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RESEARCH ON HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH A FLUORESCENT DETECTOR TO ANALYZE SIMULTANEOUSLY AFLATOXIN B1, B2, G1, G2 IN VARIOUS FOODS

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LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Nguyen Thanh Duy**, Nguyen Thuy Ngan Ha, Nguyen Lam Kieu Diem, Dang Thi Kim Hang, Ly Tuan Kiet, **Nguyen Quoc Hung**, Le Thanh Tho, Chu Van Hai, **Nguyen Quang Trung** (2022), *Building and validating the analysis process of aflatoxin B1, B2, G1, G2 in baby food powder using UPLC-FLD fluorescence detection ultra-performance liquid chromatography*, Journal of Analytical Chemistry, Physics and Biology, No. 1, Volume 27, Pages 77-81
2. **Nguyen Thanh Duy**, Nguyen Thuy Ngan Ha, Nguyen Lam Kieu Diem, Dang Thi Kim Hang, Ly Tuan Kiet, **Nguyen Quoc Hung**, Le Thanh Tho, Chu Van Hai, **Nguyen Quang Trung** (2022), *Validation of the method for analyzing aflatoxin B1, B2, G1, G2 in corn by UPLC-FLD fluorescence detection liquid chromatography and survey of some corn samples and corn products in Ho Chi Minh City*, Journal of Chemical, Physical and Biological Analysis, No. 2, Volume 27, pp. 183-188.
3. **Thanh Duy Nguyen,** Thuy Ngan Ha Nguyen, Tuan Kiet Ly, **Quoc Hung Nguyen,** Thanh Tho Le, Van Hai Chu, **Tien Dat Nguyen,** Dinh Vu Le **(**2023), *A high-performance method for quantitation of aflatoxins B1, B2, G1, G2: Full validation for raisin, peanut matrices, and survey of related products at Ho Chi Minh City***,** Food Science and Nutrition, Vol 11, Issue10, pp, 5639-6707, (https://doi,org/10,1002/fsn3,3594).
4. **Thanh Duy Nguyen,** Thuy Ngan Ha Nguyen, Tuan Kiet Ly, **Quoc Hung Nguyen,** Thanh Tho Le, **Tien Dat Nguyen,** Ngoc Minh Trương (2024), *Health risk assessment of dietary aflatoxin B1 exposure in common food matrices in Vietnam*, Acta Chromatographica (https://doi.org/10.1556/1326.2024.01286).
5. **Nguyen Thanh Duy**, Nguyen Thuy Ngan Ha, Nguyen Lam Kieu Diem, Dang Thi Kim Hang, Ly Tuan Kiet,(2024), *Process for simultaneous quantitative analysis of aflatoxin B1, G1, B2, G2 in chili powder by non-derivative method with high-performance liquid chromatography with fluorescence detection*, Exclusive utility solution patent No. 3872, granted by the National Office of Intellectual Property under Decision No. 142123/QĐ-SHTT, issued on December 4, 2024.

INTRODUCTION

Vietnam is a country with a high proportion of agricultural production, with many valuable export products. However, one of the challenges for this industry is that products can be contaminated with mycotoxins, including aflatoxin (AF). *Aspergillus fungi* are the main agents producing AF, a toxin that can cause serious harm to human and animal health, including DNA damage, cancer, and developmental defects. Long-term exposure to AF can lead to acute poisoning, causing severe liver damage. Infants and young children are the most vulnerable groups due to their immature metabolism and higher absorption rates than adults. Common aflatoxins include aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2), of which AFB1 is classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC). Foods such as oilseeds (soybeans, peanuts, sunflower seeds, and cottonseeds), cereals (corn, sorghum, wheat, and rice), spices (chili, black pepper, coriander, turmeric, and ginger), and dried fruits (raisins, pistachios, almonds, and walnuts) are commonly contaminated with aflatoxins produced by the fungus *Aspergillus spp*. In addition to its impact on human health, AF also leads to significant economic losses due to widespread food contamination.

Among the foods with a high risk of AF contamination, raisins, chili powder, corn kernels, and peanuts are popular products with high nutritional value and play an important role in the daily diet. Raisins are rich in nutrients and energy, often consumed directly or used in food processing. Chili powder is an important spice, contributing to the characteristic flavor of many dishes. Corn kernels are popular foods, used in a variety of processing from fresh foods to processed industrial products. Peanuts are a familiar nutritious nut, widely consumed in many forms such as direct consumption, peanut butter, candy, and cooking oil. In addition to their importance in food and diet, all four of these products belong to the food group with maximum allowable limits according to Vietnamese and international standards that need to be strictly controlled. Therefore, the detection and quantification of AF in this food group by a green, sensitive, and efficient method is essential to ensure food safety and prevent potential risks to human health.

Currently, the analytical methods commonly used to determine AF are thin-layer chromatography, ELISA, high-performance liquid chromatography with fluorescence detection (HPLC-FD) combined with pre-column or post-column derivatization, and liquid chromatography coupled with mass spectrometry (LC-MS/MS). However, these methods still have one or more limitations, such as low sensitivity, false positives, complicated procedures, or high costs. Meanwhile, ultra-performance liquid chromatography with fluorescence detection (UPLC-FD) is designed with a large-flow fluorescence signal measurement chamber and a mercury-xenon lamp, optimizing sensitivity without the need for derivatization. This method is notable for its time-saving, solvent-reducing, and environmental friendliness. However, studies on the simultaneous analysis of aflatoxins (B1, B2, G1, and G2) by UPLC-FD in Vietnam are still very limited.

Therefore, the thesis topic "Research on high-performance liquid chromatography with a fluorescent detector to analyze simultaneously aflatoxins B1, B2, G1, G2 in various foods" is carried out with the desire to contribute to the development of an effective analytical method to simultaneously determine AFB1, AFB2, AFG1, AFG2 in four foods that are often at risk of exposure: peanuts, chili powder, raisins, and corn. The thesis was carried out with the following objectives:

➢ Research and development of a method for simultaneous analysis of aflatoxin (B1, B2, G1, and G2) in peanuts, chili powder, raisins, and corn using UPLC-FD, without derivatives, helping to save time, reduce solvents, and simplify the process.

➢ Research to determine the content of aflatoxin (B1, B2, G1, and G2) in samples of peanuts, chili powder, raisins, and corn kernels collected at markets in Ho Chi Minh City in 2020-2022.

➢ The study assessed the hazard of aflatoxin toxicity through the daily intake index (EDI) and the margin of exposure (MOE) for both male and female adults according to 3 body types: lean, normal, and overweight.

New contributions of the thesis

- For the first time in Vietnam, a study has standardized a method for the simultaneous analysis of aflatoxins B1, B2, G1, and G2 in peanuts, chili powder, raisins, and corn using ultra-performance liquid chromatography with fluorescence detection (UPLC-FD) without derivatization. This method offers several outstanding advantages: a simple procedure, high sensitivity (LOD 0.1 µg/kg; LOQ 0.3 µg/kg), time and solvent efficiency, environmental friendliness, health safety, and compliance with the permissible limits set by QCVN 8-1/2011/BYT. It is also highly applicable to laboratory settings.
- An initial assessment of the presence and distribution of aflatoxins in food samples collected in Ho Chi Minh City revealed a strong correlation between AFB1 and total aflatoxins, leading to the proposal of a predictive model for toxin contamination risk.
- For the first time, dietary exposure to AFB1 was evaluated across three body condition groups (lean, normal, overweight) among Vietnamese adults, providing essential data for public health risk assessment.

CHAPTER 1. OVERVIEW

1.1. Introduction of aflatoxin

Aflatoxins are commonly found in agricultural products. Currently, about 18 different types of aflatoxins have been found: B (B1, B2); B2a; B3 (Parasiticol); G (G1, G2); G2a; M (M1, M2); M2a; GM1, GM2, GM2a; P1, Q1, Q2a; Aflatoxicol R0; Aflatoxicol M1; Aflatoxicol H1; Aspertoxin. However, there are 4 main types most commonly found, including 4 compounds of the bis-furanocoumarin group, which are metabolic products created by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, named B1, B2, G1, and G2. The four substances are distinguished based on their fluorescent color. The symbol "B" stands for Blue under UV light, and the letter G stands for Green.

Aflatoxin G1 has a structure very similar to that of AFB1: it has two lactone functions, while AFB1 has only one (Figure 1.1). By dehydrating the double bond in the terminal hydrofuran nucleus of aflatoxin B1 and G1, two other toxic products, aflatoxin B2 and G2, are obtained.

