

Research Article

Determination of Aflatoxin B1 Contents in Peanut Protein Snack Bars

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Peanuts, which are rich in nutrients, are used in many products and are often a primary ingredient of protein bars. However, when the necessary production and storage conditions are not met, mycotoxins, and particularly aflatoxin B1 (AFB1), which is the most toxic and most common mycotoxin, may pose a great risk. This study was undertaken to indirectly examine the appropriateness of storage conditions for peanut protein bars sold at different supply points and to identify the presence of AFB1 in compliance with the relevant legal limitations. In February and March 2022, different varieties of peanut protein bars without added sugars were obtained from local markets and nonmarket store chains (places where sports products, cosmetic products, and protein bars are sold) in Ankara, Turkey. AFB1 contents were analysed by the enzyme-linked immunosorbent assay (ELISA) method. The limit imposed by the Turkish Food Codex regulation on contaminants is 5 ppb. While 38.3% of the samples were under that limit, 61.7% were above. No significant difference was found for the place of sale ($p = 0.542$ and $\chi^2 = 2.150$), selling conditions ($p = 0.497$), product ingredients, or remaining shelf-life ($p = 0.804$) regarding the level of AFB1 in the samples. However, it was determined that samples with peanut percentages lower than 17.0% had higher amounts of AFB1 ($p < 0.001$), and other available ingredients might affect the AFB1 content of peanut bars. It was concluded that most samples ($n = 37.0$, $p < 0.001$, $t = -8.607$) posed a risk in terms of AFB1. Considering the shelf-life of such products and that peanuts can produce AFB1 during their shelf-life, it would be beneficial to monitor the frequency of supervision and prevent the sale of peanut bars with AFB1 contents higher than the limits.

1. Introduction

Mycotoxins are toxic metabolites produced by fungal species that may pose a risk to human life, and they can be carried by moulds or wind and air circulation and can endure drought and various other environmental conditions [1, 2]. Mycotoxins have more varied chemical structures and biological activities compared to bacterial toxins. Approximately 400 mycotoxin types have been identified to date and the ones encountered most are aflatoxins, trichothecenes, ochratoxins, and fumonisins [1, 2]. Aflatoxins (AFs) are mycotoxins that can be commonly found in cereals, oilseeds, spices, meats, and many other foods and fodder including dairy products [1]. AFs, which are toxic and carcinogenic polyketide secondary metabolites, are mycotoxins produced by

Aspergillus flavus, *Aspergillus parasiticus*, *Aspergillus nomius*, and *Aspergillus pseudotamarii* strains [3–5]. There are a total of 18 AF types, the most common of which are B1, B2, G1, G2, M1, and M2 [6]. Among them, while the most toxic are AFB1 and AFB3, AFG2 and AFM2 are known to have the lowest levels of toxicity. It has been previously suggested that the order of toxicity of AFs commonly found in foodstuffs and fodder is $AFB1 > AFM1 = AFG1 > AFB2 > AFG2 > AFM2$ [6]. AFB1 is the most powerful hepatocarcinogenic mycotoxin listed as a Group I carcinogenic by the International Agency for Research on Cancer (IARC) [7].

It is known that peanuts and peanut-containing products are the riskiest foods in terms of mycotoxin and especially AF contamination [8, 9]. The amount of toxins present in

peanuts is closely related to the kind, type, and strain of mould as well as the durability and type of peanut, the production method, drying and storing conditions, kernel moisture, and environmental conditions such as temperature, moisture, and precipitation during and after harvest [10, 11]. One of the main food sources of AFs is peanuts as AFs are produced by the fungal species *Aspergillus flavus*, which may be found in peanuts. Faulty agricultural applications during the production of peanuts enable the spread of fungus and other harmful microorganisms. The moisture in the environment before and after storage, which is an important step in the production process, may provide the optimum conditions for the development of toxins [12]. Due to the increasing popularity of peanut-based protein bars in recent years, it is obvious that consumers will be adversely affected due to more frequent consumption [13, 14]. Therefore, the number of studies in the literature addressing this topic and raising awareness is steadily increasing. The present study makes an important contribution by observing whether the presence of AFB1 due to the storage conditions of various peanut protein bars available in markets is within the legal limit or not while also addressing the ensuing health effects.

