257 indicated that the effect of the Ochratoxins on the FC of the grains was very low. In other words, 25.7% variation in the FC of the grains was caused by the Ochratoxins production, while the remaining 74.3% may be due to other factors.

Bulk density: Table 7 indicated a high degree of correlation, with R value, 0.738. The R^2 value of 0.545 indicated that the effect of the Ochratoxins on the bulk density of the grains was slightly above average. Bulk density could be enhanced when the particles are small, compactible, appropriately tapped and properly vibrated and with a suitable packaging material (Machuka et al., 2000).

Water Absorption Capacity (WAC): From Table 7, the R value indicated the degree of correlation between the Ochratoxins and water absorption capacity (WAC) of the grain flours. An R value of 0.682 indicated a slightly high degree of correlation. Ochratoxins are produced by species of the *Penicillium* and the *Aspergillus* (*Bayman and Baker, 2006).* The R^2 value indicated how much of the total variation in the WAC of the flours was explained by the Ochratoxins. The R^2 value of 0.465 indicated that the effect of the Ochratoxins on the WAC of the grains was slightly or moderately high. In other words, 46.5% change in the WAC of the grains was caused by the Ochratoxins production. WAC is imperative in products bulking and stability as well as in baking applications (Lorenz & Collins, 1980).

Emulsion capacity (EC): Table 7 showed the degree of correlation between the Ochratoxins and emulsion capacity of the grain flours. Protein can form and stabilize the emulsion by creating electrostatic repulsion on the surface of oil droplet (Kaushal *et al.,* 2012). An R value of 0.498 indicated a moderate degree of correlation, while the R^2 value of 0.248 indicated that the effect of the Ochratoxins on the EC of the flours was very low. In other words, 24.8% variation in EC of the flours was caused by the presence of the Ochratoxins, while the remaining 74.8% may be due to other factors.

3.5.3. Influence of Patulin on the functional properties of the grain samples

Swelling index: Result in Table 7 showed an R value of 0.507, which indicated a moderate correlation of swelling index (SI) and the Patulin. Patulin is regularly seen in apples as well as apple products such as jams, juices, and ciders (Llewllyn *et al.,* 1998). It has been spotted in foods like bananas, grains, strawberries, cherries, blueberries, plums, and grapes (Llewllyn *et al.,* 1998). The R^2 value of 0.257 indicated that the effect of the Patulin on the SI of the grains flours was low. It showed that 25.7% variation in the SI of the flour was due to presence of the Patulin, while the remaining % may be due to other causes.

Oil Absorption Capacity (OAC): Result in Table 7 showed an R value of 0.601, which indicated a moderate correlation of oil absorption capacity (OAC) and the Patulin. Patulin cut sperm count as well as changed sperm morphology in rat studies (Selmanoglu, 2006). The R^2 value of 0.361 indicated that the effect of the Patulin on the OAC of the grains flours was slightly low. It showed that 36.1% variation in the OAC of the flour was due to presence of the Patulin, while the remaining % may be due to other causes. This shows that the presence of the Patulin had slightly low influence on the Oil Absorption Capacity of the grains. Patulin caused abortion of F1 litters in mice after i.p. injection (Puel *et al.,* 2010).

Foaming capacity (FC): From Table 7, the R value indicated the degree of correlation between the Patulin and foaming capacity of the grain flour. The provisional tolerable daily intake for patulin was kept at 0.43 µg/kg bw by the FDA based on NOAEL of 0.3 mg/kg bw a week. An R value of 0.458 indicated a moderate degree of correlation. The R square value indicated how much of the total variation in the FC could be explained by the Patulin. The R^2 value of 0.210 indicated that the effect of the Patulin on the FC of the grains was very low. In other words, 21.0% variation in the FC of the grains was caused by the Patulin production, while the remaining 79.0% may be due to other factors.

Bulk density: Table 7 indicated a high degree of correlation, with R value, 0.726. The R^2 value of 0.527 indicated that the effect of the Patulin on the bulk density of the grains was slightly above average. Patulin has shown immunotoxicity. It was seen to be immunotoxic in animal and even human studies. Decreased cytokine secretion, oxidative burst inside macrophages, amplified splenic T lymphocytes, and amplified neutrophil numbers are few endpoints observed (Puel *et al.,* 2010).