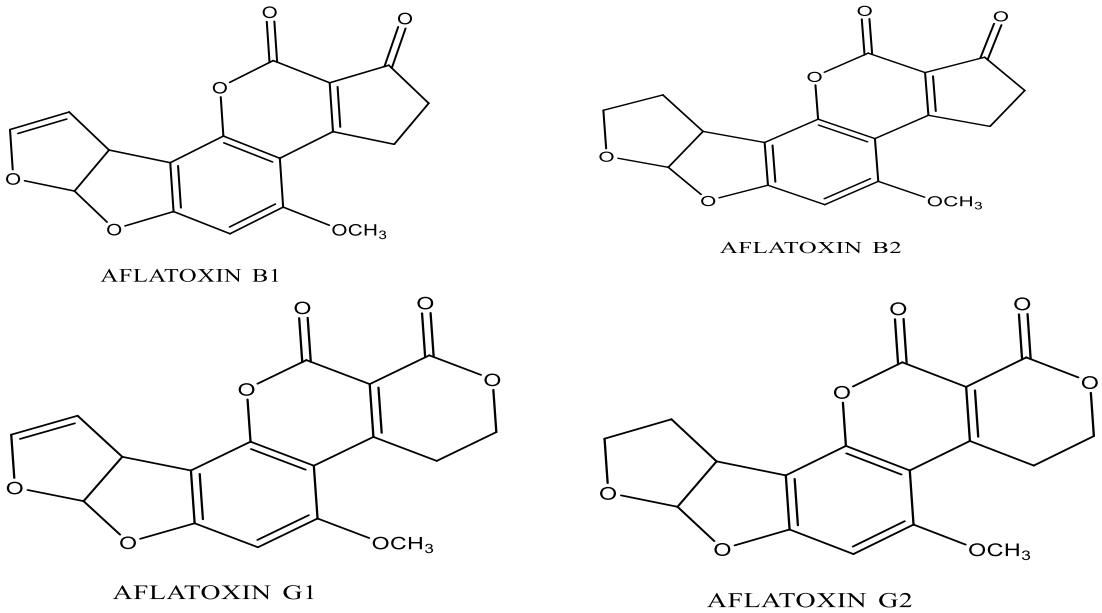


Figure 1.1: Molecular structure of the four main types of aflatoxins

1.2. Toxicity and permissible limits of aflatoxin

1.2.1. Toxicity of aflatoxin

The International Agency for Research on Cancer (IARC) classifies aflatoxin B1 as a group I carcinogen. Diseases caused by AF consumption are called aflatoxicosis. Chronic aflatoxicosis leads to cancer, immunosuppression, and other diseases with slow disease progression. Meanwhile, acute aflatoxicosis can lead to death.

1.2.2. Permissibility of aflatoxin

Due to AF's high toxicity and carcinogenicity, strict maximum allowable limits (ML) have been established to minimize exposure. According to QCVN 8-1:2011/BYT standards, the permissible levels for AFB1 and total AF vary by food type: peanuts and corn have MLs of 2 µg/kg and 4 µg/kg,

respectively, while chili powder and raisins are allowed higher limits of 5 µg/kg and 10 µg/kg, reflecting differences in contamination risks and food matrices.

1.3. Human exposure and toxicity to aflatoxins

1.3.1. Risk of aflatoxin contamination in food

Aflatoxin is one of the most dangerous mycotoxins, commonly found in food due to the invasion of *Aspergillus* species, especially *A. flavus* and *A. parasiticus*. Of these, *A. flavus* mainly produces AFB1 and AFB2, which are commonly found in cereals, peanuts, and spices. Meanwhile, *A. parasiticus* not only produces AFB1 and AFB2 but also synthesizes AFG1 and AFG2, which mainly contaminate oilseeds and dried fruits.

Environmental conditions, such as high humidity, warm temperatures, and improper storage methods, are key factors that promote the growth of mold and the formation of aflatoxin. In the supply chain, from harvesting to storage, unsecured conditions can promote the growth of *Aspergillus spp*., the primary agent responsible for producing AF.

1.3.2. Assessment of aflatoxin exposure and impact on human health

The average daily consumption of peanuts, chili powder, raisins, and corn kernels in Vietnam during the period 2019 - 2021 according to the report of the Food and Agriculture Organization of the United Nations is 0.01493 kg/person/day; 0.00029 kg/person/day; 0.000030 kg/person/day and 0.02219 kg/person/day.

According to the results of the National Nutrition Survey 2019 - 2020 published by the Ministry of Health in 2021, the average height for men is 168.1 cm and for women is 156.2 cm and based on the BMI index for thin people (16 - 18.4); normal (18.5 - 24.9); obese people (25 - 40) corresponding to the average body weight (kg b.m/person) for men 48.60; 61.32; 91.84 and for women 41.96; 52.94; 79.29

The evaluation parameters to assess the level of harm of AF to human health are the estimated daily intake (EDI), the exposure margin (MOE), and the risk index (HI).

1.3.3. Aflatoxin contamination situation abroad

The analysis of AF content in food has always been an important part of countries' annual food safety assessment plans. This is demonstrated by 53 scientific studies from more than 30 countries, studying 17 types of food, published by authors Mahato (2005–2017) and Jallow (2015–2020). Among them, peanuts, nuts, figs, corn, rice, spices, chili, and dried fruits were products containing high levels of AF.

1.3.4. Aflatoxin contamination situation in Vietnam

Research on AF in food was published with three typical studies by authors Mai Huong (2016), Thanh Xuan (2019), and Huu Tuan (2020). These studies were conducted in Hanoi, Ha Giang, Lao Cai, and Thanh Hoa, showing that corn, peanuts, and spices are products with high levels of AF contamination.

In addition, several surveys on AF toxin contamination have been presented at domestic scientific conferences and reported through newspapers and radio, highlighting the potential risk of AF contamination in various foods sold at traditional markets and market-based businesses.

1.4. Methods of analysis of aflatoxin in food

1.4.1. Method of extraction and clean up of aflatoxin

Various methods are used for extraction and cleanup, including Liquid-Liquid Extraction (LLE), Liquid-Solid Extraction (LSE), Ultrasonic Extraction, Supercritical Fluid Extraction (SFE), and Solid Phase Extraction (SPE). Among these, SPE stands out as the most accurate and reliable technique for preparing mixtures before qualitative and quantitative analysis. SPE allows for isolating the target analyte while effectively removing interfering components.

A common application of SPE is the use of immunoaffinity columns (IAC), cleanup and multifunctional cleanup (MFC) columns, such as MultiSep®, MycoSep®, and Myco6in1. Cleanup steps, including IAC and MFC techniques, are typically performed before aflatoxin extraction. These methods are highly effective for isolating aflatoxin analytes and removing impurities, making them ideal for subsequent quantification using high-performance liquid chromatography (HPLC).

1.4.2. Methods for detection and quantification of aflatoxin

Thin-layer chromatography (TLC), Mini-Column, and ELISA Kits: These methods are commonly used for the qualitative determination of the total content of the four main aflatoxins (B1, B2, G1, and G2). They are fast and simple but often prone to false positives, making it challenging to accurately quantify individual aflatoxins in a sample.

Liquid Chromatography with Fluorescence Detection (HPLC-FLD): This technique is highly accurate and suitable for quantitative analysis, meeting stringent AF limits set by the Ministry of Health and international regulatory agencies. However, the method requires the use of environmentally hazardous solvents, such as chloroform and benzene, as well as toxic derivatizing agents for pre- or post-column treatment, which pose environmental and safety concerns.

Liquid Chromatography with Mass Spectrometry (LC-MS): LC-MS is another reliable quantitative method offering exceptional accuracy. However, its high cost and the complexity of equipment operation make it less accessible compared to HPLC-FLD.

❖ ***From the information and data presented in the overview section, it can be seen that:***

Previous studies have underscored the risk of AF contamination in nuts such as peanuts, raisins, corn, and chili powder, raising serious food safety concerns, particularly regarding traditional markets and market vendors, where most food is distributed to consumers, but storage conditions and quality control are often lacking. In Ho Chi Minh City, traditional markets play a key role in supplying daily food. However, storage practices in these markets frequently fall short of food safety standards, with poor temperature and humidity control creating conditions that can lead to cross-contamination between batches and increase the risk of mycotoxin contamination, including aflatoxins. Given these risks, it is essential to conduct a survey of AF content and assess contamination levels in peanuts, chili powder, raisins, and corn to evaluate both exposure levels and the potential health risks associated with aflatoxin intake.

Currently, standard analytical methods such as TCVN 7930:2008, TCVN 9522:2012, TCVN 10638:2014, AOAC 2005.08, and AOAC 994.08 recommend the simultaneous detection of aflatoxins (B1, B2, G1, G2) in common sample matrices like peanuts, corn, pistachios, chili powder, and cereals using high-performance liquid chromatography with fluorescence detection (HPLC-FD). These methods typically involve pre- or post-column derivatization to enhance signal detection. While they meet the required standards for accuracy and sensitivity, they also present several drawbacks,

including lengthy analysis times, high consumption of organic solvents, and the need for specialized derivatization equipment, which can reduce efficiency and increase environmental impact. An alternative study explored a derivatization-free UPLC-FD method applied to cereals, though its detection limit for total aflatoxins exceeded the threshold set by QCVN 8-1:2011/BYT. Building on previous research, the current study adapted the UPLC-FD system and sample preparation protocol, optimizing the method for a wider range of matrices - including peanuts, chili powder, corn kernels, and raisins - without the use of derivatization. This updated method not only meets the sensitivity requirements of QCVN 8-1:2011/BYT but also supports environmentally friendly practices by reducing analysis time, minimizing solvent usage, lowering pollution, and simplifying sample preparation, all while safeguarding analysts' health. Furthermore, the study aims to estimate hazard levels and assess cancer risk associated with AF exposure, offering a more comprehensive understanding of the public health implications of aflatoxin contamination.

Therefore, the main contents of the thesis need to focus on research, including:

- Research on suitable chromatographic conditions for simultaneous analysis of aflatoxin (B1, B2, G1, and G2) on ultra-performance liquid chromatography with fluorescence detection (UPLC-FD) system and research on the extraction process of aflatoxin (B1, B2, G1, and G2) in nuts including peanuts, chili powder, raisins, and corn kernels.

- Research to confirm the validity of the method for analyzing aflatoxin (B1, B2, G1, and G2) in peanuts, chili powder, raisins, and corn kernels.

- Research to determine the aflatoxin content (B1, B2, G1, and G2) in samples of peanuts, chili powder, raisins, and corn kernels collected at markets in Ho Chi Minh City in 2020-2022.

– Study to assess the hazard of aflatoxin toxicity through daily intake index (EDI) and exposure margin (MEO) for both male and female adults according to 3 body types: lean, normal, and overweight.

CHAPTER 2. RESEARCH OBJECTS AND METHODS

2.1 Research object

The toxins AFB1, AFB2, AFG1, AFG2, and total AF (sum of AFB1, AFB2, AFG1, and AFG2) in peanuts, chili powder, raisins, and corn.

