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Aflatoxin B_1 Measurement in Traditional Kermanshah Cookies and Risk Assessment in Dietary Exposure

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HIGHLIGHTS

- Nankhormaii had the highest amount of aflatoxin B_1 among cookies studied.
- The lowest amount of aflatoxin B1 was found in Nanroghani.
- The age group studied was at risk of cancer due to consuming traditional cookies.
- Monitoring the quality of traditional cookies' flour and raw materials was deemed essential.

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Abbreviations
AF=Aflatoxin
EDI=Estimation Daily Intake
EFSA=European Food Safety Authority
FFQ=Food Frequency Questionnaire
HPLC=High Performance Liquid Chromatography
LADD=Lifetime Average Daily Dose
MOE=Margin of Exposure
Ri=Excess individual lifetime risk of cancer

ABSTRACT

Background: Aflatoxins (AFs), especially the B_1 subtype, present a significant threat to public health. Chronic exposure to AFB_1 has been associated with the development of serious diseases, such as cancer. Therefore, detecting and controlling its presence in food is crucial for preventing long-term health issues.
Methods: In the present study, we collected 40 samples of four types of traditional Kermanshahi cookies from a local market at random intervals throughout 2023 (Nanbernji, Kak, Nankhormaii, and Nanroghani). These samples were examined for AFB_1 contamination using High Performance Liquid Chromatography. The risk of exposure to this toxin was then calculated by utilizing a Food Frequency Questionnaire and various parameters (Estimation Daily Intake, Lifetime Average Daily Dose, Margin of Exposure, excess individual lifetime risk of cancer) were calculated using Crystal Ball software. Statistical analysis was conducted using a completely randomized design with three replications.
Results: The concentration of AFB_1 in Nanbernji, Kak, Nanroghani, and Nankhormaii (traditional Kermanshah cookies) was 3.12, 2.99, 1.64, and 3.95 µg/kg, respectively. The AFB_1 contamination levels in Kermanshah's traditional cookies exceeded the European Union's limit of two ng/g. The Margin of Exposure for all cookie samples in both adult and teenage age groups was higher than 10,000 except for Nanroghani consumption in individuals under 18 years old. Based on health evaluation results, all age groups in Kermanshah were found to be at risk of cancer.
Conclusion: Considering the consumption of these traditional sweets by individuals and the risk of cancer in the study population, competent authorities must adopt a supervisory approach and develop a documented national program.

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Introduction

Mycotoxins are produced by fungi species (*Penicillium*, *Alternaria*, *Fusarium*, and *Aspergillus*) and the contamination of agricultural products by them has caused concern about food security (Soltani et al., 2022). The main mycotoxins in food are zearalenone (ZEN), Aflatoxin (AF), deoxynivalenol (DON), and ochratoxin A (OTA) (Medina et al., 2021).

The climate in Kermanshah is conducive to the accumulation of mycotoxins, especially AFB_1. Improper storage and hot weather can lead to the growth of fungi (Alvito and Assunção, 2022; De Barros et al., 2020; Licona-Aguilar et al., 2023; Tueller et al., 2023).

Aspergillus flavus and *A. parasiticus* can produce AFB_1 and they contaminate food items containing grains, rice, corn, and peanuts (Chaharaein et al., 2021). AFB_1 is the most carcinogenic fungal metabolite, particularly linked to liver cancer, with the International Agency for Research on Cancer (IARC) categorizing it as a group one carcinogen (Sahin et al., 2022). The maximum allowable residue of AFB_1 in cereals is set at 2 µg/kg by the European Commission (EC) and 4 µg/kg by the American Food and Drug Administration (FDA) (Brinda et al., 2013; Joubrane et al., 2020).

Wheat is one of the most consumed grains in Iran and many countries. This grain is a source of bioactive and nutritious compounds. However, it may be contaminated by AFB_1 during cultivation, harvesting, and storage (Gómez et al., 2020). Products made from wheat and rice flour (bread, biscuits, cakes, and cookies) are part of the diet in Iran and all over the world (Andrade et al., 2012; Gonçalves et al., 2019; Jahanbakhsh et al., 2019; Noroozi et al., 2020).

AFB_1 contamination in rice and rice flour poses a significant food safety issue. These toxins can be produced in warm and humid environments, particularly during the stages from planting to storage of rice. As a result, rice flour is also at risk of contamination. Research indicates that in certain Asian regions, particularly in countries that produce rice, levels of AFs in rice and its derivatives can sometimes exceed the allowable limit (e.g. four µg/kg according to European Union standards). To address this problem, it is advised to utilize rapid detection methods like High Performance Liquid Chromatography (HPLC) (Gonçalves et al., 2019).

