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Research Article

Evaluation and Assessment of Aflatoxin M1 in Milk and Milk Products in Yemen Using High-Performance Liquid Chromatography

Safwan Murshed [1,2]

[1]Center of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan
[2]Yemen Standardization, Metrology and Quality Control Organization, Sana'a, Yemen

Correspondence should be addressed to Safwan Murshed; safwan_1001@yahoo.com

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Aflatoxin M1 is one of the major fungal contaminants found in dairy products around the globe. The objective of this study was to investigate the incidence and occurrence of aflatoxin M1 (AFM1) in samples of milk and milk products in Yemen. The tested dairy product samples were collected from different sources for aflatoxin M1 (AFM1) in Yemen. A total of 250 local and imported samples consisting of 38 liquid milk, 60 powder milk, 62 yogurt, and 90 cheese samples which are marketed throughout Yemen were tested by using high-performance liquid chromatography (HPLC) along with a fluorescence detector and immunoaffinity column purification for detection of AFM1. High levels of AFM1 were detected in preserved milk (77.24%), ranging from 0.021 μg/L to 5.95 μg/L. On the other hand, AFM1 was detected in 66.66% and 68.42% in powdered milk and liquid milk samples, respectively. Among dairy products, 87.09% of yogurt and 81.39% of cheese samples were found contaminated with AFM1. The AMF1 values were higher than the acceptable range for humans set by the European Union. So, we concluded that dairy products used in Yemen showed an AFM1 content beyond the acceptable range, and this is a major factor for causing health-related complications including cancer. In the present study, we reported for the first time the presence of mycotoxins especially AFM1 in dairy products used in Yemen.

1. Introduction

Aflatoxins (AF) are highly carcinogenic toxins [1–3] produced by different groups of fungi, especially *Aspergillus flavus* and *Aspergillus parasiticus*. These two strains *A. flavus* and *A. parasiticus* produce different types of aflatoxins including aflatoxin B1, B2, G1, and G2 [4]. *A. parasiticus* produces aflatoxin B1, B2, G1, and G2, while *A. flavus* merely produces aflatoxin B1 and B2 [5, 6]. Among these toxins, AFB1 is of great interest to livestock and dairy industry [7]. Toxigenic fungi are able to infect animal feed during plant growth, harvesting, and storage [8–11]. Aflatoxin M1 (AFM1), which is found in the milk of animals that have consumed feeds contaminated with AFB1, is the hydroxylated metabolite of aflatoxin B1 (AFB1) [12–14] and it is finally excreted in urine and milk and transmits in dairy products, i.e., fresh and processed milk, cheese, and yogurt. AFM1 was the first aflatoxin detected in milk (M denotes the source, e.g., milk) of cattle and other lactating mammals [15]. Numerous studies have reported the high carcinogenic potential of AFM1 [14]; therefore, the International Cancer Research Association (IARC) has classified AFB1 as a Group 1 carcinogen while aflatoxin M1 (AFM1) is classified as a Group 2B carcinogen [16].

In Yemen, cattle, sheep, and camels account for 20% of national agriculture GDP, and dairy production accounts for 28% of total animal GDP [17]. Despite multifaceted crises, the total milk production in Yemen increased by 71% from 299,008 tons in 2005 to 417,190 tons in 2011. Cow milk accounts for 71% (301,300 tons), goat milk 13% (53,455 tons), sheep milk 10% (43,004 tons), and other sources 5%. However, the annual milk yield recorded in 2011 was extremely low, i.e., 6,086 Hg/animal (cow) as compared to developed countries [18]. Despite such a great increase by

71% in milk production, statistics from the Ministry of Agriculture and Irrigation, Yemen, reveals that the production of milk can only meet one-third of domestic demand. Thus, the country spends a huge amount of revenue on importing long life and powdered milk. Furthermore, more than 95% of processed dairy items are imported from neighboring and developed countries. There are main types of local dairy products such as fresh milk, laban (liquefied yogurt), and cheese from cattle while industrial dairy products (local industries and/or imported) include ultra-high-temperature- (UHT-) treated milk, yogurt, laban (liquefied yogurt), cheese, ice cream, cream, and flavored milk.