2.2. Chemical, equipment, and material

2.2.1. Chemical

The chemicals and materials used in the study included HPLC grade acetonitrile (ACN, ThermoFisher, USA) with a purity of ≥ 99.9% and water content ≤ 0.02%, formic acid (HCOOH, Merck, Germany) with a purity of ≥ 99.9% and acetic acid content ≤ 0.05%, and HPLC grade methanol (MeOH, Merck, Germany) with a purity of ≥ 99.9% and water content ≤ 0.02%. Aflatoxin immunoaffinity column (IAC, Vicam, USA) was used at a flow rate of 1-3 mL/min, cross-contamination level < 0.01%, and column volume of 3 mL. Double-distilled deionized water was obtained from a Milli-Q Direct 8 apparatus from Merck Millipore. Sodium chloride (NaCl, Merck, Germany) with purity ≥ 99% and moisture ≤ 0.5%; Phosphate buffer solution pH 7.4 (PBS). Aflatoxin standard solution (LGC standards GmbH, Germany) includes B1: 2 µg/mL, B2: 0.5 µg/mL, G1: 2 µg/mL, and G2: 0.5 µg/mL in ACN solvent.

Preparation of AF standard solution was prepared in the following order: Stock standard C0 (5 µg/mL), consisting of 2 µg/mL AFB1, 0.5 µg/mL AFB2, 2 µg/mL AFG1, and 0.5 µg/mL AFG2 in ACN solvent, stored at 2-8 °C, and used within 12 months. From the stock standard, intermediate standard 1 (CTG1, 500 µg/L) was prepared by accurately pipetting 1 mL of C0 solution into a 10 mL volumetric flask and diluting to the mark with ACN. Next, intermediate standard 2 (CTG2, 50 µg/L) was prepared by taking 1 mL of CTG1 into a 10 mL volumetric flask and then diluting it with 0.2% HCOOH/ACN (1:1) solution and intermediate standard 3 (CTG3, 10 µg/L) was prepared by taking 1 mL of CTG2 into a 5 mL volumetric flask and diluting it with 0.2% HCOOH/ACN (1:1) solution to the mark. Finally, working standards from 0.5 – 50 µg/L were prepared by taking the required volume of intermediate standard solution into a vial and adding 0.2% HCOOH/ACN (1/1) so that the total volume was 1 mL (1000 µL).

The solutions are prepared as follows:

- 0.1% HCOOH solution: Take 1 mL of pure HCOOH (≥ 99.9 %) into a 1000 mL volumetric flask, add H_2O to the mark, shake well, and sonicate for 5 minutes.

- 0.2% HCOOH solution: Take 2 mL of pure HCOOH (≥ 99.9 %) into a 1000 mL volumetric flask, add H_2O to the mark, shake well, and sonicate for 5 minutes.

- 0.2% HCOOH/ACN solution (1/1, v/v): Take 50 mL of 0.2% HCOOH solution into a 100 mL volumetric flask, add ACN to the mark, shake well, and sonicate for 5 minutes.

- ACN/H_2O solution (6/4, v/v): Take 600 mL of ACN into a 1000 mL volumetric flask, add H_2O to the mark, shake well, and sonicate for 5 minutes.

- MeOH/H_2O solution (8/2, v/v): Take 800 mL of MeOH into a 1000 mL volumetric flask, add H_2O to the mark, shake well, and sonicate for 5 minutes.

2.2.2. Equipment and material

The equipment and materials used in this study include:

- Chromatographic System: Ultra high-pressure liquid chromatography system with main components including quarterly pump (Serial No. M15QSM234A; Singapore), FTN autosampler (Serial No. M15SD1942G; Singapore), FD fluorescence detector (Serial No. M15UPF301G; Singapore), BEH C18 chromatographic column (150*2.1 mm; 1.7 µm), and pre-column of the same stationary phase from Waters.

- Other supporting equipment includes: a shaker, vortex mixer, centrifuge, ultrasonic bath, mobile phase filtration system, analytical balance with a precision of 0.0001 g, milligram balance accurate to 0.001 mg, and micropipettes with measuring ranges of 20–200 µL and 100–1000 µL. Additional laboratory glassware and tools consist of measuring cylinders (100 mL, 500 mL, and 1000 mL), volumetric flasks (5 mL, 10 mL, 25 mL, and 50 mL), 1.5 mL glass vials, 1-liter bottles with lids, 50 mL PTFE plastic centrifuge tubes, 11 cm blue filter paper, and PTFE filter membranes with a pore size of 0.45 µm and a diameter of 13 mm.

2.3. Research methods

2.3.1. Method for simultaneous analysis of aflatoxin (B1, B2, G1 and G2) on UPLC-FD system

Optimization of chromatographic conditions for simultaneous analysis of aflatoxins (B1, B2, G1, and G2), studying 3 factors including changing the mobile phase composition to improve the separation and detection of substances, assessing the compatibility of the AF analysis system, and establishing the linear range and calibration curve.

Based on published studies, the mobile phase used had a composition of H_2O/ACN/MeOH with a ratio of 64/18/18, a BEH C18 chromatographic column (150 × 2.1 mm; 1.7 µm), an excitation wavelength of Ex = 365 nm, an emission wavelength of Em = 455 nm and the column temperature was maintained at 40°C. In this study, two mobile phase conditions were investigated, including H_2O/ACN/MeOH (56/22/22), and H_2O/ACN/MeOH (64/18/18). Experiments were performed by injecting a standard solution of aflatoxin mixture (B1, B2, G1, and G2) with a concentration of 10 µg/L into the UPLC-FD liquid chromatography system. After selecting the mobile phase conditions for investigation, the study continued to adjust the percentage of HCOOH added to the mobile phase with concentrations of 0%, 0.1%, and 0.2%, respectively.

2.3.2. Method for the extraction of aflatoxins B1, B2, G1, and G2 in peanut, chili powder, raisin, and corn kernel samples

The method of extracting aflatoxin (B1, B2, G1, and G2) in food samples was carried out through 2 main surveys:

- Survey of aflatoxin extraction solvents (B1, B2, G1, and G2) evaluated based on criteria such as recovery efficiency and precision in analysis:

+ The procedure was carried out as follows: 5.00 g of sample was accurately weighed using an analytical balance (precision to 0.1 mg), then extracted with 20.00 mL of either MeOH/H_2O (8:2) or ACN/H_2O (6:4). The mixture was vortexed, subjected to ultrasonic treatment for 30 minutes, and centrifuged at 9000 rpm for 3 minutes. The supernatant was filtered through green filter paper. From the filtrate, 5.00 mL was mixed with 20.00 mL of either H_2O or PBS buffer, vortexed, and centrifuged again at 9000 rpm for 3 minutes, and 20.00 mL of the resulting solution was passed through an IAC column. The column was washed with 20.00 mL of distilled water. Aflatoxins were then eluted with

3.00 mL of MeOH, evaporated to dryness, and reconstituted in 1.00 mL of a 0.2% HCOOH/ACN (1:1) solution. The final solution was filtered through a 0.22 µm PTFE membrane filter before injection into the UPLC-FD system.

- Survey of the influence of sample matrix on the extraction process of aflatoxin (B1, B2, G1, and G2) by evaluating the matrix effect (%ME).

2.3.3. Method for validating the analytical method for aflatoxins (AFs) in peanuts, chili powder, raisins, and corn kernels

Before applying the method for aflatoxin analysis (B1, B2, G1, and G2) on real samples such as peanuts, chili powder, raisins, and corn kernels, method validation is an indispensable step.

Validation of the method through important parameters such as selectivity, method detection limit (MDL) and method quantitation limit (MQL), precision (repeatability RSDr and reproducibility RSD_R), trueness (recovery efficiency), sample dilution, sample stability, measurement uncertainty, and analytical result assurance helps ensure that the method meets the strict requirements for accuracy, reliability, and applicability on complex food sample matrices.

2.3.4. Method for assessing the hazard of aflatoxin to human health according to three body types: lean, normal, and overweight

The method for assessing the hazard level of aflatoxin to human health is through estimated daily intake (EDI), the margin of exposure (MOE), and the hazard index (HI). The method includes collecting data on AF content in peanuts, chili powder, raisins, and corn kernels. Based on the daily dietary data, the groups of subjects were divided according to their body mass index (BMI) to analyze three body types: lean, normal, and obese, applicable to both adult men and women.

The EDI, MOE, and HI indexes were calculated, and then the EDI and MOE were compared between the body types to assess the risk of aflatoxin exposure in each physical condition.

2.3.5. Sample collection and preservation method

In this study, 400 peanut samples including (370 raw peanuts and 30 processed peanuts); 400 chili powder samples; 400 raisin samples, and 400 corn kernel samples (100 white corn kernels, 100 yellow corn kernels, and 200 processed corn kernels) were collected in 12 districts (District 1 - District 12), Tan Binh district, Tan Phu district, Thu Duc district, Phu Nhuan district, Go Vap district, Binh Thanh district, Binh Tan district and Hoc Mon district of Ho Chi Minh City by random sampling method.

Peanut samples and chili powder samples were collected from January to April 2021 and from January to May 2022 for raisin and corn kernel samples. After collection, the samples will be transferred to the laboratory's sample receiving department for coding and sample homogenization. Samples are stored at ≤ -20 °C if not analyzed on the same day.

