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LEVELS OF AFLATOXINS IN NON-PROCESSED AND PROCESSED DIARY PRODUCTS

BY

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DECLARATION

I NAMBAJIMANA Marie Claire hereby declare that this report is original and it has not been submitted elsewhere in any form for any word of academic qualification. .

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APPROVAL

This is to certify that NAMBAJIMANA Marie Claire of Registration Number 16/X/2344/PS carried out this research and it is not duplicated and it is now ready for submission to the University Supervisor as well as the Department of Chemistry, Makerere University

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DEDICATION

I dedicate this work to all my friends and family and the entire chemistry department staff.

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My heartfelt gratitude goes to my supervisor Mr Maiki Ernest and head of department Dr John Wasswa for their professional guidance, supervision and patience as I carried out the study. I am grateful to my family that gave me moral support and encouragement during the study. I am indebted to every person who contributed in any way, however small may God richly bless you.

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ABSTRACT

Aflatoxins are a group of structurally related mycotoxin compounds produced by Aspergillus fungi that grow on wide variety of crops. Aflatoxins are naturally occurring carcinogenic substances. High level of aflatoxins exposure has been shown to cause acute aflatoxicosis in human and animals. Cases of aflatoxicosis have been reported in many countries. The main objective of this study was qualitative and quantitative analysis of aflatoxin in processed and non- processed dairy products.10 samples of raw milk, pasteurized milk and ultra-heat treatment (UHT) milk of each 250g were collected. ELISA technique was usedfor detection and quantification of aflatoxins. The results show that All raw milk samples were positive for aflatoxin M1 with a mean value of 346.15±49.12, Pasteurised milk with 202.26±110.39 and ultra-heat treatment UHT milk with 49.08±18.67.Aflatoxin M1 concentration in 100% raw milk samples, 80% of pasteurized milk samples and 40% of UHT milk samples with mean values of 346.15±49.12, 241.14±87.6 and 65.5±8.8 respectively were above EU's legal limit of 50ng/L but below US Food and Drug administration legal limit of 500ng/L.

CHAPTER ONE: INTRODUCTION

1.0 BACKGROUND

Aflatoxins are a group of mycotoxins produced as secondary metabolites by three species of fungi (mould) of the genus aspergillus, namely, Aflavus, Aparasiticus and Anomius (Nidhina, 2017)There are four main types of aflatoxins: aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2). AFB1 is considered the most toxic aflatoxin and the most potent carcinogenic substance, thus classified as Group 1 human carcinogen by the International Agency of Research on Cancer (IARC, 2002)). AFG1, AFB2 and AFG2 are less carcinogenic and less mutagenic than AFB1 (Bbosa GS, 2013).Aflatoxin metabolism differs between children and adults (Dohnal V, 2014). Liver is the dominant site of aflatoxin. When cows, sheep, goats or other ruminant animals have consumed feeds contaminated with aflatoxins B1 and B2, aflatoxins M1 and M2 will be formed as a result of the metabolic process in liver of ruminants and excreted in milk (Xiong, 2015). Aflatoxins are produced most commonly in moist products stored under conditions that are favourable for growth of moulds. The favourable conditions include high moisture content (at least 70 %), range of temperature (10-40˚C), pH of 4-8, and capability of growing on dry surface (Bryden, 2001). Stress such as drought, insect infestation, damage and broken grain kernels also contribute to mould colonization of the food and feed by Aspergillus (Jacques, 1988). Aspergillus species are wide spread in nature and can colonize and contaminate before harvest and during storage (Kang'ethe, 2007). Aflatoxins exposure to human and animal is through consumption of fungal contaminated foods and feeds (Nelson, 1993). Chronic dietary exposure to aflatoxin is a major risk factor for hepatocellular carcinoma, especially in areas where hepatitis B virus infestation is endemic. Metabolism Children are particularly affected by aflatoxin exposure which leads to stunted growth and delayed development (Gong Y. Y., 2002). An outbreak of human acute aflatoxin poisoning involving 317 cases with 125 deaths was reported in Kenya in 2004. The epidemiologic investigation attributed this to consumption of contaminated maize (FAO, 2004)various regulatory authorities have set legal limits for AFM1 in milk to decrease the health risk, given the high milk consumption by humans, especially children, and unfavourable effects of AFM1 on human health. The USA food and drug administration has established a maximum level of 500ng/L for milk AFM1 (Administration(FDA), 2005) and European commission is 50ng/L (Europeancommission(EC), 2006).