The problem of food contaminated with AFM1 is a real concern and acts as a double-edged sword in zones of conflicts that are facing both humanitarian crises and food insecurities. The crisis in Yemen has put the lives of more than 24 million dwellers at risk [19] with approximately 15 million requiring humanitarian assistance, while about 11 million are facing malnutrition and food insecurity [20]. In the ongoing humanitarian crises in Yemen, one of the most vulnerable groups are children under five years [19–21]. Therefore, due to ongoing chronic food insecurities and malnutrition, alternative sources of feeding such as milk and milk products are sought for infants and children. However, the risk of contaminated milk and its products in markets is widespread, and the humanitarian crisis in conflict zones can exacerbate the food insecurities and health risks for the younger population [12, 22]. So far, no data are available on the current status of mycotoxins, especially in dairy products.

In the current study, we aimed to investigate AFM1 in different types of dairy products marketed in Yemen. These findings will provide a baseline for a detailed assessment of risks associated with AFM1 and can help the country to develop policies for protecting its population from health-associated risks caused by AFM1.

2. Materials and Methods

2.1. Sample Collection and Preparation. A total of 250 samples of commercial milk and milk products were purchased from different markets in Yemen. The samples consisted of 38 liquid milk (18 local samples and 20 imported samples), 60 powder milk, 62 yogurt (38 local samples and 24 imported samples), and 90 cheese (20 local samples and 70 imported samples) samples. All samples were refrigerated at 4°C until use. All samples were prepared for downstream analysis in aseptic conditions using sterilized ingredients.

2.1.1. Milk Sample Preparation. Liquid milk samples were placed at 35–37°C in a water bath. Samples were then manually shaken for 5 min to ensure sample homogeneity followed by centrifugation at 2000 rpm for 15 min to separate and discard the fat layer using a spatula. The fatless samples were filtered using Whatman filter paper, and 50 mL of sample was transferred to the cleanup step. For powder milk samples, 10 g of each sample was initially diluted in 50 mL water (preheated in the water bath to 50°C) with continuous mixing using a glass rod until a homogeneous mixture was prepared. The beaker was placed in a water bath at 50°C to ensure complete dissolution. Samples were cooled down to 20°C to 25°C and transferred to a 100 mL volumetric flask. After the mixing process, the volume of each sample was adjusted to 100 mL with water. Filtration was done, and 50 mL of the prepared milk powder sample was transferred to the cleanup step. Artificially contaminated milk samples were prepared from aflatoxin-free milk and used as control.

2.1.2. Cheese and Yogurt Sample Preparation. Twenty-five grams of cheese and yogurt samples were added into a 250 mL volumetric flask or half-pint blender jar (in case samples contain butter melt sample) and 15 g of celite was added. 100 mL of methanol/water (80/20) (v/v) was added to each sample in a stopper flask or seal blender jar. Samples were mixed in a gyratory shaker for one hour or blended for 2 minutes at high speed. The solutions were filtered through filter paper (Whatman No. 4). In 10 mL of each extract, 40 mL of deionized water was added and transferred to the cleanup step.