This study aims to examine the presence of AFB1 in peanut protein snack bars sold in markets and compliance with the legal limit of <5 ppb. In addition, the study indirectly examines the appropriateness of the storage conditions for peanut protein bars sold at different supply points and compares the AFB1 amounts (ppb) of various bars according to their peanut contents and other variables.

2. Materials and Methods

2.1. Sample Selection and Supply. In this study, a variety of ready-to-eat peanut protein bars ($n = 60$) without added sugars available for sale were collected. Samples were obtained from local food markets and nonmarket chain stores (places where sports products, cosmetic products, and protein bars are sold) in Ankara, Turkey.

For the standardization of samples and the generalization of the results, attention was paid to ensuring that at least three samples were obtained for the same product and same brand from different supply points. Samples were supplied from special supply points as soon as possible in special dry sample collection boxes. Additionally, descriptive information about the peanut protein bars was noted, such as the product name, other nutritional elements included in the bars besides peanuts, the supply point, the place of production, the expiry date, and selling conditions. The design of the study is summarized in Figure 1.

2.2. Analysis of Samples. This study was conducted in the Food Chemistry and Analysis Laboratory of the Department of Nutrition and Dietetics, Faculty of Health Sciences, Gazi University. All analyses were carried out in duplicate.

2.2.1. Preparation of Samples for Analysis. The samples supplied were prepared and analyzed without waiting. Samples were homogenized before conducting the enzyme-

linked immunosorbent assay (ELISA) analyses, which were performed using the extraction processes advised by the kit's manufacturer. Accordingly, portions of the samples were granulated and made into a paste. The paste was weighed to 20 g in a blender container, and 100 mL of 70% methanol extraction solution was added such that the ratio of sample to extraction solution was 1:6. The container holding the sample was tightly closed and shaken vigorously for 3 min. The sample was then left to subside, remnants of the protein bars were filtered through a Whatman No. 1 filter paper, and the filtered material was collected. The sample extracts had a pH of 6–8 and were diluted with a buffer at a 1:1 ratio. Samples were thus prepared for analysis.

2.2.2. Aflatoxin B1 Analyses. The AFB1 analyses of the samples were conducted by ELISA using a commercial kit (AgraQuant®, Austria). The kit protocol was followed in the analysis. According to this, the Aflatoxin B1 low-matrix test is based on the principle of solid-phase competitive enzyme inhibition. In this test, polystyrene microwells are partially filled with an antibody unique to AFB1. Diluted samples and standards are pipetted onto the plate, and then, horseradish peroxidase (HRP), a conjugate that can bind AF, is added. If AF is present in the samples or standards, it is bound to the antibody. After a certain incubation period, the ingredients of the microwells are emptied and washed, and HRP substrate, which creates a blue colour in the presence of enzymes, is added. The intensity of the emerging colour is directly proportional to the amount of the conjugate and indirectly proportional to the standard or AF and their amounts in the sample. Acidic stop solution is then added, the conversion of the colour from blue to yellow is prevented, and the absorbance is read at 450 nm. The results obtained from the calibration curve are multiplied by the dilution factor and given in ppb.

2.2.3. Statistical Analyses. While conducting the statistical analyses, the amount of AFB1 and compliance or non-compliance of that amount (ppb) with the legal limit were considered as dependent variables. While evaluating AFB1 in peanut protein bars according to the legal limit, the value of 5 ppb determined by the Turkish Food Codex regulation on contaminants was used as a threshold. The normal distribution of the data was determined using visual (histograms and probability graphs) and analytical (Kolmogorov–Smirnov/Shapiro–Wilk tests) methods. Values were given as numbers (n) and arithmetic means \pm standard deviation ($\bar{x} \pm SD$) in the statistical analysis of the variables. One-way analysis of variance (ANOVA) was used in comparisons of AFB1 amounts of the peanut bars according to the stores in which they were sold and their locations within the stores, and chi-square tests were used for comparisons of the distribution according to the legal limits. Additionally, the percentages of peanuts found in the samples were separated into tertiles and AFB1 amounts were compared with one-way ANOVA according to those tertiles. Binary logistic regression analysis was used to compare the effects of other ingredients in peanut protein bars on the risk