1.1 PROBLEM STATEMENT

There is a need to investigate the levels of aflatoxins in dairy products in stores and on the markets in order to reduce the risk of aflatoxicosis. Detection and quantification of aflatoxins levels is important in order to compare levels of contamination with the recommended maximum residue limit so that appropriate remedial action of aflatoxin contamination can be taken, and appropriate preventive practices of aflatoxin contamination during handling and storage are implemented.

1.2 OBJECTIVE

The objective of this work is to determine aflatoxins in non-processed and processed dairy products.

1.3 SIGNIFICANCE

Milk is highly consumed especially by children therefore it's important to establish its level of aflatoxin contamination. The results from this study can be used to get information on the levels of aflatoxins in milk and compare with the recommended maximum residue limit so that appropriate remedial action of aflatoxin contamination can be taken, and appropriate preventive practices of aflatoxin contamination during handling and storage are implemented.

CHAPTERTWO: LITERATURE REVIEW

2.0 Introduction

Aflatoxins are a group of structurally related toxic compounds produced by Aspergillus species of fungi that grow on wide variety of grains and nuts (Nidhina, 2017). Aflatoxins were discovered in 1960 when 100,000 turkey poultry died from eating fungus- infested peanut meal. A. flavus was found in the infested peanut meal together with alcohol extractable toxins termed aflatoxins(Joanne, 2008). The native habitat of Aspergillus is in soil, decaying vegetation, hay and grain undergoing microbiological deterioration. Fungi in the genus Aspergillus invade all types of organic substrates whenever conditions are favourable for its growth. Human foods which are frequently affected include cereals like maize, sorghum, pearl millet, rice and wheat. Oil seeds like peanuts, Soya bean, sunflower, and cotton seed, spices and tree nuts also support the growth of the fungus (Massey, 1995). There are 18 different aflatoxins with aflatoxins B1, B2, G1 and G2 being the major ones. Aflatoxin B1, which in its pure form is a pale-white to yellow crystalline and odourless solid, is found in large amount in cultures and food products and is considered the most toxic (CAST, 2003). Different species of Aspergillus may produce specific aflatoxins. A. parasiticus may produce aflatoxins B1, B2, G1 and G2, whereas A. flavus produces only B1 and B2 (Moss, 1989). Aflatoxins M1 and M2 are metabolic products of aflatoxin B1 and B2 produced by animals following ingestion of B1 and B2, and they are secreted in milk of both animal and human, and excreted in urine and faeces(Kang'ethe, 2007). Aflatoxin B2A and G2A which may be produced in minor amounts have been isolated from A. flavus and A. parasiticus(Reddy, 2000) . Aflatoxicol is a reductive metabolite of Aflatoxin B1 and is normally secreted in milk and excreted in urine of dairy cattle and other mammalian species that have consumed aflatoxin B1(Peraica, 1999). Other compounds closely related to aflatoxins such as aflatoxin GM1, and parasiticol are produced by Aspergillus flavus (Reddy et al., 2000). The mycotoxins produced by fungi, are not required for the growth or the development of the fungi, but serve as protective mechanism for the fungi. They weaken the receiving host and may use the host as a strategy to better their environment for further fungal proliferation (Fox E. M., 2008).

2.1 Conditions that favour aflatoxin contamination in foods and feeds

Mycotoxin contamination of foods and feeds depends on environmental conditions that lead to mould growth and toxin production (Pittet, 1998). Commodities can be contaminated at any time from growth in the field through harvesting, processing, storage and shipment (Pittet, 1998). Aspergillus parasiticus is well adapted to a soil environment and is prominent in peanuts, whereas A. flavus seems to be adapted to active development on the aerial parts of a plant (such as leaves and