2.3.6. Data processing method

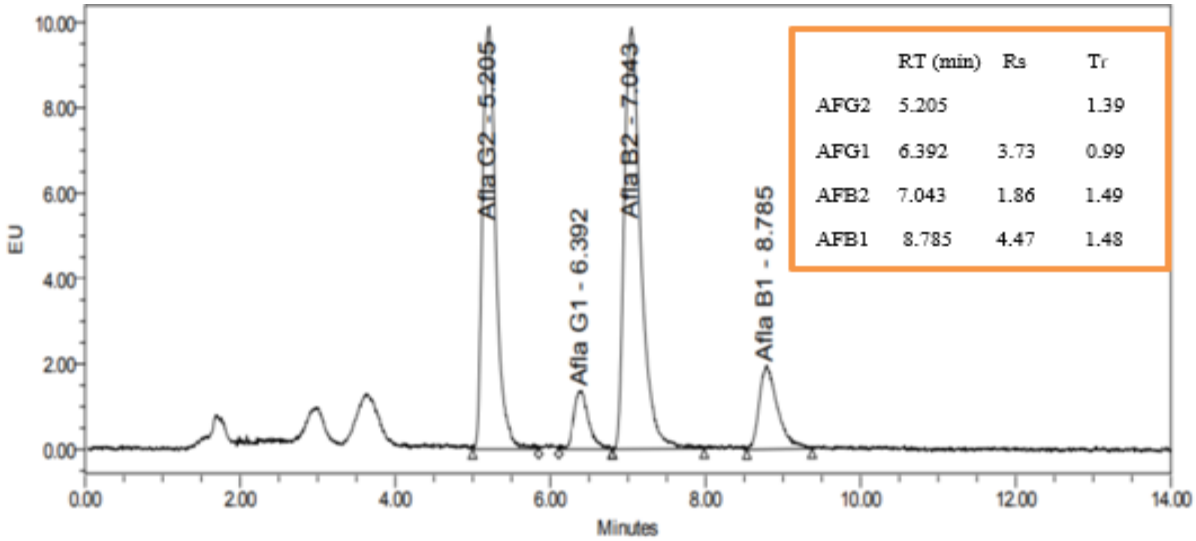
Data were processed and statistically analyzed using Microsoft Excel 2019 software and Empower 3 software, calculated from the UPLC-FD Waters analyzer. Samples with aflatoxin concentrations (B1, B2, G1, and G2) below the detection limit (lower than LOD) were considered zero. Pearson correlation analysis, ANOVA, t-test, and linear regression analysis (SPSS 22.0 software) were performed to evaluate the relationship between aflatoxin content (B1, B2, G1, and G2) and total AF in peanut, chili powder, raisin, and corn kernel samples.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Optimization and survey of chromatographic conditions for simultaneous analysis of aflatoxin (B1, B2, G1, and G2) using UPLC-FD

The results of chromatographic conditions, including optimization of mobile phase composition, signal stability of aflatoxin peaks (B1, B2, G1, and G2), and linear range of the standard curve, are summarized in Table 3.21.

A representative chromatographic analysis of 10 µg/L in ACN/0.2% HCOOH is conducted using a C18 analysis column (150 × 2.1 mm; 1.7 µm) at 40 °C. The flow rate is set at 0.2 mL/min, with an injection volume of 5 µL. Detection is performed at a stimulation wavelength of 365 nm and an emission wavelength of 455 nm. The resolution (R_S) values obtained are 3.8 for AFG1, 1.9 for AFB2, and 4.5 for AFB1, as presented in Figure 3.1



Hình 3.1: Standard chromatogram 10 µg/L in ACN/0.2% HCOOH with mobile phase composition 0.1% HCOOH/ACN/MeOH (64/18/18)

3.2. Survey of the extraction process for aflatoxins (B1, B2, G1, and G2) in peanut, chili powder, raisin, and corn kernel samples

3.2.1. Survey on solvents for extracting aflatoxin (B1, B2, G1, and G2) from peanut, chili powder, raisin, and corn kernel samples

The extraction process of aflatoxin (B1, B2, G1, G2) in the sample matrix of peanuts, chili powder, raisins, and corn kernels is summarized as follows:

Corn kernel: Accurately weigh 5.00 g of the sample using an analytical balance (precision to 0.1 mg), then add 20.00 mL of MeOH/H_2O (8/2). Extraction was performed under ultrasound for 30 min, centrifuged at 9000 rpm for 3 min, and filtered through Ω 110 mm filter paper. Take 5,00 mL of the filtrate, and add 20,00 mL of distilled water. Vortex and centrifuge at 9000 rpm for 3 min. Add 20,00 mL of the solution after centrifugation to the IAC. Wash impurities with 20 mL of distilled water. Elute with 3,00 mL of MeOH, dry under nitrogen flow, and add 1.00 mL of ACN/H2O with

0.2% of HCOOH (1/1), vortex. Filter through a 0.45 µm PTFE filter before injection into the ultra-performance liquid chromatography coupled with fluorescence detection (UPLC-FD) system.

Chili powder and raisin: Accurately weigh 5.00 g of sample using an analytical balance (precision to 0.1 mg), then add 20.00 mL of MeOH/H_2O (8/2). Extraction was performed under ultrasound for 30 min, centrifuged at 9000 rpm for 3 min, and filtered through Ω 110 mm filter paper. Take 5,00 mL of the filtrate, and add 20,00 mL of PBS buffer. Vortex and centrifuge at 9000 rpm for 3 min. Add 20,00 mL of the solution after centrifugation to the IAC. Wash impurities with 20 mL of distilled water. Elute with 3,00 mL of MeOH, dry under nitrogen flow, and add 1.00 mL of ACN/H2O with 0.2% of HCOOH (1/1), vortex. Filter through a 0.45 µm PTFE filter before injection into the ultra-performance liquid chromatography coupled with fluorescence detection (UPLC-FD) system.

Peanut: Weigh 5.00 g of sample on an analytical balance (precision to 0.1 mg), then add 20.00 mL of ACN/H_2O (6/4). Extraction was performed under ultrasound for 30 min, centrifuged at 9000 rpm for 3 min, and filtered through Ω 110 mm filter paper. Take 5,00 mL of the filtrate, and add 20,00 mL of distilled water. Vortex and centrifuge at 9000 rpm for 3 min. Add 20,00 mL of the solution after centrifugation to the IAC. Wash impurities with 20 mL of distilled water. Elute with 3,00 mL of MeOH, dry under nitrogen flow, and add 1.00 mL of ACN/H_2O with 0.2% of HCOOH (1/1), vortex. Filter through a 0.45 µm PTFE filter before injection into the ultra-performance liquid chromatography coupled with fluorescence detection (UPLC-FD) system.

Extraction efficiency and repeatability for each sample matrix are summarized in Table 3.21.

3.2.2. Survey on the influence of sample matrix on the extraction of aflatoxin (B1, B2, G1, and G2) from peanut, chili powder, raisin, and corn kernel samples

The matrix effect (% ME) in the sample matrices of peanuts, chili powder, raisins, and corn kernels were all lower than 20%, so the standard curve in the solvent was used to confirm the value of the method instead of constructing a standard curve on the sample matrix which is laborious, time-consuming and chemical-intensive. (see summary in Table 3.21).

3.3. Method validation

After the survey and validation of the method for simultaneous analysis of aflatoxin (B1, B2, G1, and G2) in 4 sample matrices of peanuts, chili powder, raisins, and corn kernels using a non-derivative method with UPLC-FD technique, the results are summarized in Table 3.21.

Table 3.21: Summary of research results on simultaneous aflatoxin (B1, B2, G1, and G2)

process on the UPLC-FD system

No	Research content	Acceptance criteria	Results achieved
1	Survey on chromatographic conditions for analysis of simultaneous aflatoxin (B1, B2, G1, and G2)		
1.1	Optimizing mobile phase composition	Resolution (Rs) ≥ 1.5 Drag coefficient (T_f): $0.8 \leq T_f \leq 1.5$	Rs: 1.9-4.5 T_f: 0.9-1.5 Mobile phase selection 0.1%HCOOH/ACN/MeOH (64/18/18)

No	Research content	Acceptance criteria	Results achieved
1.2	Investigation of signal stability on UPLC-FD	-Retention time (% RSD <= 2%) -Peak area (% RSD <= 2%) -Resolution $R_s \geq 1.5$ -Drag coefficient $0.8 \leq T_f \leq 1.5$	% RSD (RT, n=6) =0.07-0.11% % RSD (Area, n=6) =0.18-0.59% R_s = 1.98-4.98 (n=6) T_f = 1.28-1.5 (n=6)
1.3	Linear range and standard curve	Standard curve: $0.99 \leq R^2 \leq 1$, Deviation: calculated concentration at each reference point ≤ 15% compared to the actual value (except concentration at MQL point ≤ 20%	Achieved standard curve correlation coefficient: R^2 = 0.9999, with concentration range 0.2 - 20.0 µg/L for AFB1 and AFG1 and 0.05 - 5.0 µg/L for AFB2 and AFG2, and % deviation: 15-18%
2	Survey of the extraction process for aflatoxins (B1, B2, G1, and G2) in peanut, chili powder, raisin, and corn kernel samples		
2.1	Survey on solvents for extracting aflatoxin (B1, B2, G1, and G2)		
2.1.1	Peanut	- H% extraction is 60-115% - Repeatability RSDr ≤ 21%	%H = 85.7 - 9 9.2% %RSDr < 6% Selection of extraction solvent ACN/H_2O (6/4) – H_2O
2.1.2	Chili powder	- H% extraction is 60-115% - Repeatability RSDr ≤ 21%	%H = 70.1 - 97.8% %RSDr < 6% Selection of extraction solvent MeOH/H_2O (8/2) - PBS
2.1.3	Raisin	- H% extraction is 60-115% - Repeatability RSDr ≤ 21%	%H = 72.4 – 95.4% %RSDr < 3% Selection of extraction solvent MeOH/H_2O (8/2) - PBS
2.1.4	Corn	- H% extraction is 60-115% - Repeatability RSDr ≤ 21%	%H = 90.3 – 99.8% %RSDr < 2% Selection of extraction solvent MeOH/H_2O (8/2) - H_2O
2.2	Survey on the influence of sample matrix on the extraction of aflatoxin (B1, B2, G1, and G2) from peanut, chili powder, raisin, and corn kernel samples		
2.2.1	Peanut	Matrix effect (%ME) is considered minimum (%ME < 20), medium (20≤ %ME≤ 50), and maximum (%ME >50).	Peanut: 2.81 – 8.04%
2.2.2	Chili powder		Chili powder: 13.9 – 18.1%
2.2.3	Raisin		Raisin: 5.63 – 11.4%
2.2.4	Corn		Corn: 6.1 – 11.3%
3	Validation of the method		
3.1	Selectivity	No interference appeared at the retention time of AFB1, AFB2,	Good selectivity

