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Research article

Assessment of aflatoxin B1 contamination in rice and maize

Evaluación de la contaminación por aflatoxina B_1 en arroz y maíz

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ABSTRACT

Introduction: Aflatoxins B_1 are among the most common poisonous mycotoxins produced by certain fungi that harm animals and crops. Mycotoxins can cause a variety of adverse health effects and pose a serious health threat to humans. The Maximum Residue Limits of aflatoxin B_1 in processed cereals and ingredients are 2 parts per billion (ppb) and 5 ppb, respectively.

Objectives: To evaluate the status of aflatoxin B_1 contamination in rice, corn and staple food produced in Ha Giang province compared with the maximum permitted levels.

Methods: A total of 210 rice and maize samples were analyzed to quantify the level of aflatoxin B_1. Analysis of mycotoxins was conducted by High Performance Liquid Chromatography using a fluorescence detector.

Results: It was found that rice, rice products, maize, and maize products had a mean aflatoxin B_1 content of 1.79 ppb, 2.55 ppb, 2.19 ppb, and 6.35 ppb, respectively. The results also showed that 71.9% of samples were contaminated with mycotoxins, and 14.28% of samples exceeded the maximum allowable limit.

Conclusion: The concentration of aflatoxin B_1 in 14.28% of the samples are over permissible limits by nationwide regulations.

Keywords: aflatoxin B_1; mycotoxins; rice; maize; chromatography; high pressure liquid.

RESUMEN

Introducción: La aflatoxina B_1 se encuentra entre las micotoxinas más comunes y venenosas producidas por ciertos hongos que dañan a los animales y los cultivos. Las micotoxinas pueden causar una variedad de efectos adversos para la salud y representar una grave amenaza para la salud de los seres humanos. Los límites máximos de residuos de aflatoxina B_1 en cereales e ingredientes procesados son de 2 ppb y 5 ppb, respectivamente.

Objetivos: Evaluar el estado de contaminación por aflatoxina B_1 en arroz, maíz y alimentos básicos producidos en la provincia de Ha Giang, en comparación con los niveles máximos permitidos.

Métodos: Se analizaron un total de 210 muestras de arroz y maíz para cuantificar el nivel de aflatoxina B_1. El análisis de micotoxinas se realizó mediante cromatografía líquida de alta resolución, utilizando un detector de fluorescencia.

Resultados: Se encontró que el arroz, los productos de arroz, el maíz y los productos de maíz tenían un contenido medio de aflatoxin B_1, de 1,79 ppb, 2,55 ppb, 2,19 ppb y 6,35 ppb, respectivamente. Los resultados también mostraron que el 71,9 % de las muestras estaban contaminadas con micotoxinas y el 14,28 % de las muestras excedieron el límite máximo permitido.

Conclusión: La concentración de aflatoxina B_1 en el 14,28% de las muestras está por encima de los límites permisibles por la norma nacional.

Palabras clave: aflatoxina B_1; micotoxinas; arroz; maíz; cromatografía líquida de alta presión.

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INTRODUCTION

Among mycotoxins, aflatoxin B_1 (AFB_1) is considered the most common and also the most harmful found in food. Mycotoxins are naturally occurring toxins produced as secondary metabolites of diverse fungi such as *Aspergillus flavus*, *Aspergillus parasiticus*, and occasionally *Aspergillus nominus.* AFB_1 is most toxic chemical agents found in food, posing the greatest threat to human health.(1)

Most mycotoxins are chemically and thermally stable, so even at temperature ≥ 100 ºC they are not decomposed by heat. This is a major obstacle in reducing contaminants in food, as high-temperature cooking and cold storage cannot degrade AFB_1.(2)

Aflatoxin B_1 is of particular interest because of its effects on the human immune system and its carcinogenic potential.(3) In terms of toxicity, aflatoxin is teratogenic, mutagenic and carcinogenic. The International Agency for Research on Cancer (IARC) has classified AFB_1 as group 1 human carcinogen and mainly pose a threat to human liver.(4)

Aflatoxin B_1 has been implicated in acute and chronic exposure in humans. Exposure to high concentrations of mycotoxins over a relatively short period of time is considered to be the cause of acute illness that frequently occurs in developing countries. Studies have shown that exposure with high level of acute aflatoxin poisoning can cause abdominal pain, vomiting, and even death, while chronic low-dose AFB_1 exposure over long periods of time can lead to liver cancer.(5) Other health effects of chronic exposure include hepatocellular carcinoma, growth retardation in children, and impairment of immune function.(6)