flowers) and is dominant on corn, cottonseed and tree nuts (Diener, 1987). Aspergillus flavus colonization in maize and oil seeds are encouraged by high humidity (80-89 %) and heat (10-40◦C) (Lunyasunya, 2005). Drought stress has been found to increase the number of Aspergillus spores in the air (Sorenson, 1984). Nitrogen stress (low soil fertility) and other stress that affect the plant growth during pollination can increase the level of aflatoxins production by Aspergillus fungi. Mature maize that remains in the field (as dry heaps) or maize that is stored without proper drying is susceptible to Aspergillus fungi growth and aflatoxin production (Lunyasunya, 2005). Poorly stored feeds and grains can indeed become contaminated with aflatoxin (Lillehoj, 1975). Time of harvest has also been shown to have influence on aflatoxins level because Aspergillus does not compete well with other mould when maize is below 20 % moisture content. Thus harvesting maize with moisture content of above 20 % and then drying down to at least a moisture content of 15 % within 24 to 48 hours of harvest will keep Aspergillus fungi growth and toxin production at minimum(Lunyasunya, 2005). Mould growth and toxin formation require a moisture content of the substrate greater than 14 % and a temperature of approximately 25 %. Reduced oxygen content diminishes aflatoxin formation(Diener, 1987). Damage of cereals by insects such as weevils and physical damage can greatly increase Aspergillus infection and the levels of aflatoxins. Protein supplements such as cotton seed cakes, sunflower cakes, fish meal and other oil seed by products which are often poorly stored are the primary source of the mould found in home-made dairy concentrates on small hold farms (Lunyasunya, 2005). Although maize is traditionally stored in granaries, storage inside homes occurs during periods of shortage. This may favour growth of moulds and subsequent contamination of maize with aflatoxins. The warm environment inside windowless homes and storage of maize on dirty floor may promote fungal growth in wet maize kernels(Eduardo, 2005). Traditional methods of drying and storing maize in elevated granaries are protective against aflatoxicosis. The granaries with elevated platform isolate the maize from the spores and insect on the ground (Eduardo, 2005). It is important to note that, although drying feeds and foods has been shown to reduce mould count, many moulds spores remain in the feeds and foods after they have been dried. These spores can grow if conditions are right (Lunyasunya, 2005).

2.2 Aflatoxin management strategies applicable in Africa

Factors fundamental to a country's ability to protect its population from mycotoxins include political will to address mycotoxins exposure and support capacity for testing commodities, which determines whether requirements can be enforced (Wagacha, 2008). Therefore, it is very important for African countries to take into consideration the prevention of exposure to aflatoxins, possible decontamination and surveillance and monitoring of moulds in contaminated food and feedstuff for

effective mycotoxins management (Kabak, 2006).To ensure that food have the lowest aflatoxins concentration possible prevention of exposure is required. Good practices during production, harvesting, storage, transportation, marketing, processing and regulation need to be observed. Cultural practices during production including crop rotation, tillage, choice of planting date, and management of irrigation and fertilization can limit infection and subsequent mycotoxins accumulation(Munkvold, 2003)(Champeil, 2004).

Biological strategies have been developed, such as a toxigenicfungi, which out compete their closely related strains thus reducing the levels of mycotoxins in the crops. Less toxigenic competitive exclusion have been isolated from Nigerian soils (Atehnkeng, 2008) and given approval for test releases. Such a toxigenic strains of A. flavus and A. parasiticus upon introduction to soil of developing crops have resulted in aflatoxins contamination in peanuts in the United States ranging from 74.3% to 99.9% of the original seen contamination (Dorner, 2002).post-harvest (storage) aflatoxin contamination has been reduced by 95.9% through field application of non-toxigenic strains of A. flavus and A. Parasiticus (Dorner, 2002).