Water Absorption Capacity (WAC): From Table 7, the R value indicated the degree of correlation between the Patulin and water absorption capacity (WAC) of the grain flours. Water Absorption Capacity is a vital functional property essential in food preparations especially those involving

dough handling (Lorenz & Collins, 1980). It shows the ability of the flour to retain moisture. An R value of 0.611 indicated a slightly high degree of correlation. The R^2 value indicated how much of the total variation in the WAC of the flours was explained by the Patulin. The R^2 value of 0.373 indicated that the effect of the Patulin on the WAC of the grains was slightly or moderately high. In other words, 37.3% change in the WAC of the grains was caused by the Patulin production.

Emulsion capacity (EC): Table 7 showed the degree of correlation between the Patulin and emulsion capacity of the grain flours. The high emulsion capacity of cowpea samples may be due to its relative high protein content (Kaushal *et al.,* 2012). An R value of 0.483 indicated a moderate degree of correlation, while the R^2 value of 0.233 indicated that the effect of the Patulin on the EC of the flours was very low. In other words, 23.3% variation in EC of the flours was caused by the presence of the Patulin, while the remaining 76.7% may be due to other factors. Patulin is a type of mycotoxin made by various molds, in particular, *Aspergillus, Penicillium*, & *Byssochlamys* (Puel *et al*., 2010).

3.5.4. Impact of penicillic acids on the functional properties of the grain samples

Water Absorption Capacity (WAC): From Table 7, the R value indicated the degree of correlation between the penicillic acids and water absorption capacity (WAC) of the grain flours. Some biomelecules like proteins are both hydrophilic and hydrophobic, and therefore can interact with moisture in foods (Suresh *et al.,* 2014). An R value of 0.682 indicated a slightly high degree of correlation. The R^2 value indicated how much of the total variation in the WAC of the flours was explained by the penicillic acids. The R^2 value of 0.465 indicated that the effect of the penicillic acids on the WAC of the grains was slightly or moderately high. In other words, 46.5% change in the WAC of the grains was caused by the penicillic acids production.

Bulk density: Results in Table 7 indicated a high degree of correlation, with R value, 0.646. Penicillic acid is a mycotoxin produced by *Penicillium requeforti* mold (El-Hawary *et al.,* 2017). The R^2 value of 0.417 indicated that the effect of the penicillic acids on the bulk density of the grains was slightly moderate. It is the major product of acid degradation of penicillins (El-Hawary *et al.,* 2017).

Swelling index: Result in Table 7 showed an R value of 0.487, which indicated a moderate correlation of swelling index (SI) and the penicillic acids. Penicillic acid and ochratoxin A are synergistic toxic fungal metabolites (Parker *et al.,* 1982). The mode of toxic interaction of the 2 mycotoxins may have been due to impaired detoxification of ochratoxin A through penicillic acid depletion of carboxypeptidase A activity (Parker *et al.,* 1982). The R^2

value of 0.237 indicated that the effect of the penicillic acids on the SI of the grains flours was low. It showed that 23.7% variation in the SI of the flour was due to presence of the penicillic acids, while the remaining % may be due to other causes. This showed that the presence of the penicillic acids had low influence on the SI of the grains.

Emulsion capacity: Table 19 4.6d showed the degree of correlation between the penicillic acids and emulsion capacity (EC) of the grain flours. An R value of 0.514 indicated a moderate degree of correlation, while the R^2 value of 0.264 indicated that the effect of the penicillic acids on the EC of the flours was very low. The effect of penicillic acid on the pancreatic enzyme carboxypeptidase A in vitro was decreased conversion of parent ochratoxin A to alpha-ochratoxin, a non-toxic metabolite (Parker *et al.,* 1982). It was observed that 26.4% variation in EC of the flours was caused by the presence of the penicillic acids, while the remaining 73.% may be due to other factors.

Foaming capacity: From Table 7, the R value indicated the degree of correlation between the penicillic acids and foaming capacity (FC) of the grain flour. An R value of 0.509 indicated a moderate degree of correlation. In vivo, penicillic acid inhibits mouse and chicken pancreatic carboxypeptidase A after multiple oral exposures (Parker *et al.,* 1982). The R square value indicated how much of the total variation in the FC could be explained by the penicillic acids. The R^2 value of 0.259 indicated that the effect of the penicillic acids on the FC of the grains was very low. In other words, 25.9% variation in the FC of the grains was caused by the penicillic acids production, while the remaining 74.1% may be due to other factors. This showed that the presence of penicillic acids was not sufficient to account for the variation in the results of the FC of the grain flours.