Cookies are pastry products with a crunchy texture, and traditional cookies are popular snack foods all over Iran, especially in Kermanshah, a western province. Some traditional cookies in Kermanshah, where flour is the main ingredient, include Nanbernji, Kak, Nanroghani, and Nankhormaii.

Food products should be checked for the risk of AFB_1 contamination to minimize the damage of this toxin (Broekaert et al., 2015). Risk assessment is a process that identifies adverse factors and human exposure to dangers like toxins and food pollutants. It has four stages: risk identification, risk description, exposure assessment, and risk probability description. However, the last two stages are more effective in the risk assessment process (Bashiry et al., 2021; Bevilacqua et al., 2023). Food consumption and toxin exposure can be investigated using the Food Frequency Questionnaire (FFQs) and the risk is evaluated by combining the obtained data (Bailey, 2021; Tucker, 2007).

Several methodologies, including the point estimate approach, the semi-probabilistic technique, and Monte Carlo Simulation (MCS), have been developed for analyzing AFs exposure. MCS is an efficient method that can identify uncertainties in predicting the risks associated with long-term exposure to contaminated food (Nematollahi et al., 2020). Therefore, the present study examined the AFB_1 concentration in traditional cookies of Kermanshah city, description of exposure, and risk assessment.

Material and methods

Standard of AFB_1 (A6636, Sigma-Aldrich, UK), HPLC grade solutions (100030, Merck Company, Germany) including methanol, n-hexane, acetonitrile, chloroform, and water were purchased to determine the AFB_1 concentration in four types of cookies. Phosphate buffer tablets (P4417, Gibco company, US) and sodium chloride (NaCl; 106404, Merck Company, Germany) were used for pH adjustment and extraction samples. The purity of all consumables was 99.9%, and a paper filter (No.1) with a suitable quality was bought from the Whatman company, U.K. for smoothing the samples. The immunoaffinity column (AflaStar and OchraStar, Romers, USA) was used for analyte purification, extraction, and concentration.

Sampling and extraction

Initially, 40 samples of Kermanshah's traditional cookies, representing different brands-Nanbernji, Kak, Nankhormaii, and Nanroghani (10 samples each)-were obtained from the market. Subsequently, three samples of each brand were purchased at monthly intervals in 2023 and transported to the laboratory, where they were kept at 20 °C until analysis. For HPLC analysis, the samples from each brand were combined and injected in triplicate. The main ingredients needed to prepare the sweets are listed in Table1.

For extraction, 50 g of the sample and 5 g NaCl were accurately weighed using a scale (AND, GH202 model, Japan) and transferred to a blender (waring company, 8011ES model, US) for homogenization (5/18,000

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min/rpm). Then, 100 ml of solvent (80% methanol: 20% water) was added to the sample and stirred for five min. The mixture was filtered using filter paper and 20 ml of it was transferred to a phosphate buffer solution (100 ml). The immunoaffinity column (IAC) was washed with 10 ml phosphate buffered saline solution and the extracted sample was passed through it. Next, the column was cleaned with 10 ml distilled water. Lastly, nitrogen gas was used to evaporate the collected solvent at room temperature and one ml methanol: water (1:1 v/v) was added to it. The volume of the sample injected into the HPLC was 100 µl (Martinez-Miranda et al., 2019; Pralatnet et al., 2016).

Table1: Main ingredients of traditional Kermanshahi cookies

	Traditional Kermanshahi cookies			
Gredient	**Nankhormaii**	**Nanroghani**	**Kak**	**Nanbernji**
Wheat flour	40-45%	50-55%	55-60%	-
Rice flour	-	-	-	50-65%
Dates (pitted)	25-30%	-	-	-
Oil (solid/butter/liquid)	10-15%	15-20%	20-25%	10-15%
Sugar (powdered/granulated)	0-5%	10-15%	10-15%	15-20%
Egg (whole/yolk)	-	5-10%	-	5-10%
Water or milk	5-10%	5-10%	5-10%	-