No	Research content	Acceptance criteria	Results achieved
		AFG1, and AFG2 in the blank sample chromatogram.	
3.2	MDL and MQL on four sample matrices: peanut, chili powder, raisin, and corn kernel	MDL: Signal to noise (S/N) meets the criterion S/N ≥ 3	MDL = 0.1 µg/kg for each AFB1, AFB2, AFG1, AFG2 on 4 sample matrices with S/N = 3.10 – 9.61
		MQL: Acceptance criteria for recovery efficiency around 70-120%, repeatability $RSD_R \leq 20\%$	% H: 94.9 - 99.4% (peanut) % H: 81.6 - 94.3% (chili powder) % H: 86.4 – 96.9% (raisin) % H: 71.8 – 85.7% (corn) % RSD_R < 3% (peanut) % RSD_R < 7% (chili powder) % RSD_R < 3% (raisin) % RSD_R < 7% (corn) Quantitation limit MQL = 0.3 µg/kg for each AFB1, AFB2, AFG1, and AFG2 on 4 sample matrices of peanuts, chili powder, raisins, and corn kernels.
3.3	Precision (repeatability RSDr and reproducibility RSD_R) and trueness of the method	Precision (repeatability RSDr% and reproducibility RSDR%) should not exceed 22%, recovery should be within the range of 60-115%	Peanut % H: 76.5-99.8% % RSDr and % RSD_R < 5% Chili powder % H: 81.2 - 87.2% % RSDr and % RSD_R < 3% Raisin % H: 80.9-97.3% % RSDr and % RSD_R < 2% Corn % H: 80.4-99.2% % RSDr and % RSD_R < 2%
3.4	Sample dilution factor	Recovery efficiency and reproducibility meet AOAC 2019 criteria	The recovery efficiency was from 80.3 – 103.8 %, and the reproducibility was < 10%. Diluting the sample with 0.2% ACN/HCOOH with different dilution factors did not affect the trueness and precision of the method.
3.5	Measurement uncertainty	Expanded measurement uncertainty % Uexp < 44 % at concentration < 100 µg/Kg	%Uexp: 7.2 - 28.6% (peanut) %Uexp:28.1-31.7%(chili powder) %Uexp : 12.8 – 24.6% (raisins)

No	Research content	Acceptance criteria	Results achieved
			%Uexp : 25.6 – 37.8% (corn)
3.6	Sample stability:	Difference % D ≤ 15%	% D: 0.1 – 15.6% (peanut) % D: 0.1 – 10.9% (chili powder) % D: 0.4 – 16.5% (raisin) % D : 0.1 – 10.4% (corn)
		Coefficient of variation % CV ≤ 20%	% CV: 0.1 – 2.3% (peanut) % CV: 0.1 – 2.8% (chili powder) % CV: 0.1 – 3.5% (raisin) % CV: 0.1 – 3.9% (corn)
3.7	Ensure analytical results	% RSD_R repeatability assessment did not exceed 22% of 3 labs at concentrations < 100 µg/kg	% RSD_R < 6% (peanut) % RSD_R < 6% (chili powder) % RSD_R < 11% (raisin) % RSD_R < 12% (corn)
		Participate in proficiency testing under the AF program in corn (CSSMY021 – M21411AF), Criteria (- 2 ≤ Z-score ≤ 2)	AFs (25.8 µg/kg, Z-score = -0.9), AFB1(20.9 µg/kg, Z-score = -0.9) AFB2 (4.9 µg/kg, Z-score = -0.7), Meets criteria (- 2 ≤ Z-score ≤ 2)
		Daily QC sample control at MQL concentration level for AFB1 according to the Control Chart	Meet recovery efficiency at MQL concentration level according to AOAC 2019 criteria and data statistics, control chart monitoring

In summary, the simultaneous analysis of aflatoxins B1, B2, G1, and G2 using a non-derivatized method with the UPLC-FD technique has demonstrated several advantages, including reduced analysis time, lower solvent consumption, minimized environmental impact, improved operator safety, simplified sample preparation, and enhanced overall analytical efficiency. The method achieved method detection limits (MDLs) that were either lower than or comparable to those obtained using pre- or post-column derivatization techniques. These improvements can be attributed to several key factors:

High-sensitivity detection system: The UPLC-FD system, equipped with a mercury-xenon fluorescence detector and a high-flow fluorescence measurement chamber, offers optimized sensitivity without the need for derivatization.

Mobile phase optimization: Adjustments to the mobile phase composition improved both chromatographic separation and fluorescence response. Notably, the inclusion of HCOOH enhanced peak sharpness, thereby increasing method sensitivity.

Efficient sample preparation: The extraction and dilution solvents (PBS buffer or H_2O) were carefully optimized to improve aflatoxin solubility, facilitating effective cleanup using immunoaffinity columns.

3.4. Research on the contents of aflatoxin (B1, B2, G1, and G2) in peanut, chili powder, raisin, and corn kernel samples collected from the market

3.4.1. ***Results of aflatoxin (B1, B2, G1, and G2) analysis in peanut, chili powder, raisin, and corn kernel samples***

Peanut, chili powder, raisin, and corn kernel samples were collected from markets in Ho Chi Minh City and analyzed simultaneously for aflatoxins (B1, B2, G1, G2) using the non-derivatized UPLC-FD method. Detailed results on the number of samples contaminated with each AF type and total AF are presented in Table 3.23 and Figure 3.11.

Table 3.23: Detailed summary table of survey results of aflatoxin B1, B2, G1, and G2 content in food in Ho Chi Minh City

Matrix	Number of infected samples (percent, %) Min-max (µg/kg)					Number of samples exceeding the AFB1 threshold, rate (%)	Number of samples exceeding total AF threshold, rate (%)
	AFB1	**AFB2**	**AFG1**	**AFG2**	**Tổng AF**		
Raw and processed peanuts (n = 400)	117 (29.3%) 0.31 - 554	66 (16.5%) 0.31 – 110	25 (6.3%) 0.30 – 12.5	-	117 (29.3%) 0.31 – 668	62 (15.5%)	59 (14.8%)
Raw peanuts (n = 370)	107 (28.9%) 0.31 – 554	60 (16.2%) 0.31 – 110	23 (6.2%) 0.30 – 12.5	-	107 (28.9%) 0.31 – 668	57 (15.4%)	54 (14.6%)
Processed peanuts (n = 30)	10 (33.3%) 0.32 – 130	6 (20.0%) 0.43 – 24.5	2 (6.7%) 0.35 – 0.62	-	10 (33.3%) 0.32 – 155	5 (16.7%)	5 (16.7%)
Chili powder (n = 400)	286 (71.5%) 0.30 – 121	152 (38.0%) 0.30 – 8.18	91 (22.8%) 0.30 – 8.54	1 (0.3%) 0 – 0.37	286 (71.5%) 0.30 – 127	132 (33.0%)	99 (24.8%)
White, yellow and processed corn (n=400)	104 (26.0%) 0.32 - 494	83 (20.8%) 0.34 – 29.7	49 (12.3%) 0.32 – 4.87	1 (0.3%) 0 – 0.37	104 (26.0%) 0.32 – 525	86 (21.5%)	79 (19.8%)
White corn kernels (n = 100)	64 (64.0%) 0.38 - 494	61 (61.0%) 0.34 – 29.7	43 (43.0%) 0.32 – 4.87	1 (1.0%) 0 – 0.37	64 (64.0%) 0.38 – 525	62 (62.0%)	60 (60.0%)
Yellow corn kernels (n = 100)	5 (5.0%) 0.50 – 6.23	2 (2.0%) 0.43 – 0.5	2 (2.0%) 0.49 – 0.67	-	5 (5.0%) 0.5 – 7.34	2 (2.0%)	2 (2.0%)
Processed corn (n = 200)	35 (17.5%) 0.32 – 46.8	20 (10.0%) 0.37 – 4.02	4 (2.0%) 0.34 – 1.21	-	35 (17.5%) 0.32 – 50.8	22 (11.0%)	17 (8.5%)
Raisins (n = 400)	-	-	-	-	-	-	-

(-): No samples were contaminated with aflatoxin, MDL = 0.1 µg/kg for all substances; Min – Max: only accurately quantified samples have specific measured values above the MQL level

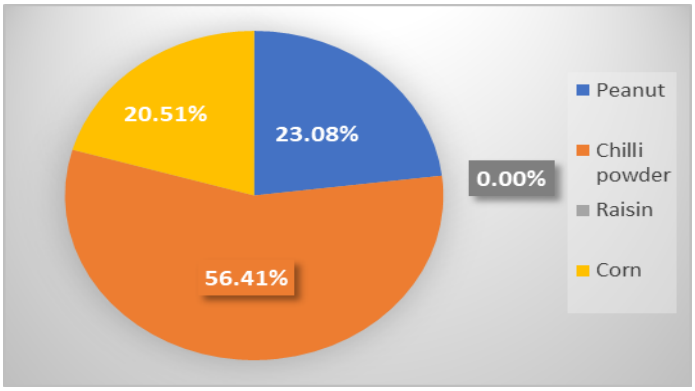


Figure 3.11: Percentage of samples contaminated with aflatoxin in 4 matrices of peanuts, chili powder, raisins, and corn kernels in Ho Chi Minh City

According to the results presented in Table 3.23 and Figure 3.11, the rate of aflatoxin contamination in chili powder was the highest (56.41%), followed by peanuts (23.08%), corn kernels (20.51%), and raisins (0.0%). Among the aflatoxins, the level of AFB1 contamination was the highest, followed by AFB2, AFG1, and AFG2. Furthermore, if the sample was not contaminated with aflatoxin B1, the remaining three aflatoxins would also not be present.