Chemical control is another tool. Appropriate use of pesticides during the production process could help in reducing the fungal infection or insect infestation and subsequent mycotoxins contamination. Fungicides such as itraconazole and amphotericin B have been shown to effectively control the aflatoxins producing Aspergillus species (Ni, 2005).Use of fungicides is often not an option in Africa due to economic reasons and growing concerns about environmental and food safety(Ni, 2005). Lowering of the overall contamination level of a production batch may be achieved by mixing with a non- or less-contaminated batch. This approach is forbidden through legislation in a number of countries such as in the EU (De Koe, 1999). Theoretically, also decontamination, e.g., by treatments with ammonia is a possibility for aflatoxins. (Bagley, 1979) reports that corn containing aflatoxin can be decontaminated by treatment with gaseous ammonia at atmospheric pressure. Toxicity feeding trials with ducklings, broiler chicks, and trout confirmed that the process inactivates aflatoxin. The process was shown to reduce aflatoxin levels from 1,000 parts per billion (ppb) to within the FDA action level of 20 ppb (Bagley, 1979). Ammoniation is not useful for reducing aflatoxin contamination in food products since they would be unpalatable and in the EU decontamination by chemical means is not allowed for food products (De Koe, 1999). Reasons for not allowing batch mixing or chemical treatments as means of lowering the content of a mycotoxin in foods are multiple. First it may render traceability more difficult in case of problems, secondly a non homogenous mixing may still mean that some consumers would face unacceptable high exposure levels ,thirdly relying on chemical detoxification may result in unsafe products and unknown

compounds may be formed which are either toxic or compromise the approved systems of analysis for control. Breeding for resistance is one of the most promising long term strategies for mycotoxin management in Africa. Potential biochemical and genetic resistance markers have been identified in food commodities, especially maize which are being utilised as selectable markers in breeding for resistance of aflatoxin contamination (Wagacha, 2008).gene clusters housing genes that govern the formation of aflatoxin have been elucidated and being targeted in strategies to interrupt the biosynthesis of these mycotoxins (Cleveland, 2003). To come up with effective strategies to control fungal infection and minimize mycotoxins production in host plants, a better understanding of genetic variability and population structure at the intraspecific level and ability to detect cryptic populations or lineages which might arise that possess significant features in terms of toxins profile or host preferences are necessary (Mul´e, 2005).

There are also simple management strategies that can significantly reduce toxin levels of crops. Early harvesting reduces fungal infection of crops in the field for harvest. Even though most farmers in Africa are well aware of the need for early harvesting, weather changes, labour constrait, need for cash, threat of thieves, rodents and animals compel farmers to harvest at inappropriate time (Amyot, 1983). For instance, early harvesting and threshing of groundnuts has proven to lower aflatoxins levels and increase the gross returns with 27% as compared to delayed harvesting (Rachaputi, 2002).

2.4 Aflatoxicosis

Aflatoxicosis is poisoning that result from ingestion of aflatoxins in contaminated foods in human and feeds in animals. It manifests as chronic or acute aflatoxicosis (Lunyasunya, 2005). Chronic aflatoxicosis results from ingestion of low to moderate levels of aflatoxins. Chronic dietary exposure to aflatoxins is a major factor for hepatocellular carcinoma (Fung, 2004). The effects are subclinical and are difficult to recognize. Common symptoms are impaired food conversion and slow rate of growth with or without the production of an overt aflatoxin syndrome. Ingestion of higher doses of aflatoxin can result in an acute aflatoxicosis which manifest as hepatotoxicity or in severe cases, fulminant liver failure (Fung, 2004). Acute symptoms include haemorrhage, acute liver damage, odema, alteration in digestion, absorption and/or metabolism of nutrients and possibly death. No animal species is resistant to the acute toxic effect of aflatoxins (Lunyasunya, 2005). The biological effects of aflatoxin can be grouped into four general categories: acute and chronic liver damage, reduced growth rate, impairment of immunologic and innate defense mechanisms and carcinogenic and tetragenic effects. Many of these effects of aflatoxins relate to their reaction with cellular protein and maintenance of cellular integrity (Patterson, 1982). Animal species respond differently in their susceptibility to chronic and acute toxicity of aflatoxins. This toxicity can be influenced by

environmental factors, exposure level and duration of exposure, age health and nutritional status of diet(Dohnal V, 2014). Aflatoxin B1 is a very potent carcinogen in many species including non-human primates, birds, fish and rodents. In each species, the liver is the primary target organ of aflatoxin toxicity and carcinogenicity in acute injury (Xiong, 2015).

Exposure to aflatoxins is widespread in many African countries. Blood tests have shown that a high percentage of West Africans are exposed to aflatoxins. Studies reported by (Wild, 1996) and carried out in Gambia, Guinea, Nigeria, and Senegal, up to over 98% of subjects tested positive to aflatoxins markers. In Benin 99% of the children had aflatoxin markers in their blood with some of the highest aflatoxin levels in humans ever observed (Gong Y. Y., 2002).