HPLC conditions and validation

The mobile phase consisted of HPLC grade acetonitrile and water (90:10). In this research, an HPLC device (Knauer AZURA, Germany) and photochemical derivative (UV/LCTech, Germany) were used to measure AFB_1. The excitation wavelength was 329-460 nm and the column (Knauer C18) temperature was set at 40 °C. The flow rate of the mobile phases was 1.5 ml/min. Concentrations of standard calibration solutions (0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 3, 5, 10, 20, and 50 ppb) were prepared in methanol-water solvent (40/60 v/v) and kept at four °C to draw the calibration curve and determine the regression equation (y=37/367x+29/235). To determine the recovery percentage, five and 10 ppb of AFB_1 was added to the non-contaminated cookies and injected into the HPLC in three repetitions (after the extraction process). In addition, the Limit of Detection (LOD) and the Limit of Quantification (LOQ) were determined (Bol et al., 2016; Chaharaein et al., 2021).

The Food Frequency Questionnaire (FFQ)

A FFQ comprising 165 food item questions (accuracy: 0.5; confidence level: 0.95) was developed by a team of trained nutritionists and epidemiologists to assess the exposure level of the Kermanshah population to AFB_1 through traditional cookies (Nanbernji, Kak, Nanroghani, and Nankhormaii). The questionnaire's content validity was evaluated by a panel of experts in food safety and public health, and its reliability was confirmed using a test-retest method on a subsample of participants, yielding a Cronbach's alpha, indicating good internal consistency. The study population was randomly selected from both teenagers (under 18 years) and adults (over 18 years). The administration of the FFQ and data collection were carried out by nutritionists to ensure accuracy and consistency (Batal et al., 2021; Mohammadifard et al., 2021).

Risk assessment and exposure

The following formulas were used to evaluate the exposure to AFB_1 by consuming traditional Kermanshah cookies for two age groups (teenagers and adults). We needed two types of data to Estimated Daily Intake (EDI) of AFB_1: occurrence data (AFB_1 concentration) and consumption data (the intake rate of traditional cookies by a certain population obtained through questionnaires) (Mohamed et al., 2019).

$$EDI = \frac{C_i \times IR_i}{BW} \quad \text{(Gustafsson et al., 2022)} \quad (1)$$

EDI is the estimation daily intake (µg/kg bw/day), Ci is the average concentration of AFB_1 in traditional cookies (µg/kg), IRi is the average amount of cookies consumed (gr/day), and BW is the body weight (kg).

$$LADD = \frac{Ci \times IR_i \times ED \times EF}{BW \times LT} \quad \text{(González-Martínez et al., 2018)} \quad (2)$$

LADD is the Lifetime Average Daily Dose, ED is the exposure duration (70 years), EF is the exposure frequency (365 days/year), and LT is the lifetime (25,550 days).

The Margin of Exposure (MOE) proposed by European Food Safety Authority (EFSA)'s Scientific Committee to identify the genotoxic and carcinogenic potential of AFB_1 (Benford et al., 2010) was also calculated using the following formula:

$$MoE = \frac{BMDL10}{EDI} \quad \text{(Taghizadeh et al., 2020)} \quad (3)$$

In formula 3 BMDL10 is the 10% extra risk for characterizing the MOE that was attained based on data on liver tumor incidences in rats and it is equal to 400 ng/kg bw/day for AFB_1 (Esposito et al., 2017). MOE<10,000 indicates a higher risk of AFB_1 and MOE>10,000 leads to less concern in terms of exposure risk (Udovicki et al., 2021).

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To estimate the lifetime risk of cancer from consuming AFB_1 through food products, the equation 4 was used (Taghizadeh et al., 2020). In this formula, the carcinogenicity Slope Factor (SF) is adjusted based on hepatitis B surface antigen (HBsAg) status.

$$Ri = LADD \times SF \quad (4)$$

Ri: Excess individual lifetime risk of cancer /SF (mg/kg/day): Cancer potency SF for HBsAg+ (0.3) and HBsAg- (0.01).

Sensitivity determination and statistical analysis

Using the probabilistic Monte Carlo simulation method by Crystal Ball software (V.11.1.2.3, Oracle, Inc., USA), the risk of food contaminants exposure can be assessed. In this study, according to the formulas 1 to 4, the risk of exposure to AFB_1 was calculated for the age group of teenagers and adults, and variance analysis (ANOVA) was performed by SPSS software (v. 26) in a completely randomized design (with three replication). Moreover, sensitivity analysis and effect of each hypothesis such as Body weight (BW), Concentration of AFB_1 ($CAFB_1$), and Ingestion Rate (IR) were done using Crystal Ball software.