➢ ***Aflatoxin content in raisin samples***

Of the 400 raisin samples, including black, yellow, and green raisins imported from the United States, Australia, South Korea, Chile, and some other unlabeled raisins, none of the remaining samples were found to contain AFB1, AFB2, AFG1, and AFG2. Raisins are one of the high-value imported and exported foods, so technologies such as drying, packaging, and preservation are focused on to significantly reduce the occurrence of AF toxins.

➢ ***Aflatoxin content in peanut samples***

The results are presented in Table 3.23, showing the percentage (%) of samples contaminated with AFB1, AFB2, AFG1, and AFG2. The percentage of samples exceeding the allowable levels for AFB1 (ML = 2 µg/kg) and total AFs (ML = 4 µg/kg) were almost similar in both raw and processed peanuts. When comparing the contamination rates between raw and processed peanuts, the percentages were as follows: AFB1 (28.9% vs. 33.3%), AFB2 (16.2% vs. 20.0%), AFG1 (6.2% vs. 6.7%), and AFG2 (0.0% vs. 0.0%). In addition, the percentage of samples exceeding the permissible limit for AFB1 was 15.4% for raw peanuts and 16.7% for processed peanuts, while for the total they were 14.6% and 16.7%, respectively. These results suggest that processing is not the main factor in reducing the level of aflatoxin contamination in peanuts.

➢ ***Aflatoxin content in chili powder samples***

The results presented in Table 3.23 show that for chili powder samples, 286 samples were contaminated with AFB1 with concentrations ranging from 0.3 to 121 µg/kg, accounting for 71.5% of

the samples, 152 samples contained AFB2 (0.30 to 8.18 µg/kg), accounting for 38.0% of the samples, and 91 samples were contaminated with AFG1 (0.30 - 8.54 µg/kg), accounting for 22.8% of the samples. Only one sample was found to contain AFG2 (0.37 µg/kg). It is noteworthy that the percentage of samples exceeding the allowable threshold for AFB1 (ML = 5 µg/kg) and total AF (ML = 10 µg/kg) prescribed by the Ministry of Health (QCVN 8-1:2011/BYT) was 33.0% for AFB1 and 24.8% for AF. Improper storage conditions can significantly contribute to mold growth in chili and chili powder.

- ***Effect of sample storage in sealed and unsealed packaging on aflatoxin contamination***

Independent-sample T-Test results showed that there was no significant mean difference in AFs content between sealed (7.02 µg/kg) and unsealed (9.80 µg/kg) chili powder samples.

In total 400 chili powder samples including 200 chili powder samples stored in sealed packaging, and 200 samples in unsealed packaging, with the results Figure 3.13, showing the AF contamination rate between unsealed and sealed chili powders as follows: AFB1 (75.5 vs. 67.5%), AFB2 (44.0 vs. 32.0%), AFG1 (24.5 vs. 21.0%) and AFG2 (0.5 vs. 0.0%). This shows that the proportion of samples detected AF in unsealed chili powder is higher than that in sealed chili powder. Moreover, the proportion of samples exceeding the permissible limits for AFB1 and total AF in unsealed chili powder is higher (38.0% and 28.5%, respectively) than in sealed (28.0% and 21.0%, respectively). These findings indicate that chili powder stored in sealed packaging partially reduced the risk of *Aspergillus growth*, thereby preventing aflatoxin production. This risk reduction may be due to limiting the product's exposure to oxygen and moisture in the air, thereby reducing the likelihood of mold growth.

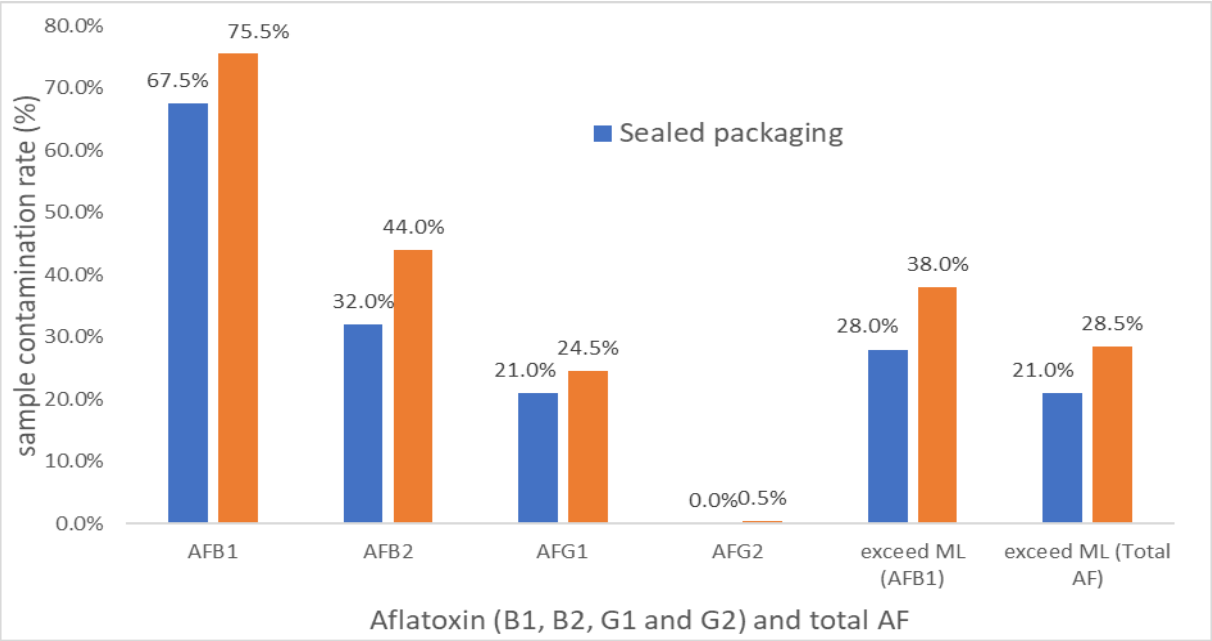


Figure 3.13: Comparison chart of the percentage of AFB1 contaminated samples, exceeding the allowable AFB1 level, total AF in sealed (blue) and unsealed (orange) packaging

➢ ***Aflatoxin content in corn samples***

The results of the survey of 200 corn kernels including fresh yellow and white kernels and 200 processed corn including popcorn, corn cakes, corn snacks, fried corn, popcorn, corn flour, and corn milk are presented in Table 3.23 showing the percentage (%) of samples contaminated with AFB1, AFB2, AFG1, and AFG2. According to the survey, the percentage of samples exceeding the standards for AFB1 (ML = 2 µg/kg) and total AF (ML = 4 µg/kg) in corn kernels was 2.0 - 11.0 times higher than that in processed corn. A comparative analysis of the AF contamination rate between corn kernels and processed corn gave the following results: AFB1 (34.5 vs. 17.5%), AFB2 (31.5 vs. 10.0%), AFG1 (22.5 vs. 2.0%), and AFG2 (0.8 vs. 0.0%). Furthermore, the percentage of samples exceeding the permissible level for AFB1 was found to be 32.0% in grain corn and 11.0% in processed corn, while for total AF, these values were 31.0% and 8.5%, respectively. These findings indicate that both grain corn and processed corn are contaminated with aflatoxins. However, grain corn is more susceptible to aflatoxin contamination than processed corn.

- ***Differences in Aflatoxin Content in Raw (Unprocessed) Corn Varieties***

Results Table 3.23 shows 200 corn kernel samples consisting of 100 fresh white kernels and 100 dry yellow kernels. The results indicate that white corn varieties may be more susceptible to aflatoxin contamination, as they had a significantly higher contamination rate (64.0%) than dry yellow corn kernels (5.0%). Furthermore, the percentage of samples exceeding the allowable limits for AFB1 and total AF was significantly higher in white kernels (62.0% and 60.0%, respectively) than in yellow kernels (5.0% and 2.0%, respectively). Environmental conditions and the presence of pests may contribute to fungal contamination in kernels. Therefore, it is important to implement strategies that combine resistant corn varieties with recommended agricultural practices. In this way, food safety can be ensured, and the occurrence of aflatoxin contamination in food crops can be prevented.

3.4.2. Comparison between the rate of aflatoxin B1 exceedance and the rate of total aflatoxin exceedance

Figure 3.16 shows that when the number of peanut, chili powder, and corn samples contaminated with total AF exceeded the permissible level, the number of samples contaminated with AFB1 exceeding the prescribed level was at least equal to the number of samples contaminated with total AF exceeding that level. This can be concluded that if a sample is contaminated with aflatoxin exceeding the AF threshold, that sample also exceeds the AFB1 threshold.