2.2. Cleanup/Purification and HPLC Condition. The extraction of AFM1 from milk and milk products was performed according to the instructions enclosed with the test kit of immunoaffinity columns and the method described by Iqbal, Asi, and Jinap, 2013, with few modifications [23]. The samples are prepared as mentioned in Section 2.2. The extract was then filtered with Whatman No. 5 filter paper, and about 50 mL of the sample was passed through an AflaTest immunoaffinity column at a rate of 1–3 mL/minute and washed with water (10 ml) twice at the same flow rate. The bound AFM1 was eluted with HPLC grade acetonitrile (assay, 99.8%) with 1.5–3.0 mL (i.e., 3×0.5 ml) of solvent and collected in a vial. Finally, the residue was evaporated with nitrogen stream at 40°C. The samples were placed in the dark place (15 min) at room temperature and the caps of the vials were tightly closed. Then, 200 μL of acetonitrile was added to the vials. A 20 μL portion of the solution was subjected to LC analysis. The HPLC system used in the current study was a Shimadzu Class vp, equipped with a multi-λ fluorescence detector (FD) with an excitation wavelength of 365 nm and an emission wavelength of 435 nm. The chromatographic column was C18 5 mm (4.6×250 mm) (HS, Bellefonte, USA). The mobile phase (water: acetonitrile: methanol; 68 : 24 : 8, v/v/v) was run for 15 min at 30°C with a flow rate of 1 ml/min. Calibration curve was prepared from either peak heights or peak areas by injecting 20 μL of a series of standard solutions of AFM1 with concentrations of 0.05, 0.1, 0.5, 1.0, 2.5, and 10 μg/L to ensure linear relationship. The retention time for AFM1 was 6.2–6.7 min.

2.3. Recovery Check. The percentage of recovery (RC) determines the effectiveness of the analysis method that was spiked with known amounts of aflatoxins.

Internationally, it is recommended that accepted RC be between 70 and 120%. To prepare a spiked sample, an aflatoxin-free sample was taken and artificially contaminated samples (spike) were prepared by pipetting 5 μL of 10 μg/mL standard to 100 mL of milk sample. Spiked milk was transferred to a beaker for mixing. 50 mL of the solution was placed into the immunoaffinity column carefully for analysis as described in the method mentioned previously. This sample solution was prepared which would test the method at levels that have been reported in commercial milk. The recovery of spiked samples was calculated for AFM1. The calculations were made on the basis of the following equation:

$$W_m = W_a \times \left(\frac{V_f}{V_i}\right) \times \left(\frac{1}{V_s}\right), \quad (1)$$

where W_m = amount of AFM1 in the test sample in μg/L; W_a = amount of AFM1corresponding to the area of AFM1 peak of the test extract (ng); V_f = the final volume of redissolved eluate (μL); V_i = volume of injected eluate (μL); and V_s = volume of test portion (milk) passing through the column (mL).

2.4. Statistical Analysis. The data were analyzed and calculated for all statistical values, and the mean and standard deviation of all variables were calculated by using Microsoft Excel® 2013 and SPSS application (Statistical Package for Social Sciences, version IBM 23). Descriptive statistics were performed to get frequencies and charts. For each sample of a given matrix, a series of three analyses are performed, and the coefficient SD represents the variability that can occur between the simultaneous analyses of the same samples.

2.5. Data Validation. Before analyzing the samples for AFM1, known AFM1 concentration was added to mycotoxin-free milk to determine recovery results. The result of the recovery experiment is shown in Table 1. Accuracy and validation of our work were assessed by spiking samples using three concentration levels, such as 0.05, 0.1, and 0.5 μg/L. The recoveries were to some extent depending on spike levels but in any case better than 90% and precision is expressed as relative standard deviation percentage (RSD%). The recovery at added level 0.5 ppb was ranging from 102.94% to 108.31% and RSD was <10% ($n = 3$). Figure 1 shows the standard curve of aflatoxin M1 by the HPLC technique. The standard curve was linear ($y = 649.56 + 12828.88\ x$, y = area and x = amount), and the standard concentrations were ranging from 0.05 μg/L to 10 μg/L AFM1 concentrations. The coefficient of determination (R^2) was 0.99995, and the limit of detection (LOD) was 0.002 μg/L for AFM1 in dairy products.