Results and discussion

Determining the AFB_1 concentration in traditional cookie samples

Due to the health risks associated with AFB_1, focusing on the contamination of commonly consumed foods and increasing public awareness can effectively help reduce chronic diseases like cancer. This study measured the incidence of AFB_1 in traditional cookies from Kermanshah.

These cookies (Nanbernji, Kak, Nanroghani, and Nankhormaii) are consumed daily as a snack and are even taken as souvenirs to other cities in Iran. Therefore, the level of their contamination by mycotoxins (especially AFB_1) is important. At first, the accuracy of the test was proven with the percentage of recovery (83%) in the concentration of 5 and 10 µg/kg. Relative Standard Deviation (RSD=5.3%) and the level of LOD (0.2 µg/kg) and LOQ (0.5 µg/kg) were determined based on the analysis of each sample in three replicates. The calibration curve was linearly and the confidence coefficient (R2=0.997) was observed at an acceptable level. The chromatogram obtained from a spiked and non-spiked sample of traditional cookies (Nankhormaii) is shown in Figure 1. After extracting, the four types of Kermanshah traditional cookies were injected into the HPLC and Figure 2 shows the AFB_1 concentration in these samples. The AFB_1 in Nankhormaii (3.95 µg/kg) was higher than other cookies and the lowest contamination was observed in Nanroghani (1.64 µg/kg). Nanbernji (3.12 µg/kg) and Kak (2.99 µg/kg) contained more AFB_1 after Nankhormaii, respectively ($p \leq 0.01$). Except for Nanroghani, the contamination of the samples exceeded the European standard (two µg/kg).

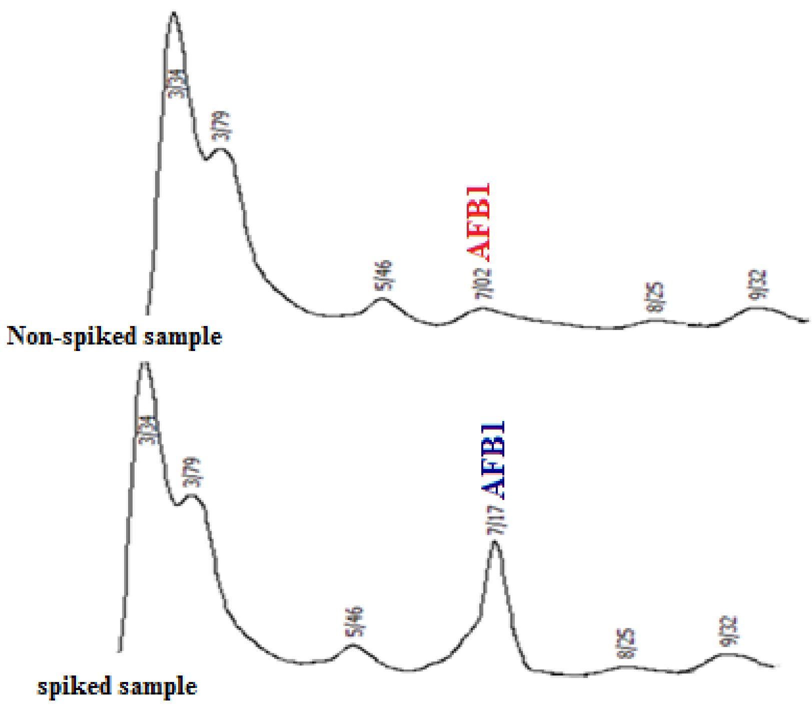

Figure 1: Spiked and non-spiked chromatogram of Aflatoxin B_1 (AFB_1) related to samples of Kermanshah traditional cookies injected into High Performance Liquid Chromatography (HPLC)

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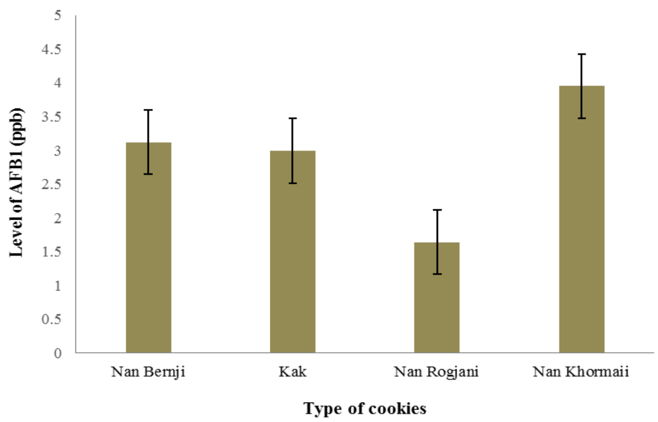