Human exposure to mycotoxins may result from consumption of plant-derived foods that are contaminated with toxins, the carry-over of mycotoxins and their metabolites in animal products. Lifetime exposure to AFB_1 may start from a fetus in the womb through the placenta. When a mother ingests feed contaminated with aflatoxin they metabolize and excrete the metabolite, aflatoxin M_1 (AFM_1), in milk. Therefore, it reflects the possible mother-to-infant contamination occurring through breast milk although aflatoxin M_1 is considered to be less toxic than AFB_1. There has been evidence linking childhood aflatoxin exposure to growth retardation.(7)

It is impossible to totally prevent the contamination of several foods, including rice, maize, peanuts, and oilseeds, with AFB_1, especially in tropical regions where the humidity and temperature are conducive to the formation of fungi. Fungi can grow before and after harvest in an area with a hot, humid atmosphere. Mycotoxin contamination is more likely when inappropriate harvesting, storage, and transportation conditions are used.(8)

Aflatoxin B_1 and food safety issues are caused by high humidity and inadequate food storage conditions. Moreover, due to a scarcity of water during the summer in the hot province of Ha Giang, maize produced in the mountains may be contaminated with AFB1. Additionally, the researchers *Palumbo R* et al.(9) have shown that numerous other mycotoxins are also present in food, in addition to AFB_1. Several mycotoxins present at once can make food more hazardous.

The present study aims to determine the level of AFB_1 contamination in maize and rice samples and compare the findings to Vietnam's maximum permitted levels.

METHODS

Research location

Samples of maize and rice were sampled from households and business households in Quan Ba, Hoang Su Phi and Bac Quang districts of Ha Giang province (the northern mountainous province of Vietnam).

Samples

A total of 210 samples including 105 corn and 105 rice samples were drawn at random from homes, commercial buildings, shops, and marketplaces in 2021.

One kilogram sample, is put in a plastic bag after being tested for temperature and humidity. Samples were stored at Department of Military Hygiene, Military Medical Academy at a temperature of -4 °C until they were analyzed.

Aflatoxin B_1 in samples were analyzed in laboratory of Department of Military Hygiene, Vietnam Military Medical University.

Variables

The mean, maximum and minimum of aflatoxin B_1 in corn and rice. The percentage of samples exceeding the permitted level were measured, and also the percentage of contaminated samples with aflatoxin B_1, and the number of samples which were below the limit of detection (LOD) of the instrument.

Chemicals

All reagents and solvents used in the experiment are of analytical grade. AFB_1 standard solution was purchased from Sigma-Aldrich. Metanol (CH_3OH), Acetonitrile (CH_3CN), Acid acetic (CH_3COOH), Sodium chloride (NaCI), Sodium phosphate ($NaHPO_4$), Disodium hydrogen phosphate (Na_2HPO_4), Monopotassium phosphate (KH_2PO_4), Sodium hydroxide (NaOH), Potassium chloride (KCI), and Kali bromua (KBr) were all purchased from Merck.

Analysis of AFB_1

Mycotoxins in food were quantified by High Performance Liquid Chromatography (HPLC) machine combined with fluorescence detector. The analytical procedure consists of 3 main steps: sample preparation, immunoaffinity chromatography, and HPLC analysis.

Sample preparation

An effective extraction stage is necessary for the quantification of AFB_1 samples in foods. Methanol, a solvent, provided the analyte with the greatest recovery, and was selected as the extraction solvent.

Five grams of sample after finely ground were blended with 1 gram of NaCl and 25 mL of methanol and natri bicacbonat 0,5%; Shaked thoroughly to combine the material and solvent. After centrifugation, 7 mL of the solution was diluted with 28 mL of 0.1 M PBS buffer pH= 7.4. Through the glass microfiber filter, the extract was purified, and collected 25 mL of the filtrate for immunoaffinity column chromatography (IAC).

Extraction by IAC

Aflatoxin B_1 was extracted from the sample using an immunoaffinity column after sample processing. The raw material was injected into the column, and because the AFB_1 antibody had a high affinity for it, the antigen was retained on the column during migration. After removing the complex from the column with 1 mL of methanol, samples of AFB_1 were gathered in different vials for chromatographic analysis. Cleaning is a crucial step in mycotoxin detection because it enables the removal of materials that might impede mycotoxin analysis, thereby increasing accuracy.