3. Results and Discussion

Amid security situation and ongoing conflict, Yemen, a developing country in the south of the Arabian Peninsula, is facing several health issues [24, 25]. Among these issues, cancer is a major public health issue and the underlying risk factors are not yet well understood in the south of Yemen. The most common cancers include breast cancer, leukemia, non-Hodgkin's lymphoma, brain cancer, and Hodgkin's disease [26]. Furthermore, the World Health Organization (WHO) estimates a total of 10,000 cancer deaths in Yemen in 2005 with 8000 of these occurring before the age of 70 [27]. Cancer deaths in Yemen accounted for about 5.6% of all deaths in 2005 and are projected to reach 8.4% in 2030. AFM1 is one of the major factors associated with various types of cancers and health issues in humans and livestock [28]. AFM1-contaminated milk is one of the main sources of carcinogens; therefore, we investigated milk and dairy products for the contamination of AFM1 in different regions of Yemen during 2016–2017.

The AFM1 concentrations in milk (liquid and powder), yogurt, and cheese samples are summarized in Table 2 and illustrated in Figure 2. Overall, 250 samples of dairy products were tested for the prevalence of AFM1. Results described that out of 250 samples, 190 (76%) were contaminated with AFM1. Positive samples were 26 (68.42%) liquid milk samples, 40 (66.66%) powder milk samples, 54 (87.09%) yogurt samples, and 74 (82.22%) cheese samples. A total of 130 (52 %) were contaminated at levels above the legal limits of the European Commission, i.e., 0.050 μg/kg for milk and 0.250 for cheese (Figure 3). On the other hand, the incidence of AFM1 contamination in local samples was 88.33%, whereas 77.55% of imported samples were contaminated with AFM.

3.1. Milk Samples. A total of 98 samples (38 liquid milk and 60 powder milk samples) purchased from the markets of Yemen were analyzed for AFM1 by HPLC.

3.1.1. Liquid Milk Samples. Out of 38 liquid milk samples, 18 samples were local and 20 were imported samples. Of the total samples, 26 samples (68.42%) were contaminated with AFM1 ranging from 0.021 to 0.418 μg/L, and the average was 0.085 μg/L. A total of 14 (36.84%) samples had concentrations above the permissible limit (0.05 μg/L) set by EU regulation (Table 3). In our study, out of 18 local samples, 14 (77.77%) were contaminated with AFM1 with an average of 0.047 μg/L, ranging between 0.021 and 0.123 μg/L while 4 (22.22%) of the local milk samples had concentrations above the permissible limit set by the EU regulation (0.05 μg/L). Among the total of 20 imported samples, 12 (60%) samples were contaminated with an average of 0.113 μg/L, ranging from 0.022 to 0.418 μg/L. Among imported samples, 10 (50%) samples had concentrations above the permissible limit set by the EU regulation (0.05 μg/L).

The high percentage of contaminated samples with AFM1 in the current study could be due to the use of contaminated batches of milk powder or contaminated raw milk for producing these samples. Many authors have observed a higher prevalence of contamination with AFM1 in liquid milk (89%), of which 7.4% exceeds the threshold set by European regulations [29]. Similarly, in Iran, studies have evaluated the level of AFM1 in reconstituted milk and

Table 1: Validation of detection protocol through recovery of known amount of mycotoxins in AFM1-free milk. AFM1-free samples were mixed with a known amount of AFM1 standard and analysed by HPLC.

Spike sample	HPLC peak area	Recovery (%)	AFM1 con. in milk (ppb)
Sample 1	116395	108.31	0.541333 ± 1.01
Sample 2	118984	110.73	0.553441 ± 0.97
Sample 3	110651	102.94	0.514469 ± 0.96

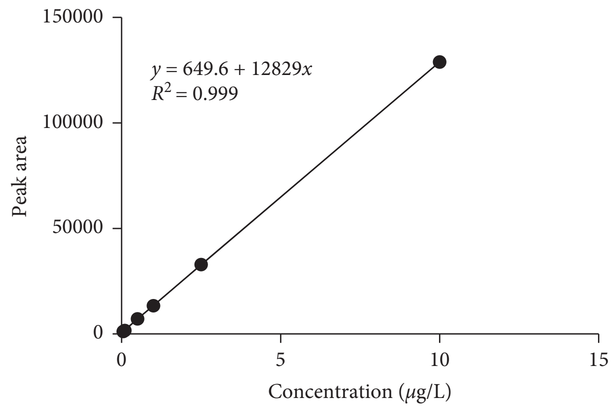

Figure 1: Standard curve of AFM1 contamination.