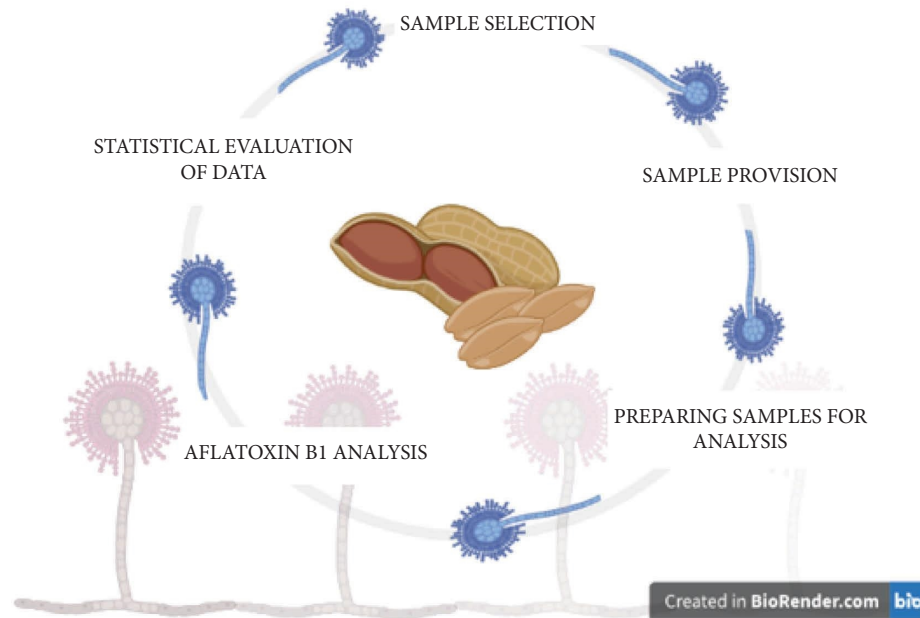


FIGURE 1: Visual representation of the research.

of exceeding the legal limit for AFB1. The independent sample *t*-test was used to compare the AFB1 amounts of the samples according to their remaining shelf-lives. The remaining shelf-lives of the samples were calculated via IBM SPSS Statistics according to their expiry dates and the date of analysis. All statistical analyses were carried out with IBM SPSS Statistics 26.0.

3. Results

3.1. Presence/Amount of Aflatoxin B1 in Peanut Snack Bars. In the scope of this study, 60 peanut snack bars were analysed by the ELISA method in terms of the presence and amount of AFB1. The arithmetic means and standard deviations of the AFB1 amounts of the snack bars (ppb) are shown in Table 1. The maximum AFB1 limit for peanuts and processed products containing them was specified as 5 ppb by the Turkish Food Codex, and 61.7% of the samples obtained in this study had AFB1 contents above 5 ppb. The AFB1 amounts of the samples varied between 2.82 and 9.61 ppb. The average AFB1 of the samples with AFB1 contents of ≥ 5 ppb was 6.52 ± 1.24 ppb, and the average AFB1 of the samples with AFB1 contents of < 5 ppb was 4.12 ± 0.62 ppb ($p < 0.001$) (Table 1).

The comparison of samples according to their place of sale is illustrated in Figure 2. Examining the average AFB1 amounts according to the place of sale, it was seen that average AFB1 levels were 5.58 ± 1.55 ppb in beauty shops, 5.49 ± 1.77 ppb in supermarkets, 5.91 ± 1.36 ppb in stores selling sports products, and 5.83 ± 1.36 ppb in coffee shops ($p = 0.940$, $F = 0.133$) (Figure 2).