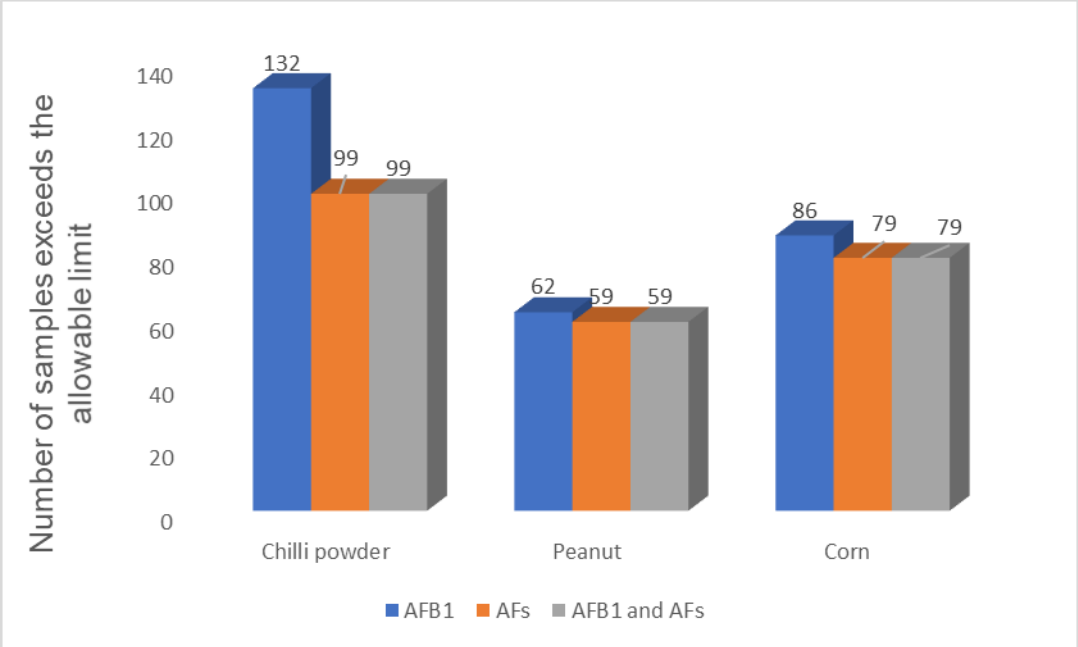


Figure 3.16: A comparison chart of the number of samples contaminated with aflatoxin exceeding the prescribed threshold between AFB1 (blue) and AF (orange) for each sample matrix; gray is the number of contaminated samples exceeding the limit for both parameters

3.4.3. Correlation between aflatoxin (B1, B2, G1 and G2) contamination levels with total AF

The results of Pearson correlation analysis, linear regression analysis, variance inflation factor (VIF) evaluation, t-test Sig value (student), ANOVA table, Model Summary table also give Durbin–Watson (DW) value to evaluate the phenomenon of first-order serial autocorrelation using SPSS 22.0 software, showing that the model predicts the correlation between AFB1; AFB2, AFG1, AFG2 contamination content with total AF on sample matrices as follows::

Total AF (peanut) = 0.925* AFB1 + 0.096 *AFB2 + 0.010*AFG1

Total AF (chili powder) = 0.931* AFB1 + 0.061 *AFB2 + 0.051*AFG1 + 0.001* AFG2

Total AF (corn) = 0.924* AFB1 + 0.076 *AFB2 + 0.014*AFG1

3.4.4. Correlation between aflatoxin B1 contamination and total aflatoxin

The results presenting the correlation between AFB1, AFB2, AFG1, and AFG2 with total AF show that AFB1 has the strongest impact on total AF (coefficient ≥ 0.92) on all three sample matrices of peanuts, chili powder, and corn. Logarithmic linear regression model was performed according to the variables y (Log(total AF) and x (Log(AFB1), both variables x, and y were transformed by Logarithm based on the available AFB1 and total AF content values in the three sample matrices to evaluate the correlation coefficient R^2, and extrapolate the results between AFB1 and total AF from the linear curve.

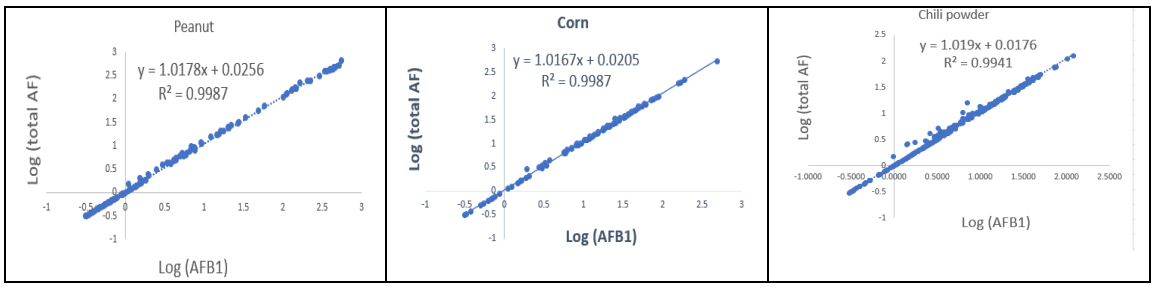


Figure 3.17: Correlation between AFB1 contamination content and total AF for peanut, chili powder, and corn

As shown in Figure 3.17, the correlation coefficient (R^2) between Log(AFB1) and Log(Total AF) was significantly high and consistent across all three sample matrices, with R^2 values equal to or greater than 0.99. Specifically, the R^2 coefficients for chili powder, peanuts, and corn were 0.9941, 0.9987, and 0.9987, respectively. These figures demonstrate a strong relationship between the known AFB1 content in a sample and the extrapolated total AF content (sum of AFB1, AFB2, AFG1, AFG2) as shown by the linear curve in Figure 3.17. These findings confirm the reliability of the equation used to extrapolate AF results from AFB1 results and vice versa, reinforcing its scientific credibility.

*3.4.5. **Assessment of AFB1 exposure risk through consumption of peanuts, corn kernels, chili powder, and raisins**.*

3.4.5.1. Assessment of daily consumption

The average peanut consumption is 0.01493 kg/day/person, and corn kernels used in food are 0.02219 kg/day/person, which is more than 50 times higher than the consumption of chili powder and raisins (0.00029 and 0.00030 kg/person/day). This value shows that the two products of chili powder and raisins are the only side dishes in the daily menu of Vietnamese people.

3.4.5.2. Assessment of carcinogenic risk based on EDI daily exposure dose

Based on the results of mycotoxin content in food and daily food consumption, the exposure dose of mycotoxin AFB1 was calculated for lean, normal, and overweight body types for adult males and females.

In this study, the average LB value was used for the sample types with detection rates above LOQ, specifically, the average LB for peanuts, corn kernels, and chili powder was 18.5; 7.60; 7.73 µg/kg, respectively, and the UB for the samples where AFB1 toxin was not detected in the sample, in this case raisins with an average UB of 0.1 µg/kg.

Table 3.34: Estimated daily dietary exposure to AFB1 in lean, normal, and overweight adult males

	AFB1 tb (µg/kg)	Average consumption (kg/person/ day)	Physical condition	Average body weight (kg b.m/person)	Exposure (EDI) (µg/kg/day)	Carcinogenicity	Population risk (per 100,000 people/year)	BMDL10 (µg/kg,bw/ day)	MOE	TD50 of AFB1 (µg/kg,bw/day)	Uncertainty factor	HI
Peanuts (n=400)	18.5	0.01493	Thin	48.60	0.00568	0.068	38,616	0.17	29.9	3.2	50000	103
	18.5	0.01493	Normal	61.32	0.00450	0.068	30,606	0.17	37.8	3.2	50000	70,3
	18.5	0.01493	Fat	91.84	0.00301	0.068	20,435	0.17	56.6	3.2	50000	47,0
Corn (n=400)	7.60	0.02219	Thin	48.60	0.00347	0.068	23,598	0.17	49.0	3.2	50000	54,2
	7.60	0.02219	Normal	61.32	0.00275	0.068	18,703	0.17	61.8	3.2	50000	43,0
	7.60	0.02219	Fat	91.84	0.00184	0.068	12,488	0.17	92.6	3.2	50000	28,7
Chili powder (n=400)	7.73	0.00029	Thin	48.60	0.00005	0.068	0.311	0.17	3715	3.2	50000	0,71
	7.73	0.00029	Normal	61.32	0.00004	0.068	0.247	0.17	4688	3.2	50000	0,57
	7.73	0.00029	Fat	91.84	0.00002	0.068	0.165	0.17	7021	3.2	50000	0,38
Raisins (n=400)	0.1	0.00030	Thin	48.60	0.00000	0.068	0.004	0.17	271777	3.2	50000	0,01
	0.1	0.00030	Normal	61.32	0.00000	0.068	0.003	0.17	342908	3.2	50000	0,01
	0.1	0.00030	Fat	91.84	0.00000	0.068	0.002	0.17	513579	3.2	50000	0,01

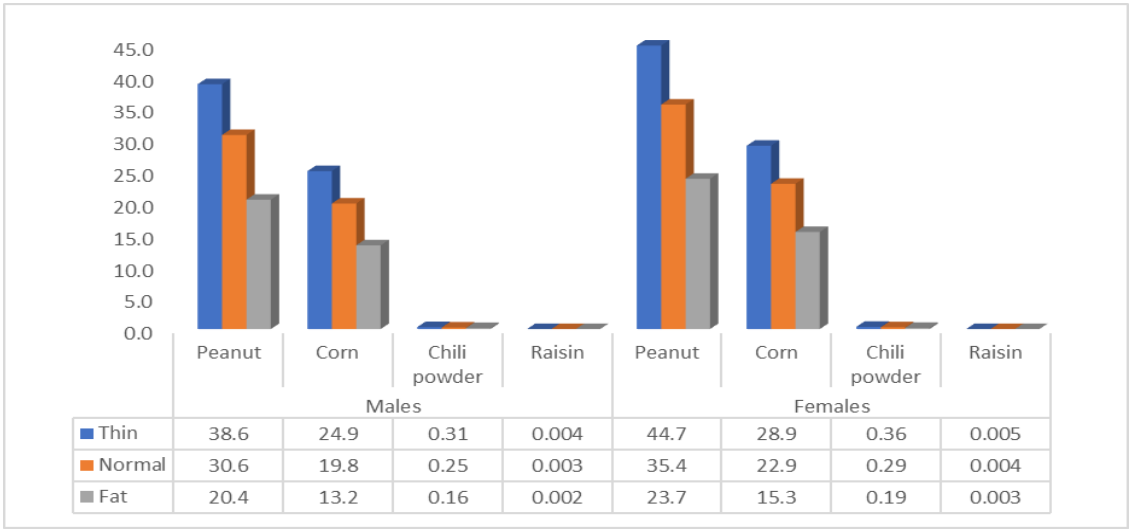
Table 3.35: Estimated daily dietary exposure to in lean, normal, and overweight adult females