Table 2: AFM1 in different types of milk products determined by HPLC.

Milk products	Number of samples	Contaminated samples		Exceeding EC regulations >0.05 (μg/L)				
		No.	(%)	No.	(%)	Range	Average	SD[a]
Liquid milk	38	26	68.42	14	36.84	0.065–0.418	0.183	0.144
Powder milk	60	40	66.66	26	43.33	0.051–2.89	0.635	0.952
Yogurt	62	54	87.09	52	83.87	0.053–.893	0.399	0.310
Cheese	90	74	82.22	38	42.2	0.255–5.955	1.198	0.114
Total	250	190	76	130	52	0.051–5.955	0.604	0.380

[a]SD = standard deviation.

reported the presence of AFM1 in milk. The contamination rate varies from 0.45 ng/L to 528.5 ng/L [30,31]. In Syria, Ghanem and Orfi reported that pasteurized milk was contaminated ranging from 8 to 765 ng/kg [32]. On the other hand, in Lebanon, where pasteurized milk is reconstituted from powder milk imported from Arab or European countries, 68% of samples are contaminated with rates ranging from 3.27 to 84.4 ng/L [33]. Also, in Italy, the contamination of pasteurized milk with AFM1 was 1.6% of which 0.5% exceeds the standard recommended by the European Community [34].

3.1.2. Powder Milk Samples. The results of our study revealed that 66.66% of powdered milk samples were contaminated with AFM1. The levels of AFM1 ranged between 0.021 and 2.89 μg/kg and the average was 0.404 μg/kg. Out of total samples, 26 (43.33%) samples were above the permissible limit set by the EU (Table 3).

A similar study conducted in Korea revealed that 74% of powdered milk samples were contaminated with a mean concentration of 270.94 ng/kg [35]. In Lebanon, 35.7% of powder milk samples were contaminated with mean ranging from 9.18 to 16.5 ng/L and the average was 13.7 ng/L [33].

The variation in the mean of AFM1 contamination in powder milk may be attributed to differentiation in geographical regions, climatic factors, and season variability [36]. Furthermore, the differences in the origins of the feed of animals and different levels of contamination of raw milk may have affected the results. The previous studies indicated that the occurrence of AFM1 is higher in the cold season compared to the hot season because cattle are fed with a greater amount of compound feeds in cold seasons [37]. In industrialized countries, strict standards for imported products have been implemented. As a result of these measures, more contaminated products may be directed to markets where the legislation is either absent or are less restrictive [38]. All analyzed powder milk samples of our study were imported from other countries. Our finding was supported by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reports which state that the high levels of AFM1 in milk powder could be a result of complete removal of water, in order to concentrate [39].