High Performance Liquid Chromatography Analysis

After cleaning the sample with an immunoaffinity column, HPLC using a fluorescence detector was used to analyze AFB_1. This is a common method for mycotoxin analysis. The analytical procedure is as follows: the mobile phase mixture consists of water: methanol: acetonitrile (mix together in the ratio of 600:250:150), 350 µl HNO_3 4M and 20 mg KBr, phase rate is 0.8 mL/min. Remove air bubbles by ultrasonic. HPLC (Alliance^{TM} e2695, Waters, USA) is equipped with a C18 column (used as stationary phase) and a fluorescence detector. For the quantification of AFB_1, a fluorescence detector used an excitation wavelength (365 nm) and an emission wavelength (435 nm). Elution is conducted in isocratic mode (when the mobile phase has a constant concentration).

Quality control

Preparation of AFB_1 standard

The standard curves for all analytical indicators were constructed using at least 5 different concentrations, the minimum value being set as the limit of detection (LOD= 0,5 ng/mL) of the instrument. The standard concentrations for AFB_1 were 0; 0,5 ng/mL; 1 ng/mL; 2 ng/mL; 4 ng/mL. The correlation coefficient of 99.61% indicated a highly linear relationship over the concentration range being studied.

Recovery

In order to provide valid and reliable data, the percentage recoveries were implemented in the analytical process. The sample was added with different concentrations of low, medium and high concentrations of the standard solution, and the test was performed in triplicate. Spiking level for AFB_1 were 1; 5; 10 ppb. The test results for AFB_1 in samples after adding the spiked sample were calculated (table 1), ranging from 91.0% to 97.1%. The accuracy assessment was performed by the recovery rate (%), according to the guide of the Association of Official Analytical Chemists (AOAC).[10] AOAC allows 70 – 125% recovery. The values of these the percentage recoveries indicated that the method was highly accurate and could be used for the analysis of AFB_1.

Table 1 - The percentage recoveries of AFB_1 of the analytical method

Sample	**AFB_1**		
Added (µg/ l)	1	5	10
Found (µg/ l)	0.91	4.72	9.71
Recovery (%)	91.00	94.50	97.10

AFB_1: aflatoxin B_1.

Data analysis

The concentrations of AFB_1 were statistically analyzed and presented as mean ± standard deviation (SD). The data were analyzed by ANOVA. All data were analyzed using SPSS (version 19.0, IBM Corp., Armonk, NY, USA).

RESULTS

Average concentrations of AFB_1 in rice and rice products, maize and corn products

While AFB_1 mean concentrations were highest in samples of maize and corn products, rice and rice products had the lowest levels, with readings of 6.35 ppb and 1.79 ppb, respectively. Similarly, processed rice products come in second place with a value of 2.19 ppb, followed by corn and corn products at 2.55 ppb. The rate of processed maize products above the maximum allowable limit (QVCN - National Technical Regulation of Vietnam) is 57.14%, which was about 4 times higher than the rate of processed rice products. The study findings demonstrate that every sample of corn and corn-derived products conform to this regulation (table 2).

Table 2 - AFB_1 contamination levels (ppb) and the frequency of specific foods in Ha Giang Province exceeding the maximum permitted levels (QCVN)

Sample	Number of samples analyzed	Content (ppb)			Number exceeding QCVN Limit	Percentage exceeding QCVN Limit (%)	QCVN Limit (ppb)
		Min	Max	Mean ± SD			
Rice and rice products	72	-	22.12	1.79 ± 5.76	1	1.39	5
Corn and Corn based product	63	-	4.96	2.55 ± 5.59	-	-	5
Processed rice products	33	-	43.81	2.19 ± 5.89	5	15.15	2
Processed corn products	42	-	81.26	6.35 ± 7.99	24	57.14	2

The average level of AFB_1 in cereals in the research area is displayed in table 3. In descending order, processed corn has the highest mean AFB_1 concentrations in cereals, followed by maize and corn products, processed rice, and rice and rice products. Although AFB_1 contamination was discovered in current investigation, the data clearly showed that roughly a third of the samples had AFB_1 amounts below LOD. AFB_1 contamination was most prevalent in processed maize, with a prevalence of 76.19%, somewhat higher than in maize and corn products. With values of 69.44% and 66.67%, respectively, rice and rice products, processed rice, had the lowest prevalence of AFB_1 contamination.