Aflatoxins have also been detected in milk. Dairy cattle that feed on aflatoxin contaminated feeds produce contaminated milk. Approximately 1-3% of the B1 initially present in the animal feedstuff appeared as aflatoxin M1 in milk, but its carryover varies from animal to animal (Xiong, 2015). Exposure of dairy cattle to low-moderate aflatoxins concentration passes serious risks to human that depend on it for milk.

(Keven David Moreira Gonçalves, 2018) Evaluated the occurrence of aflatoxins M1 and B1 in 112 milk 32 samples (whole, skimmed, semi-skimmed, liquids and powders), collected at a local commercial establishment in southern Brazil and Assomada City, Cape Verde. AFLAM1 and AFLAB1 were determined by using high-performance liquid chromatography with fluorescence detection. For the 62 milk samples from supermarkets in the city of Rio Grande, Rio Grande do Sul, 68% were contaminated by AFLAM1 (range, 40-3670 ng/l) and 16% were contaminated by AFLAB1 (range, 40-600 ng/l), with contamination found only in liquid milk. Among the 50 samples from Assomada City, AFLAM1 was detected in 76% of the samples (range, 32-2896 ng/l), at values below the current Brazilian legislation for the maximum permitted level in milk powder (5000 ng/l) but higher than that recommended by the European Commission.

(Bilandžić N., 2016), Aflatoxin M1 (AFM1) concentrations were determined in raw and UHT cow milk samples collected in different regions of Bosnia and Herzegovina and Croatia during the autumn months of 2014. The mean AFM1 levels in the raw milk samples were (ng/kg): 6.22 in Bosnia and Herzegovina, 5.65 in Croatia. In all except one milk sample, AMF1 levels were below the LOQ value of 34.2 ng/kg (ELISA method). In four milk samples, AFM1 concentrations exceeded the EU MRL of 50 ng/kg. Samples were subjected to LC-MS/MS analysis which confirmed elevated values determined by ELISA. Elevated levels were in the range 56.6– 132.6 ng/kg. Two positive milk samples from Bosnia and Herzegovina originated from Una Sana Canton, two from Croatia

from eastern Croatia. The highest AFM1 levels of 132.6 ng/kg was measured in milk from eastern Croatia. In 214 samples of processed UHT milk from Bosnia and Herzegovina and Croatia, AFM1 ranged from 2.29 ng/kg to 21.4 ng/kg, all below the LOQ value. AFM1 exceeded the EU MRL value in only 0.62% of milk samples, indicating the sporadic use of contaminated feedstuff at farms in both countries.

2.5 Economic impact of aflatoxins

The economic impact of aflatoxins is derived directly from food and livestock losses as well as directly from cost of regulatory programmes designed to reduce risks to animal and human health (CAST, 2003). The Food and Agricultural Organization (FAO) estimates that 25% of world food crops are affected by mycotoxins of which the most notorious are aflatoxins (FAO, 1998). Aflatoxins losses to livestock and poultry producers from aflatoxin contaminated feeds include death and more subtle effects of immune system suppression, reduced growth rate and loss in feeding efficiency. Other adverse economic effects of aflatoxin include lower yields for foods and fibre crops (CAST, 2003). Aflatoxin contamination impacts on loss to farmers and traders, for instance, 32 000 bags of maize were condemned in Kenya in 2009. Aflatoxin leads to decreased production of animals, high cost of decontamination, loss of trade both locally and internationally. Human deaths could result in orphaned children creating a burden to society. The contamination also leads to reduced availability of both quantity and quality of food to people. In addition, the ability of aflatoxin to cause cancer and related diseases in human given their seemingly unavoidable occurrences in foods and feeds, make the prevention and detoxification of these mycotoxins one of the most challenging toxicology issues of the present time (Marin, 2013).

CHAPTER THREE: MATERIALS AND METHOD

3.1 Sample Selection

A total of 30 samples from various brands were collected in Kampala city, which included 10 samples of raw milk, pasteurized and ultra-heat treatment (UHT) milk.