Figure 2: Aflatoxin B_1 (AFB_1) concentration (µg/kg) in samples of Kermanshah traditional cookies

The concentration levels in these analyzed samples can be considered in relation to wheat, flour, and rice flour, as they are the basic ingredients for the production of Nanbernji, Kak, Nanroghani, and Nankhormaii. The type of climate and improper storage are key factors in the growth of AFB_1-producing fungi in flour (Asghar et al., 2022). The level of AF in some cereal products is higher due to environmental conditions and practices related to cultivation, harvesting, and storage. Factors such as physical damage to grains during harvest, pest infestation, environmental stresses like drought, and improper storage (high humidity and poor ventilation) facilitate fungal growth and toxin production. Additionally, contaminated soil and the use of poor-quality seeds can increase the risk. Controlling these factors through precise agricultural practices and proper storage is essential (Nazareth et al., 2024). In Kermanshah's traditional pastry shops, the flour used to make cookies is stored at an improper temperature in the warehouse. Additionally, the confectioners lack sufficient knowledge about the health risks of mycotoxins to society and how to reduce these toxins. Therefore, the presence of AFB_1 in confectionery products has increased, posing a long-term health risk to citizens. Various studies have been conducted on the levels of AFB_1 in traditional breads and sweets consumed in Iran and other countries. In a study by Noroozi et al. (2022) the presence of AFB_1 in flour and several samples of traditional Iranian bread, biscuits, and cakes was investigated. The results showed that AFB_1 was present in the flour samples (ranging from 0.47 to 3.38 ng/g) and that fermentation conditions and cooking temperature helped to reduce the levels of this toxin.

The smallest decrease during the fermentation process was observed in cake (8.5%) and biscuit (6.5%) samples. Overall, the levels of AFB_1 were below the acceptable limits set by both the European Union and the Iranian standard. However, given the high consumption of bread in Iran, it is recommended that more monitoring of grain and flour storage conditions be conducted (Noroozi et al., 2022). In the research of Iqbal et al. (2013) the contamination of peanut cookies with AFB_1 was investigated by HPLC and the results showed that 40% of cookies were contaminated with this toxin. In recent years, the AFs concentration and risk exposure in whole and refined flour of Iran were evaluated and the presence of AFB_1 was proved in 65 samples. Seven samples contained total AFs above the permissible limit. Therefore, the contaminated flour is consumed after being baked into bread and confectionery products, which poses a risk to health (Heshmati et al., 2021).

Risk assessment of AFB_1 in Kermanshah traditional cookies

-Risk assessment based on EDI and MOE

Assessing the risk of AFB1 exposure in individuals will help align the country's standards with the new regulations established by international organizations. Based on this assessment, risk managers can develop an effective strategy to minimize the health risks to society (Bashiry et al., 2021).

One method of AFB_1 risk assessment is probabilistic modeling based on Monte Carlo simulation. In our research, the findings related to traditional cookie consumption and the population's mean body weight were collected by providing 200 questionnaires, then EDI and MOE were calculated for people under (teenagers) and over 18 years old (adults). The reason for choosing percentiles is to achieve the best (low-exposure consumers), middle, and worst-case AFB_1 risk exposure (High-exposure consumers).

The mean body weight was 64.76±1.36 kg and this information was utilized for EDI calculation (ng/kg bw/day). Exposure evaluation was simulated by the Monte Carlo method and 100,000 times probabilistic a log-normal distribution representing 5, 50, and 95 percent of the population. Table 2 illustrates the measured EDI for AFB_1

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in four samples of traditional cookies. The mean EDI of all cookies was greater in teenagers compared to adults across the 5^{th} (4.80 ng/kg bw/day), 50^{th} (11.3ng/kg bw/day), and 95^{th} (60.6ng/kg bw/day) percentiles ($p \leq 0.01$). Generally, the highest EDI was observed in (95%: 60.6 ng/kg bw/day) adolescents, which can be attributed to the lower weight of adolescents compared to adults; and the lowest EDI was observed in adults (5%: 2.56 ng/kg bw/day). Notably, the highest consumption of cookies among adults (34/53±17/36 g/day) and teenagers (40±12/40 g/day) was for Nanroghani, owing to its popularity as a breakfast item.