Table 3 - Average concentrations of AFB_1 (ppb) in maize and rice in Ha Giang Province

Product	Rice and rice products	Corn and corn-based product	Processed rice products	Processed corn products
n	**72**	**63**	**33**	**42**
Below LOD	22	16	11	10
% samples below LOD	30.56	25.40	33.33	23.81
Number contaminated	50	47	22	32
Percentage contaminated	69.44	74.60	66.67	76.19
Mead ± SD (ppb)	1.79 ± 5.76	2.55 ± 5.59	2.19 ± 5.89	6.35 ± 7.99

LOD: limit of detection; SD: standard deviation.

Comparison of the rate of exceeding permitted requirements and the average AFB_1 concentration (ppb) in maize and rice in 3 districts of Ha Giang Province

Table 4 analyzed the level of mycotoxin contamination and the frequency of standards being exceeded in 3 districts of the province of Ha Giang. The maize examined in Quan Ba district had an AFB_1 contamination percentage of 80.00% and a mean concentration of 5.87 ppb, respectively. In contrast, rice samples collected in Bac Quang had the lowest prevalence and mean values of AFB_1. The greatest rate of maize samples above the allowable level was found in Hoang Su Phi, where it was 25.71%, followed by Quan Ba district, where it was 22.86%. The maize sample from 1 family in Can Ty commune, Quan Ba district, with a value of 81.26 ppb, had the highest concentration of AFB_1. In comparison to the rice sample, the maize sample from the province of Ha Giang exhibited a substantially greater rate of exceeding the permitted standard.

Table 4 - Concentrations of AFB_1 (ppb) in maize and rice in Quang Ba, Hoang Su Phi and Bac Quang in Ha Giang province

Commodity	**Area of Collection**	**Contaminated Samples/ Total Samples (Incidence %)**	**Mean** ± SD **(ppb)**	**Range (ppb)**	**Percentage exceeding QCVN Limit**
Rice	Quan Ba	25/ 35 (71.43)	3.28 ± 8.42	LOD – 3.81	8.57
Maize		28/ 35 (80.00)	5.87 ± 12.22	LOD - 81.26	22.86
Rice	Hoang Su Phi	26/ 35 (74.29)	1.59 ± 6.01	LOD – 4.40	5.71
Maize		26/ 35 (74.29)	4.14 ± 5.89	LOD – 46.27	25.71
Rice	Bac Quang	21/ 35 (60.00)	0.86 ± 1.39	LOD – 2.87	2.86
Maize		25/ 35 (71.43)	2.20 ± 1.49	LOD – 4.7	20.00

SD: standard deviation; LOD: limit of detection.

AFB_1 levels and comparison to the maximum permissible limit for several foods in Ha Giang

The analyte's average concentration in maize and rice was 2.99 ppb. Meanwhile, this study discovered a number of contaminated samples, the findings in table 5 clearly shows that the AFB_1 concentration in the 59 samples of maize and rice was below the LOD. The findings made it obvious that 151 samples

(71.90%) were contaminated with AFB_1, and 14.28% of those samples exceeded the maximum permitted levels. AFB_1 levels varied within a range of LOD - 81.26 ppb with an average level of 2.99 ppb.

Table 5 - Average concentration of AFB_1 (ppb) in rice and maize in Ha Giang and comparison with the Maximum Residue Limits

Fungal Toxins	**AFB1**
Min (ppb)	LOD
Max (ppb)	81.26
$\bar{X}$ ± SD (ppb)	2.99 ± 7.77
Number contaminated	151
Percentage contaminated	71.90
Percentage exceeding QCVN Limit	14.28

LOD: limit of detection; $\bar{X}$: mean; SD: standard deviation; QCVN: exceeding the maximum permitted levels.

DISCUSSION

The levels of AFB_1 in rice and rice products in current research were lower than those of the author *Hassan HF* et al.[11] and *Alwan N* et al.[12] Furthermore, current findings were significantly inferior to those of *Jayaratne WMSC* et al.[13] for samples of maize and corn-derived goods. Samples of rice and rice-related items had low level of AFB_1 contamination. To improve the quality, monitoring and preventive actions should be implemented as the concentration AFB_1 can change with the seasons.

The average amount of mycotoxins found in rice and rice products in Ha Giang was 1.79 ppb, which is significantly less than Vietnam's maximum permitted levels. The average AFB_1 content in rice in the Bac Quang district is substantially lower than in other areas. The analyte concentration in rice in Quan Ba was significantly greater than in the study areas. Poor transport and storage practice were deducted; the prolonged storage period may have contributed to the contamination.