3.2 Determination of aflatoxin M1 in milk products

The ELISA test for the analysis of aflatoxin M1 in milk was performed according to the instructions for RIDASCREEN AFM1 test kit (R1121, R-Bio Pham AG, Darmstadt, Germany). Reagents for ELISA test include coated plates, standards; conjugate substrate, wash buffer solution and a stop solution. Standard solution of 0, 5,10,20,40 and 80 ng/l were used to construct the calibration curve.

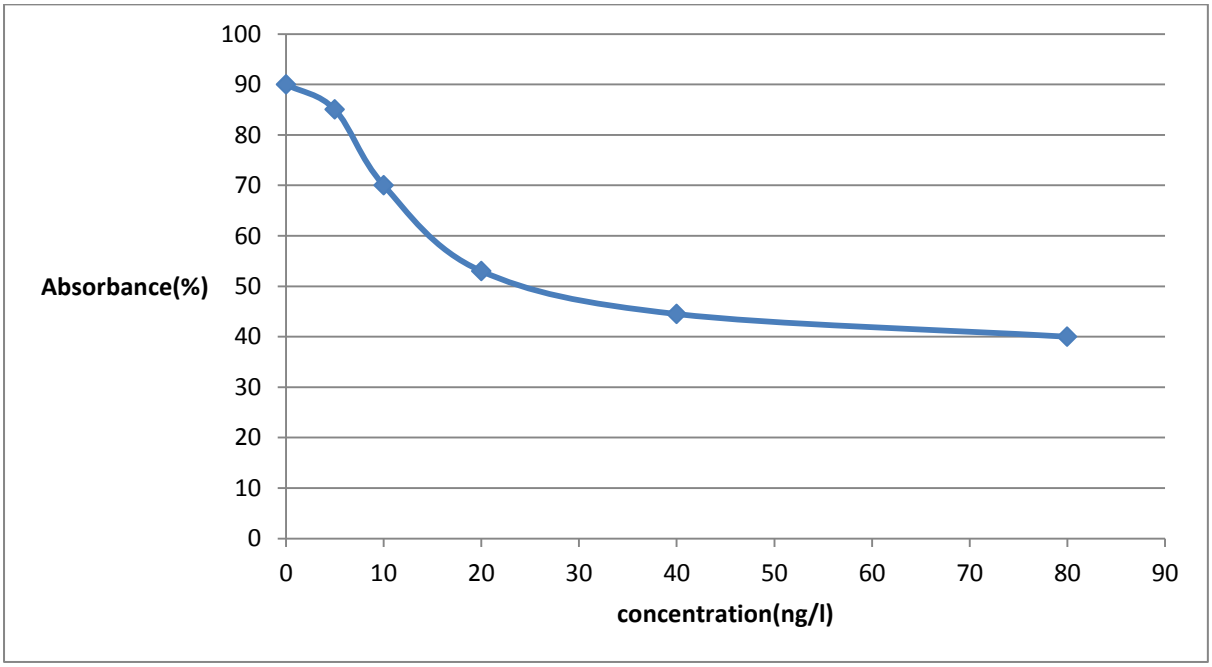

Figure 1: A graph showing the variation of absorbance with concentration

3.3 Procedure

Wells were placed into microwells holder.

1000µl of standards and samples were added into the appropriate wells in duplicate, Microwells were covered with sealing film and incubated at room temperature for 1hour and the sealing film was removed.

100µl of detection solution was added to each well Microwells were covered with the sealing film the plate was shaken manually for 30seconds and incubated at room temperature for 15minutes. Free and enzyme conjugate compete for the aflatoxin antibody binding sites and at the same time the anti-aflatoxin antibodies is bound by immobilized capture antibodies.

After incubation, the reactants were poured out of the wells and the microwell holder was taped upside down vigorously against absorbent paper to ensure complete removal of liquid from wells.

The wells were filled with three hundred microliters of wash buffer and the liquid was poured out. The washing was repeated three times. The purpose of the washing is to remove the unbound enzyme conjugate.

100µl of the enzyme substrate was added and the microwells were covered and incubated in dark at room temperature for 15minutes.

The cover was removed and100µl of stop solution was added to each well and shaken manually for few seconds. The addition of the stop solution leads to colour change from blue to yellow.