	AFB1 tb (µg/kg)	Average consumption (kg/person/day)	Physical condition	Average body weight (kg b.m/person)	Exposure (EDI) (µg/kg/day)	Carcinogenicity	Population risk (per 100,000 people/year)	BMDL10 (µg/kg,bw/day)	MOE	TD50 of AFB1 (µg/kg,bw/day)	Uncertainty factor	HI
Peanuts (n=400)	18.5	0.01493	Thin	41.96	0.00658	0.068	44,727	0.17	25.8	3.2	50000	103
	18.5	0.01493	Normal	52.94	0.00521	0.068	35,451	0.17	32.6	3.2	50000	81.5
	18.5	0.01493	Fat	79.29	0.00348	0.068	23,670	0.17	48.8	3.2	50000	54.4
Corn (n=400)	7.60	0.02219	Thin	41.96	0.00402	0.068	27,332	0.17	42.3	3.2	50000	62.8
	7.60	0.02219	Normal	52.94	0.00319	0.068	21,664	0.17	53.4	3.2	50000	49.8
	7.60	0.02219	Fat	79.29	0.00213	0.068	14,464	0.17	79.9	3.2	50000	33.8
Chili powder (n=400)	7.73	0.00029	Thin	41.96	0.00005	0.068	0.360	0.17	3208	3.2	50000	0.83
	7.73	0.00029	Normal	52.94	0.00004	0.068	0.286	0.17	4047	3.2	50000	0.66
	7.73	0.00029	Fat	79.29	0.00003	0.068	0.191	0.17	6062	3.2	50000	0.44
Raisins (n=400)	0.1	0.00030	Thin	41.96	0.00000	0.068	0.005	0.17	234644	3.2	50000	0.01
	0.1	0.00030	Normal	52.94	0.00000	0.068	0.004	0.17	296046	3.2	50000	0.01
	0.1	0.00030	Fat	79.29	0.00000	0.068	0.003	0.17	443398	3.2	50000	0.01

Results of Tables 3.34 and 3.35 show that: For men and women of lean, normal and overweight body types, the average exposure dose to AFB1 in peanuts is the highest (women: 0.00348 - 0.00658 and men: 0.00301 - 0.00568 µg/kg/day), followed by corn kernels (women: 0.00213 - 0.00402 and men: 0.00184 - 0.00347 µg/kg/day), chili powder (women: 0.00003 - 0.00005 and men: 0.00002 - 0.00005 µg/kg/day) and raisins with an average exposure dose that is too small, with a hazard index HI < 1, so it does not pose a danger to public health.

According to the results presented in Figure 3.11, the rate of aflatoxin contamination in chili powder was the highest (56.41%), followed by peanuts (23.08%) and corn kernels (20.51%). However, the average exposure dose of chili powder was the lowest, followed by corn kernels and peanuts. This is explained as follows: Comparing chili powder and corn kernels, the average LB of AFB1 was almost the same at 7.73 µg/kg (chili powder) and 7.60 µg/kg (corn kernels), but the consumption of chili powder was nearly 100 times lower than the consumption of corn kernels, so the average exposure dose of chili powder was lower than that of corn kernels. The consumption of peanuts (0.001493 kg/person/day) was not much lower than that of corn kernels (0.002219 kg/person/day), however, the LB of AFB1 of peanuts (18.5 µg/kg) was twice as high as that of corn kernels (7.60 µg/kg). This results in higher AFB1 exposure doses from peanuts than from corn kernels.

The cancer risk of AFB1 for adult men and women depends on their physical condition in order from high to low, specifically as follows: peanuts (women: 23.7 - 44.7 and men: 20.4 - 38.6 cancer cases per 100,000 people per year), followed by corn kernels (women: 14.4 - 27.3 and men: 12.5 - 23.6 cancer cases per 100,000 people per year), chili powder (women: 0.19 - 0.36 and men: 0.16 - 0.31 cancer cases per 100,000 people per year) depending on physical condition. This result shows that the risk of cancer from AFB1-contaminated diets is higher for women than for men in the case of exposure to the same physical condition. At the same time, the results show that people with weak physical conditions have the highest risk of cancer caused by AFB1 infection, followed by normal people, and finally, overweight people (Figure 3.18).



	Males				Females			
	Peanut	Corn	Chili powder	Raisin	Peanut	Corn	Chili powder	Raisin
Thin	38.6	24.9	0.31	0.004	44.7	28.9	0.36	0.005
Normal	30.6	19.8	0.25	0.003	35.4	22.9	0.29	0.004
Fat	20.4	13.2	0.16	0.002	23.7	15.3	0.19	0.003

Figure 3.18: Population risk comparison (per 100,000 people) of cancer risk from AFB1-contaminated diets between lean, normal, and obese adults.

3.4.5.3. Margin of exposure MOE and hazard index HI-Based AFB1 Exposure Risk Evaluation

Results in Tables 3.34 and 3.35 show the MOE values for AFB1 exposure for adult males and females through dietary intakes of peanuts, corn, chili powder, and raisins divided into two subgroups according to MOE recommendations as follows:

Risk groups: MOE lower than 10000 include peanuts (female: 25.8 – 48.8 and male: 29.9 – 56.6 depending on body weight), corresponding to the hazard index HI according to increasing risk (female: 102.7 – 54.4 and male: 88.7 – 46.9 depending on body weight) and corn kernels (female: 42.3 – 79.9 and male: 49.0 – 92.6 depending on body weight), corresponding to the hazard index HI according to increasing risk (female: 62.8 – 33.8 and male: 54.2 – 28.7 depending on body weight). These results show that both peanuts and corn kernels pose significant health risks due to MOE values lower than 10000 and HI values higher than 1, depending on body weight. This highlights the need for rigorous monitoring and risk management strategies for these food items.

Chili powder, although classified in the high-risk group based on MOE values (female: 3208 – 6062 and male: 3715 – 7021, depending on body type), corresponds to a hazard index HI of increasing hazard (female: 0.83 – 0.44 and male: 0.71 – 0.38, depending on body type). This suggests that while chili powder is the most contaminated with AFB1, its low consumption rate minimizes the overall risk, placing it in the lower-risk group despite its MOE value below 10000. However, if the average consumption is higher than 0.0038 kg/person/day, especially in people under 42 kg, there may be a cancer risk, suggesting the need for conscious consumption measures.

Low-risk group: Raisins are in the low-risk group, with an MOE higher than 10000 (female: 234645 – 443398 and male: 271776 – 513579, depending on body type), corresponding to a hazard index HI of 0.01 for both males and females according to body type. This indicates a negligible risk to public health and is consistent with the minimal AFB1 exposure associated with raisin consumption, reflecting the safety of this food in terms of AFB1-related cancer risk.

CONCLUSION

Through the research process, the thesis has completed the set objectives and obtained the following results:

- A method was researched and developed for the simultaneous analysis of aflatoxins (B1, B2, G1, and G2) in peanuts, chili powder, raisins, and corn kernels using ultra-performance liquid chromatography with fluorescence detection (UPLC-FD), without the use of derivatization. This approach marks a significant advancement over conventional methods, offering notable advantages such as reduced analysis time, lower solvent consumption, environmental friendliness, improved operator safety, simplified sample preparation, and high sensitivity (LOD: 0.1 µg/kg; LOQ: 0.3 µg/kg). The method meets both international and domestic regulatory standards and is easy to implement in laboratory settings.

- This study is the first to determine the prevalence of aflatoxin contamination in selected food samples in Vietnam and to develop a predictive model for total aflatoxin levels based on aflatoxin B1 concentrations. Survey results revealed that chili powder exhibited the highest contamination rate (56.41%), followed by peanuts (23.08%) and corn kernels (20.51%), while no aflatoxin was detected in raisin samples (0%). Notably, a strong linear correlation was observed between aflatoxin B1 and total aflatoxin levels across all three contaminated matrices - peanuts, chili powder, and corn kernels. The predictive models demonstrated high coefficients of determination ($R^2 \geq 0.9941$), offering a reliable tool for risk assessment and effective monitoring of aflatoxin contamination in food products.

- The study estimated daily dietary exposure to aflatoxin B1 among adults, stratified by three body types: lean, normal, and overweight. The results indicated that aflatoxin B1 exposure is associated with an elevated cancer risk, with the lean group exhibiting the highest estimated incidence, 38.6 cases per 100,000 men and 44.7 cases per 100,000 women. These findings suggest that women and individuals with lower body weight are more vulnerable and should be prioritized in preventive health strategies. Among the food groups analyzed, peanuts and corn kernels were identified as major contributors to aflatoxin B1 exposure and thus pose significant public health risks. In contrast, chili powder showed contamination potential but with comparatively lower impact, while raisins were deemed safe, with no detectable aflatoxin and minimal associated health risks.