Table 2: Estimation Daily Intake (EDI) and daily consumption for the two age groups by Monte Carlo simulation

Traditional cookies	**over 18 years old**				**under 18 years old**			
	Daily consumption (g/day)	**EDI (ng/kg bw/day)**			**Daily consumption (g/day)**	**EDI (ng/kg bw/day)**		
		5%	50%	95%		5%	50%	95%
Nanbernji	2.57±1.36	0.0031	0.32	8.17	2.52±2.11	0.0054	0.23	1.63
Kak	1.80±1.20	0.45	1.83	7.29	1.77±1.02	0.54	2.05	7.75
Nanroghani	34.53±4.36	0.31	0.91	2.57	40±3.40	0.91	2.15	5.24
Nankhormaii	2.26±1	0.39	1.26	3.89	2.15±1.06	0.7	2.75	10.2
Total cookies	10.29±1.98	2.56	6.16	29.1	11.61±1.89	4.80	11.3	60.6

The risk of AFB_1 can be determined by the MOE magnitude; the higher the MOE, the lower the risk of cancer. According to the EFSA, if the MOE is higher than 10,000, it causes less concern in terms of health and cancer, the opposite is true for values lower than 10,000 (Benford et al., 2010). The results of distribution by Monte Carlo simulation show that MOE is less than 10,000 across all age groups and percentiles, except for the 95^{th} percentile of individuals over 18 years that consumed Nanroghani (106,609/7). For some percentiles, MOE was much lower than 10,000 such as 5% for people under 18 years that consumed Nanbernji (0.73) (Table 3). The mean of MOE in all cookie types was higher for adults than for teenagers at 5 (1.24), 50 (32.6), and 95% (81); therefore, cancer risk in adults is lower than in teenagers (Figure 3B).

Table 3: Margin of Exposure (MOE) and excess individual lifetime cancer risk level (Ri) for the two age groups due to the consumption of Aflatoxin B_1 (AFB_1) contaminated traditional cookies using Monte Carlo simulation

Traditional cookies	**over 18 years old**						**under 18 years old**					
	MOE			**Ri**			**MOE**			**Ri**		
	5%	**50%**	**95%**	**5%**	**50%**	**95%**	**5%**	**50%**	**95%**	**5%**	**50%**	**95%**
Nanbernji	1.92	151.17	1.48	5.2×10^{-10}	3.7×10^{-8}	3.8×10^{-7}	0.73	61.35	756.64	5.7×10^{-10}	5.2×10^{-8}	7.3×10^{-7}
Kak	52.06	215.46	835.95	3.1×10^{-8}	2.4×10^{-7}	1.2×10^{-6}	49.6	193	707	2.5×10^{-8}	1.9×10^{-7}	9.6×10^{-7}
Nanroghani	154.35	434.37	106,609.7	1.8×10^{-8}	1.2×10^{-7}	4.7×10^{-7}	74.09	185.25	431.28	3.2×10^{-8}	2.1×10^{-7}	7.1×10^{-7}
Nankhormaii	37.99	144.21	547.26	2.3×10^{-8}	1.6×10^{-7}	6.3×10^{-7}	100.48	316.17	982.64	2.3×10^{-8}	2.1×10^{-7}	7.1×10^{-7}
Total cookies	61.58	236.30	26,998.60	$3.1\times10^{-8.5}$	$2.22\times10^{-7.25}$	$4\times10^{-6.75}$	56.23	188.94	719.39	$3.42\times10^{-8.5}$	$2.82\times10^{-7.25}$	7.77×10^{-7}

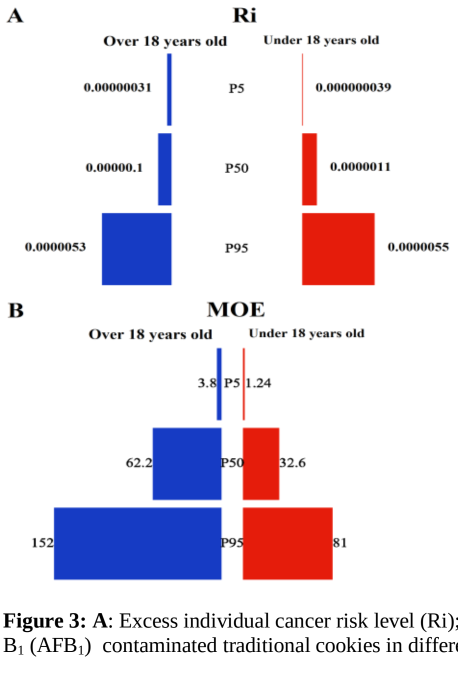

Figure 3: A: Excess individual cancer risk level (Ri); and **B**: Margin of Exposure (MOE) for the two age groups due to the consumption of Aflatoxin B_1 (AFB_1) contaminated traditional cookies in different percentiles (P)