CHAPTER FOUR: RESULTS AND DISCUSSIONS

				Distribution(ng/L)					positive samples
Milk type	Brand	positives samples	samples above EU limit	<5	5-49.9	50-99.5	>100	maximum	mean±SD
raw milk	1	2	2	0	0	0	2	360	360±0
raw milk	2	2	2	0	0	0	2	270.4	270.2±0.2
raw milk	3	2	2	0	0	0	2	390	390.05±0.05
raw milk	4	2	2	0	0	0	2	400	400±0
raw milk	5	2	2	0	0	0	2	311	310.5±0.5
raw milk	Subtotal	10	10	0	0	10	10	400	346.15±49.12
pasteurized	1	2	2	0	0	0	2	352	351±1
pasteurized	2	2	2	0	0	0	2	290.1	290.05±0.05
pasteurized	3	2	0	0	2	0	0	47	46.75±0.25
pasteurized	4	2	2	0	0	1	1	150	123.5±26.5
pasteurized	5	2	2	0	0	0	2	200	200±0
pasteurized	Subtotal	10	8	0	2	1	7	352	202.26±110.39
UHT	1	1	1	0	1	1	0	70	58.5±11.5
UHT	2	1	0	1	1	0	0	40.1	22.4±17.7
UHT	3	2	2	0	0	2	0	72.5	70.75±1.75
UHT	4	1	0	0	2	0	0	46	44.5±1.5
UHT	5	1	1	0	1	1	0	50.5	49.25±1.25
UHT	Subtotal	6	4	1	5	4	0	72.5	49.08±18.67

Table 1: occurrence of aflatoxin M1 in raw, pasteurized and UHT milk.

brands	pasteurized		raw milk		UHT milk	
1	290	290.1	360	360	70	47
2	47	46.5	270	270.4	40.1	4.7
3	150	97	390	390.1	72.5	69

4	200	200	400	400	46	43
5	352	350	310	311	50.5	48

Table 2: A table showing the concentration (ng/L) of aflatoxin in each sample

All results are expressed as mean ±standard deviation (SD), maximum concentration and distribution of aflatoxins. This study indicated high incidence of aflatoxin MI in raw, pasteurized and UHT milk samples, some of which were above the EU legal limit. In addition, this study revealed that AFM1 was less in UHT milk samples than in pasteurized and raw milk samples (Europeancommission(EC), 2006). All raw milk samples were positive for aflatoxin M1 with a mean value of 346.15±49.12, Pasteurised with 202.26±110.39 and UHT with 49.08±18.67.AFM1 concentration in 100% raw milk samples, 80% of pasteurized milk samples and 40% of UHT milk samples were above EU limit with mean values of 346.15±49.12, 241.14±87.6 and 65.5±8.8 respectively.

This is consistent with findings by (Fox E. M., 2008)who noted that mycotoxins greatly resist decomposition and being broken down by digestion. They remain in the food products, even temperature treatment such as cooking and freezing do not destroy mycotoxins.

This is further confirmed by (Yoursef, 1989) reported that aflatoxin M1 is relatively stable in raw milk and is unaffected by pasteurization or processing into cheese or yoghurt. This means that contaminated milk will give contaminated milk products.

The purpose of assessing aflatoxin contamination in milk was to highlight the existing danger of aflatoxin contamination of these foods and feeds which possibly leads to animal and human poisoning in the urban population. To decrease milk AFM1,normative procedures, including sanitation standard operation procedures, hazard analysis and critical control points ,and international standardization organization should be implemented in feed and milk processing to ensure milk safety, aflatoxin decomposing bacteria and enzymes, and mycotoxins adsorbents can be used to decompose AFB1 or prevent AFB1 absorption in intestinal tract. (Samuel, 2014).

CHAPTER FIVE: CONCLUSIONS AND RECOMMANDATION

This study demonstrated that the AFM1 content in all milk samples were above the EU legal limit 50ng/L but below the US Food and Drug administration limit 500ng/L (Administration(FDA), 2005). According to the findings of this study the following recommendations were drawn:

Studies should be done to verify whether there is a relationship between Current rising cases of liver cancer and aflatoxin contamination.

Long term solutions to food and feeds contamination should include strengthening nationwide surveillance, increase food inspection in market area to ensure their safety.

There is need to campaign for seminars and workshops by stockholders such as public health and ministry of Agriculture to create awareness among food and feed handlers against poor practices that contribute to aflatoxin contamination.

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