Oil Absorption Capacity (OAC): Result in Table 7 showed an R value of 0.551, which indicated a moderate correlation of oil absorption capacity (OAC) and the penicillic acids. The R^2 value of 0.304 indicated that the effect of the penicillic acids on the OAC of the grains flours was slightly low. It showed that 30.4% variation in the OAC of the flour was due to presence of the penicillic acids, while the remaining % may be due to other causes. It shows that the presence of the penicillic acids had slightly low influence on the OAC of the grains. The major proximate constituents affecting Oil Absorption Capacity are fat and protein (Jitngarmkusol *et al.,* 2008). Non-polar amino acid side chains can form hydrophobic interaction with hydrocarbon chains of lipids (Jitngarmkusol *et al.,* 2008).

4. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

Mycotoxins are toxic secondary metabolic products made by microorganisms of the fungus kingdom, generally known as molds. These mycotoxins are seen in grains before, during, and after harvest. The penicillic acid levels in these grains were within the permissible limit of 20 µg/Kg recommended by WHO and European Union, while most of the aflatoxins and ochratoxin levels associated with the grains were beyond the permissible limit. Some Patulin levels were within the WHO/EU limits of 50 µg/kg for adult foods, but some (especially in groundnut) were above the WHO/EU limits of 10 µg/kg for infant foods. When improperly processed, consumption of these grains may expose individuals to the risk of aflatoxicosis, liver cancer, urinary tract cancer, and kidney damage. Results showed that the presence of these mycotoxins (Aflatoxin, Ochratoxin, Patulin, Penicillic acid) have effects on the nutritional and functional properties of grains at various levels of significance. There is no significant difference in the physical characteristics of the grain samples. Mycotoxins were present in all the grains at various quantities. However, the presence of penicillic acid was not detected in all the rice samples, as well as some millet samples. Ochratoxin was not seen in some rice samples. The presence of the mycotoxins has significant effect on % carbohydrate, %protein, and %fat contents of the grains. Their presence have moderate effect – 47.2 and 54.9% respectively – on % crude fibre and % ash, and very low effect – 16.3% – on % moisture contents of the grains. Results show that the presence of the mycotoxins has moderate effect – 24.0 to 63.5% – on the functional properties (bulk density, SI, WAC, FC, EC, OAC) of the grains. The physical properties of the grains are consistent.

4.2. Contribution to Knowledge

The study revealed the levels of mycotoxins contained in various grains and their impact on the nutritional and functional properties of these grains. The penicillic acid levels in these grains were within the permissible limit of 20 µg/Kg recommended by WHO/EU. Some Patulin levels were within the WHO/EU limits of 50 µg/kg for adult foods, but some (especially in groundnut) were above the WHO/EU limits of 10 µg/kg for infant foods, while most of the aflatoxin and ochratoxin levels found in the grains are beyond the permissible limit of 15 and 5 µg/Kg for unprocessed grains. The results of regression and correlation show that the mycotoxins had impact on the nutritional and functional properties of the grains at various levels of significance. This study quantifies the amount to which the mycotoxins have impact on the nutritional and functional properties of grains. This study will be a tool for policy makers

and relevant authorities in checking levels of mycotoxins in grains sold in open markets.

4.3. Recommendation

Further research needs to be carried out to determine the effects of some commercial and traditional processing methods and techniques on the elimination of mycotoxins in foods. Since the production of these mycotoxins has an effect on the nutritional and functional properties of grains (foods), more research needs to be carried out to explore various ways – biotechnological, physical, chemical – to prevent or exterminate the presence of the molds producing these mycotoxins before, during, and after harvest. Use of permissible fungicides should be encouraged and checked. Plants and other organisms have natural chemical compounds that ensure their defense mechanism against microorganisms such as fungi. Some of these compounds – tea tree oil, cinnamaldehyde, citronella oil, monocerin, etc. – should be extracted and used as fungicides. Use of permissible fungicides – captan, sulfur, mancozeb, etc. – should be encouraged and checked.

More research ought to be carried out to determine which of the aflatoxins (B_1, B_2, G_1, etc.) and Ochratoxins (A, B, C) have higher impact on the nutritional and functional properties of foods, including grains. Also, impact of these mycotoxins on other grains (foods) should be evaluated. The levels of other mycotoxins – Citrinin, Deoxynivalenol (DON), fumonisins, ergot alkaloids (such as ergotamine), zearalenone – and their impacts should be quantified.

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