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The EDI average obtained for both age groups (teenagers and adults) and in all percentiles (5, 50, and 95%) was higher than the EFSA acceptable limit (0.017 ng/kg bw/day), which is tumor-causing (Nugraha et al., 2018). Although the daily consumption of Nanroghani was higher, due to its lower AFB_1 concentration, the MOE was higher, making it less harmful. This index shows that according to the higher AFB_1 concentration in the other traditional cookie samples (Nanbernji, Kak, and Nankhormaii) and high EDI, the consumption of these samples poses health risks to the community. The results indicate a threat to the health of people in Kermanshah, and regulatory organizations should increase their monitoring of this issue. Blanco-Lizarazo et al. (2019) investigated the exposure risk of AFB_1 in corn; the samples were collected from Columbia factories. Body weight and consumption data were collected using a questionnaire and MOE was calculated (Based on age group) by Monte Carlo simulation. Their results were consistent with our results because children and younger people were at a higher risk of exposure to AFB_1. Furthermore, the calculations showed that MOE has a potential risk for consumers because it is less than 10,000. In another research, estimation of AFB_1 intake, MOE, and risk identification were investigated by Monte Carlo simulation and it was found that children are at a higher risk of developing cancer. The mean overall EDI was 0.79-1.10 and 1.20-1.66 ng/kg bw/day for children in lower and upper bound scenarios. MOE value in the products was less than 10,000 and the health risk is directed towards the population (Udovicki et al., 2021).

-Level of cancer risk in the age groups

LADD for percentiles (5, 50, and 95%) and cookies (Nanbernji, Kak, Nanroghani, and Nankhormaii) are specified in the Table 4. The lowest amount of LADD for both age groups and all percentiles was observed in Nanbernji. The highest LADD in adults and teenagers was related to Kak (7.42 mg/kg/day) and Nankhormaii (7.17 mg/kg/day). Ri in Figure 3A shows that, generally the mean of Ri has increased in the 95^{th} percentile and the cancer risk is higher in teenagers. Table 3 shows the change of Ri in different percentiles for individuals above and under 18 years old.

Table 4: Lifetime Average Daily Dose (LADD) for the two age groups due to the consumption of Aflatoxin B_1 (AFB_1) contaminated traditional cookies using Monte Carlo simulation

Traditional cookies	over 18 years old LADD (mg/kg/day)			under 18 years old LADD (mg/kg/day)		
	5	50	95	5	50	95
Nanbernji	0.0044	0.21	61.1	0.0041	0.2	1.65
Kak	0.45	1.84	7.42	0.39	1.46	5.46
Nanroghani	0.33	0.91	2.57	0.65	1.53	3.74
Nankhormaii	0.397	1.26	3.89	0.5	1.96	7.17
Total cookies	2.68	6.93	47.5	3.44	8.13	47.2

According to the EFSA pollution committee, the carcinogenic potential of AFB_1 and AFs are reported to be the same (Taghizadeh et al., 2020). Excess individual lifetime cancer risk level (Ri) is calculated by multiplying LADD (mg/kg/day) and SF. SF was calculated for Hepatitis B virus negative ($HBsAgþ^-$) and Hepatitis B virus positive ($HBsAgþ^+$). In addition, the slope of SF for $HBsAgþ^-$ and $HBsAgþ^+$ was assumed 0.01 and 0.3 (cancer cases/year per 100,000 subjects per ng AFB_1/kg body weight (bw) per day), respectively. Calculating the LADD of cookies in general showed that the greatest AFB_1 risk is seen in people under 18 years old. Therefore, to reduce the cancer and the harmful effects of AFB_1, exposure levels should be minimized. The most effective way to prevent AFB1 production is through proper agricultural practices and storage at various stages of production. As a result of consuming four types of traditional Kermanshah cookies, high exposure levels, and the prevalence of hepatitis B in the community (1.7%), it is estimated that five new cases of liver cancer may be added in 10^5 per year (Razavi-Shearer et al., 2018). The lowest cancer risk for both age groups was observed in the 5th percentile and in higher percentiles, the risk increased. According to Bol et al. (2016), exposure to AFs from bakery product consumption was within the range of 2.3-3.2 ng/kg bw/day. It was found that bread and biscuits had the highest role in AFB_1 intake in Brazil, which indicates the significance of bakery products in controlling their AF levels. The calculated mean total AFB_1 long-term exposure from cereal-based meals was 3.38 ng/kg bw/day for the studied population, corresponding to a 1.66 case annual risk of primary liver cancer per 100,000 individuals (Bol et al., 2016). In a study by Alim et al. (2018) the mean of AFB_1 exposure in wheat flour was 1.20±0.003 ng/kg bw/day.