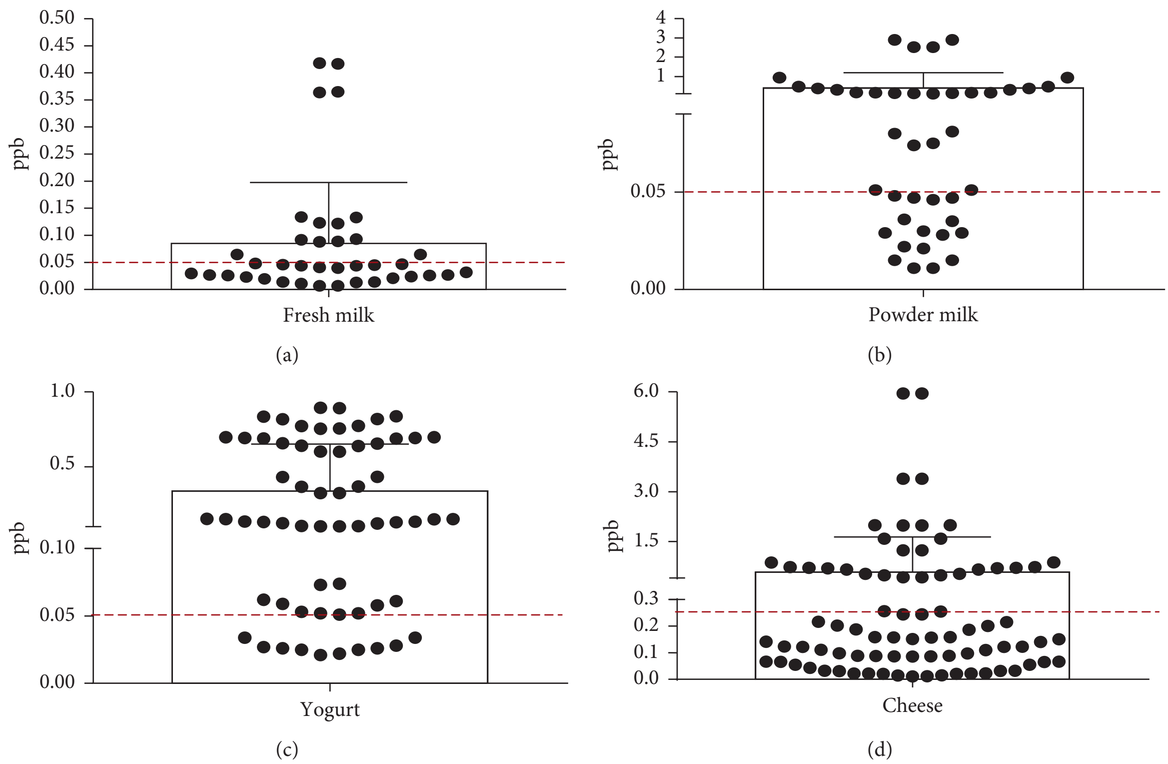

Figure 2: Distribution of aflatoxin M1 concentration in dairy product samples.

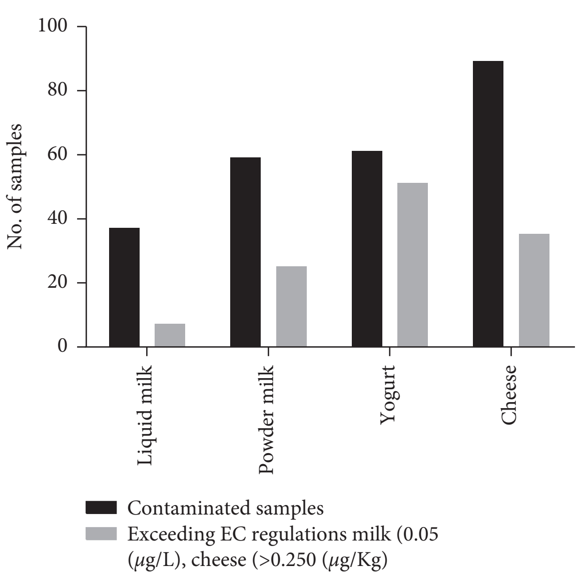

Figure 3: Aflatoxin M1 in different types of milk and milk products.

The high incidence of AFM1 in powder milk is an issue of great concern for public health especially in children as milk contains some major nutrients for the growth of children. So, we have suggested the continuous monitoring and surveillance on foodstuffs and feedstuffs to reduce consumer exposure to aflatoxins.