The comparison of AFB1 amounts of peanut protein bars according to the place of sale in terms of compliance with the legal limit is detailed in Table 2. Among the obtained samples, 31.6% were from supermarket chains, 51.6% from

TABLE 1: Evaluation of AFB1 in peanut protein bars according to the legal limits.

AFB1 amounts	<i>n</i>	%	\bar{x}	SD	Min	Max	<i>p</i>
<5.00 ppb	23	38.3	4.12	0.62	2.82	4.95	$p < 0.001$ $t = -8.607$
≥ 5 ppb	37	61.7	6.52	1.24	5.04	9.61	
Total	60	100	5.60	1.57	2.82	9.61	

beauty shops, 6.6% from coffee shops, and 10% from stores selling sports products. It was seen that 52.6% of the samples obtained from supermarket chains, 61.3% of those from beauty shops, 83.3% of those from stores selling sports products, and 75% of those from chain coffee shops had AFB1 contents above the legal limit. The compliance of AFB1 levels of the samples with the legal limit did not differ significantly according to the place of sale ($\chi^2 = 2.150$, $p = 0.542$).

The average AFB1 levels of the samples and standard deviations according to the place of sale within the store are given in Table 3. The average AFB1 of samples sold at the cash register was 4.81 ± 1.35 ppb, the average AFB1 of samples sold in aisles dedicated to snack bars was 5.92 ± 1.66 ppb, the average AFB1 of samples sold in aisles devoted to biscuits and chocolates was 4.82 ± 0.56 ppb, and the average AFB1 of samples sold on the lowest shelves of their respective aisles was 5.6 ± 1.57 ppb. The differences between AFB1 levels of samples according to their locations in stores were not statistically significant ($p = 0.086$) (Table 3).

In the examination of the remaining shelf-life of the samples, the average remaining shelf-life of those with AFB1 levels of < 5 ppb was 234.70 ± 77.897 days and the average of those with AFB1 levels of ≥ 5 ppb was 241.71 ± 111.12 days ($p = 0.804$, not shown in the table).

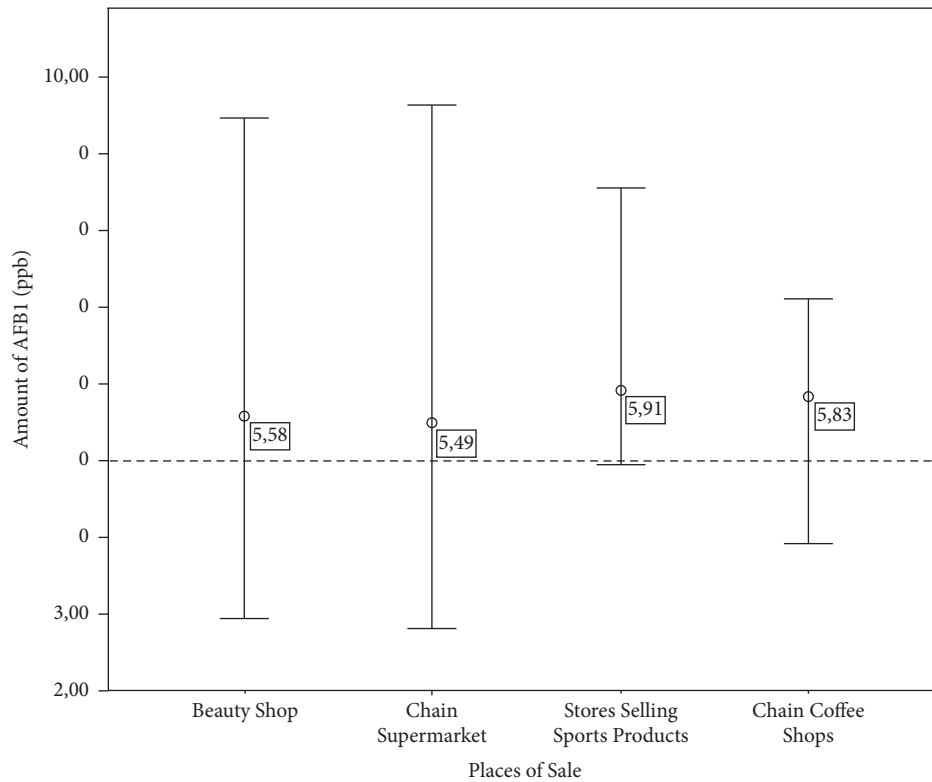


FIGURE 2: Comparison of AFB1 levels according to the place of sale.