Several regions' processed corn samples had various levels of mycotoxin. It is a fact that people in Ha Giang store food improperly. The researcher *Ting W.* et al.[14] showed that the fungus grows and produces AFB_1 best in environments with a temperature of 35 °C and a humidity of 90–94%. Residents in Ha Giang are perhaps unaware that some foods may be contaminated with mycotoxins, and can have little interest in building food drying and preservation facilities. It is necessary to use communication techniques to increase public awareness about lowering mycotoxins in food, thereby lowering health hazards for the local population.

During drying process, fungi contamination can occur, which can cause mycotoxins to be produced while the food is stored. Fungi development and the generation of mycotoxin in food are primarily caused by high humidity and inadequate ventilation in the storage area.

AFB_1 was present in the total sample examined, in 68.6% of rice samples and 75.2% of maize samples, albeit at low amounts. Because rice in the tropics is frequently contaminated with AFB_1, which is substantially higher than in temperate locations, several other scientists in Vietnam have come to the conclusion that grains are generally safe.

In Vietnam, drying maize and rice in the sun is a popular practice to preserve food. When rice is improperly dried and the moisture content is greater than 14%, fungi can grow and produce hazardous secondary metabolites.[(15)]

The food tried was in the spring, when there is a lot of precipitation and humidity, and is subject to seasonal fungi growth. The mycotoxin content of spring-harvested corn may be higher than that of summer-harvested corn.

The average mycotoxin level in the current study was 2.99 ppb, which is lower than the level in the study by *Xia L*. et al.[(16)] According to the findings of current study, 14.28% of samples surpassed the permitted standard, while 57.62% of samples were contaminated with mycotoxins but still fulfilled the norms. Current study's average AFB_1 concentration was different from that of other research, which may be the result of differing analytical procedures, sampling sites, and meteorological factors. Although, the majority of the AFB_1-contaminated samples in current study were below Vietnam's the Maximum Residue Limits, prolonged low-concentration exposure can have an impact on human health and should not be disregarded. Therefore, appropriate actions should be taken in at-risk areas to reduce health risks and a sufficient food supply.

Current findings show unequivocally that mycotoxins were present in 151 out of 210 food samples, and that more samples from maize than from rice were contaminated. Additionally, the average mycotoxin level in maize and corn products was 4.07 ppb higher than the level in the rice samples, which was 1.99 ppb.

Corn would be harder to store and more conducive to the growth of fungi because it's the amount of water is higher than that of rice. Furthermore, the climate in Ha Giang is favorable for the development of mycotoxins in maize and corn-based products.

Acute poisoning is less frequent than chronic AFB_1 toxicity, which results from repeated exposure to this mycotoxin. Mycotoxins can lower IgA antibody, impair immunity, and increase susceptibility to infectious diseases.(17) Moreover, Vietnamese citizens have a high prevalence of hepatitis B infection (HBV). The chance of developing liver cancer following mycotoxin exposure is around 30 times higher in positive individuals (HBV) than in negative individuals.(18) Human exposure to mycotoxins can be decreased by limiting the consumption of mycotoxin-tainted foods, and properly preserving foods.

Aflatoxin B_1 concentration in food can be impacted by harvesting, shipping, and storage conditions. It's very challenging to control fungus-contaminated food in the field because of a number of factors, including: ambient temperature, air humidity, soil moisture, and insect infestation. Therefore, to reduce fungal growth, public awareness campaigns should concentrate on the post-harvest and storage periods. People with good knowledge will also have good attitudes and behaviors, which will lower the risk of food contamination by fungi and fungi-related health issues.

Multiple mycotoxins may be present in food at the same time, which is a source of concern.

One limitation of current research is that the health risk posed by mycotoxins was not evaluated. The status of some mycotoxins in commonly consumed foods in Vietnam needs further study. It is necessary to investigate the Food Ingestion Rate per day, the Target Hazard Quotient and the Hazard Index, and the effect of mycotoxins in food on human health.

Aflatoxin B_1 levels in rice in Ha Giang are generally safe, but some samples of processed corn were above the maximum permitted levels. In processed corn products, current study found relatively high levels of AFB_1. In the field and during storage, maize and rice both experience significant fungi growth. Proper procedures are required to ensure that rice and corn are safe because all of the aforementioned factors pose a threat to human health. As AFB_1 concentrations in some samples can significantly affect the health of locals, regular toxicological analysis and communication to raise public awareness should be conducted.

The concentration of aflatoxin B_1 in 14.28% of the samples are over permissible limits by nationwide regulations.

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Conflict of interest

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