3.2. Yogurt Samples. A total of 62 yogurt samples, including 38 local and 24 imported yogurt samples, were analyzed. The results revealed that all local samples were contaminated with AFM1. The contamination of AFM1 ranged between 00.053 and 0.893 μg/kg with a mean of 0.515 μg/kg. All local samples were contaminated with AFM1 above the permissible limit of EU regulation (0.05 μg/kg) while 66.66% of imported samples were contaminated with AFM1, out of which 58.33% exceeded the permissible limit. In general, the yogurt samples contaminated with AFM1 ranged from 0.021 to 0.893 μg/kg with a mean of 0.339 μg/kg (Table 4). A similar study was carried out in Italy. Out of 114 samples of yogurt, 91 (80%) samples were positive for AFM1, ranging from <1 ng/L to 496.5 ng/L, with a mean level of 18.08 ng/L [40]. To put our results in perspective, it would be useful to compare them to similar reports elsewhere. The finding of our study was in agreement with those studies conducted in Iran by Barjesteh et al. [41], Behnamipour et al. [42] and Tabari et al. [43] in which all samples of yogurt were contaminated with AFM1 (100%). In contrast to our finding, Ligia and Martins [44] in Portugal showed lower AFM1 contamination which was detected in 18 (18.8%) of yogurt samples which ranged from 0.019 to 0.098 μg/kg, and 78 samples (81.2%) were not contaminated with AFM1. On the other hand, of the 48 natural yogurts tested, only 2 (4.2%) were contaminated with 0.043 and 0.045 μg/kg of AFM1. Other studies conducted in Turkey by Lan et al., 2002, Akkaya et al. 2005, and Atasever et al., 2008 revealed contamination of yogurt with AFM1 was 12.5%, 62.83%, and

Table 3: AFM1 in liquid and powder milk samples through HPLC.

Type of sample		No. of samples	Contamination samples				Exceeding EC regulations >0.05 (μg/L)	
			No. (%)	Range	Mean ± SE	RSD	No. (%)	Range
Liquid milk	Local	18	14 (77.77)	0.021–0.123	0.047 ± 0.172	0.098	4 (22.22)	0.087–0.123
	Imported	20	12 (60)	0.022–0.418	0.113 ± 0.19	0.13	10 (50)	0.065–0.418
	Total	38	26 (68.4)	0.021–0.418	0.085 ± 0.182	0.112	14 (36.84)	0.065–0.418
Powder milk	Total samples	60	40 (66.6)	0.021–2.89	0.404 ± 0.08	0.681	26 (43.33)	0.051–2.89

Table 4: AFM1 in yogurt samples through HPLC.

Type of sample	No. of samples	AFM1 contamination				Exceeding EC regulations >0.05 (μg/kg)	
		No. (%)	Range	Mean ± SE	RSD	No. (%)	Range
Local	38	38 (100)	0.053–0.893	0.515 ± 0.07	0.31	38 (100)	0.053–0.893
Imported	24	16 (66.66)	0.021–0.130	0.134 ± 0.09	0.32	14 (58.33)	0.059–0130
Total	62	54 (87.0)	0.021–0.893	0.339 ± 0.09	0.313	52 (83.8)	0.053–0.893

Table 5: AFM1 in cheese samples through HPLC.

Type of sample	No. of samples	Contamination samples				Exceeding EC regulations >0.250 (μg/Kg)	
		No. (%)	Range	Mean ± SE	RSD	No. (%)	Range
Local	20	16 (72.97)	0.022–3.38	0.279 ± 0.12	1.087	10 (50)	0.323–3.387
Imported	70	58 (82.85)	0.022–5.95	0.635 ± 0.098	1.011	28 (40)	0.255–5.955
Total	90	74 (82.22)	0.022–5.95	0.567 ± 0.11	1.049	38 (42.2)	0.255–5.955