Analysis of parameters influencing sensitivity

Figure 4 presents the sensitivity analysis results generated by Crystal Ball software, highlighting the factors influencing the outcomes. The lowest and highest hazard levels of AFB_1 were observed for teenagers in Nanroghani (37.7%) and Nanbernji (98.3%), respectively. Body weight showed the least sensitivity for teenagers (-27.6) and the

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lowest sensitivity was observed in Ingestion Rate (IR) for adults (-0.9). The relationship between the parameters, including the AFB_1 concentration, the amount of consumption, and people's weight, was investigated with the exposure level. The study by Taghizadeh et al. (2020) evaluating cancer risk from AF exposure through walnuts consumption in Iran yielded different results from ours, because half of the walnuts were contaminated with AFB_1 (0.8-14.5 mg/kg). Additionally their sensitivity analysis showed that body weight is an important factor influencing risk exposure.

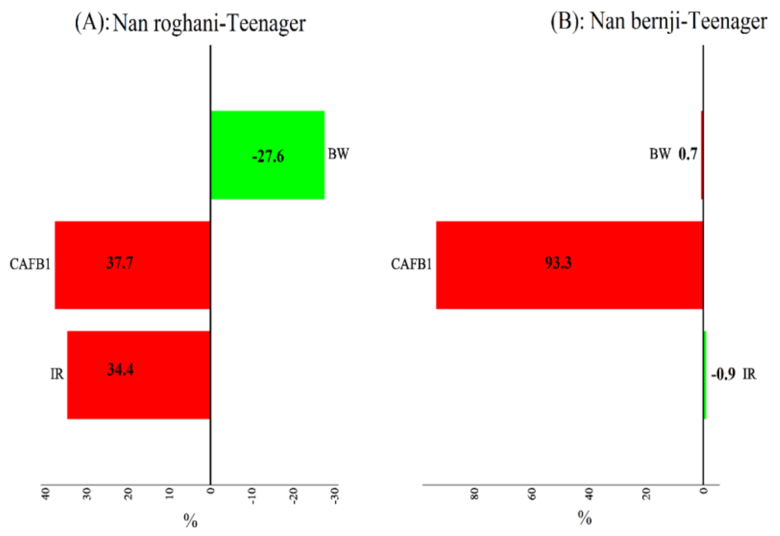

Figure 4: Parameters sensitivity with the highest and lowest influence on excess individual lifetime cancer risk level (Ri) due to the consumption of traditional cookies. **A**: Nanroghani in which the parameters are less sensitive for teenager (Nanroghani-Teenager); **B**: Nanbernji in which the parameters are more sensitive for teenager (Nanbernji -Teenager)
BW=Body weight; $CAFB_1$=Concentration of Aflatoxin B_1; IR=Ingestion Rate

Conclusion

In this research, the AFB_1 concentration in Kermanshah traditional cookies was investigated using HPLC, and the associated risk from the consumption of these cookies was evaluated using the Monte Carlo uncertainty approach. AFB_1 was detected in all samples, and the risk assessment results raised concerns about public health. Furthermore, AFB_1 concentration was identified as the most significant factor contributing to cancer risk. Therefore, the determination of AFB_1 is crucial for risk assessment and quality control in food products. Reducing AFB_1 levels in cookies may be achievable by modifying and improving processing conditions and flour storage, particularly controlling temperature and humidity. Currently, governments prioritize public health and disease prevention while also promoting industrial food processing. Consequently, it is prudent for regulatory agencies to develop innovative strategies to integrate Kermanshah's traditional cookies into the modern food industry.

Author contribution

Methodology and analysis by software were conducted by Z.J.; the project was designed by E.S. and K.A.; project administer was M.S.; other activities including formal analysis, investigation, and project administration were carried out by S.D., S.M., N.F., M.R.G., and M.M. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Ethical consideration

Not applicable.

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