80%, respectively [45]. Several studies were carried out concerning the effect of yogurt manufacturing on AFM1 content, whereas some projects were reported with no influence on aflatoxin M1 concentration [46]. In contrast to our study which found a high frequency of AFM1 contamination in all yogurt samples, the previous studies discovered variable concentrations of aflatoxin M1 in yogurt compared to the milk. This variation of AFM1 content was evaluated due to the effect of fermentation. It was reported that aflatoxin M1 concentration in most yogurt samples showed a significant increase. This increase in aflatoxin M1 was ascribed to different factors such as the formation of organic acids, low pH, other fermentation processes, or the existence of lactic acid bacteria [47]. This high percentage of contamination in yogurt may be attributed to manufacturers who usually used the imported dry milk for producing yogurt that was contaminated with AFM1 or used milk from animals grazing composite and stored fodder that may be contaminated with AFB1 [48]. AFM1 is resistant to thermal inactivation, pasteurization, autoclaving, and other different food processing procedures [48, 49]. To reduce this toxin in dairy products, it is essential to keep feedstuffs free from contamination by AFB1. The concentration of AFB1 in feedstuffs can be reduced by good manufacturing practices (GMP) and good storage practices. It can also be reduced by chemical, physical, or biological treatment [50]. The current study reveals a high concentration of AFMI in all yogurt samples. Yogurt is the most popular dairy product consumed in Yemen, so AFM1 contamination in this product poses a high risk to public health.

3.3. Cheese Samples. The analysis of cheese samples showed that 82.22% of samples were contaminated with AFM1 (74 out of 90) including 72.97% local cheese samples and 82.85% imported cheese samples. The AFM1 contamination ranged from 0.022 to 5.955 μg/kg with an average of 0.567 μg/kg. Among the total cheese samples, 42.2 % of cheese samples were above the permissible limit of the EU regulation which ranged between 0.255 and 5.955 μg/kg (Table 5). The high concentration of AFM1 may be attributed to its environmental condition, bad storage condition, and high relative humidity [51]. Depending on the previous study conducted in 2016, there is a positive connection amid the AFM1 level, average rainfall, and humidity [52]. The values detected in this study were lower than those reported in a study conducted in Turkey which showed that 99% of cheese samples were contaminated with AFM1 [53]. Likewise, in Malaysia 2017, Nadira et al. reported that all samples of cheese were contaminated with AFM1 [54]. In agreement with our present study, in Turkey, 82.4% of white cheese samples were contaminated with AFM1 [55]. The variation of AFM1 presence in cheese samples is generally affected by different factors such as type of cheese studied, cheese-making procedures, geographical region, and cheese ripening conditions (e.g., humidity and temperature) [52]. The presence of aflatoxin M1 in cheese may be due to many reasons, i.e., AFM1 in cattle milk (raw milk) as a result of aflatoxin B1 from contaminated feedstuffs to milk, synthesis of aflatoxins (B1, B2, G1, and G2) by fungi *A. flavus* and *A. parasiticus* that grow on cheese samples, and cheese produced and manufactured from powdered or dried milk contaminated with AFM1 [56]. Since the contamination of feedstuffs with AFB1 plays a major role in the contamination of cheese and other dairy products, feedstuffs must be controlled and analyzed for AFB1 periodically. Also, the dairy product needs stringent quality control during processing and distribution, and this can be achieved by implementing good manufacturing practices (GMP).

Finally, we have found a high level of incidence of AFM1 in all samples of dairy products in Yemen. The outcome of the current study attracts great attention towards very significant health and food security risks for consumers in Yemen, especially to children.

Such observations prompted us to study dairy products for mycotoxins in Yemen and make an attempt to explain the high incidence of cancer in Yemen. Though our study is the first attempt, we believe that systematic research should be carried out to assess the mycotoxins in all consumed food and to study the risk associated with mycotoxins with support from public health authorities.

4. Conclusion

The current study revealed a high frequency of AFM1 contamination in milk and milk products. Therefore, all dairy products have to be controlled under the permissible limit. Since the AFM1 is considered as a significant risk for dairy products, stringent measures should be taken by health and food safety authorities through standard regulations and specifications such as inspection and surveillance on the dairy product and feedstuffs in regard to AFM1 and other aflatoxins. Moreover, imported dairy products should be screened at the entry point to the country. We further recommend that campaigns and strategies should be implemented for public awareness.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Acknowledgments

This paper is dedicated to the fond memory of Dr. Nafees Bacha (1979–2019) my dear friend and PhD supervisor, who passed away during the course of this research.

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