TABLE 2: Compliance of AFB1 levels of peanut bars with the legal limit according to the place of sale.

Place of sale	<5 ppb		≥5 ppb		Total		Statistical analysis
	N	%	N	%	n	%	
Chain supermarket	9	47.4	10	52.6	19	31.7	$\chi^2 = 2.150$ $p = 0.542$
Beauty shop	12	38.7	19	61.3	31	51.7	
Store selling sports products	1	16.7	5	83.3	6	10.0	
Chain coffee shop	1	25.0	3	75.0	4	6.6	
Total	23	38.3	37	61.7	60	100.0	

The average AFB1 levels and standard deviations of samples according to their peanut contents (%) are given in Table 4. The average AFB1 (6.43 ± 1.62 ppb) of the products with less than 17% peanut contents ($n = 21$) was significantly higher ($p < 0.05$) compared to those with more than 25% peanut contents (4.79 ± 1.42 ppb) (Table 4).

In terms of ingredients other than peanuts, 15.0% of the snack bars ($n = 9$) contained sweetening agents, 10.0% ($n = 6$) honey, 30% ($n = 18$) nuts other than peanuts, 25.0% ($n = 15$) cereals and cereal products, 10.0% ($n = 6$) coconut, 45.0% ($n = 27$) milk and dairy products, 60.0% cacao ($n = 36$), 30.0% ($n = 18$) fruit concentrates, 15.0% ($n = 9$) dried fruits other than dates, and 70.0% ($n = 42$) dates. The evaluation of samples according to ingredients other than peanuts in terms of the risk of the AFB1 level exceeding the legal limit is provided in Table 5. While peanut bars containing cereal products (RR: 19.75, 95% CI: 1.95–200.35), coconut (RR: 20.26, 95% CI: 1.17–349.00), and dairy products (RR: 10.82, 95% CI: 1.28–91.18) had significantly

increased risk of AFB1 exceeding the legal limit, peanut bars containing dried fruits had decreased risk (RR: 0.02, 95% CI: 0.001–0.444) ($p < 0.05$).

4. Discussion

AFB1 is the most commonly encountered type of AF and has one of the highest levels of toxicity. Dried fruits, cereals, spices, and other similar foods widely consumed in Turkey are potentially risky in terms of AF contamination [15, 16]. Studies in the literature have shown that peanuts pose a particularly high risk for the production of AFB1 [1, 17–20]. The 60 peanut snack bars examined in this study had AFB1 levels varying between 2.82 and 9.61 ppb and 61.7% of them had AFB1 levels of ≥ 5 ppb, above the legal limit, which supports the previous findings regarding peanuts and the risk of AFB1 contamination. In a comparison of 112 peanut, almond, and dried fig samples from Algeria in terms of AFB1 contents, it was found that the dried fig and hulled peanut samples contained high amounts of AFB1

TABLE 3: AFB1 levels of samples according to the place of sale within the store ($\bar{x} \pm SD$).

	<i>n</i>	\bar{X}	SD	Min	Max	Statistical analysis
Products sold at cash register	11	4.81	1.35	2.93	7.10	p = 0.086 <i>F</i> = 2.310
Products sold in aisles dedicated to bars	40	5.92	1.65	2.82	9.61	
Products sold in biscuit/chocolate aisles	7	4.82	0.55	4.25	5.85	
Products sold on bottom shelf of the aisle	2	6.20	1.04	5.46	6.94	
Total	60	5.59	1.56	2.82	9.61	

TABLE 4: AFB1 levels of samples according to peanut contents (%) (ppb).

Peanut percentage (%)	<i>n</i>	<i>x</i>	SD	Min	Max	Statistical analysis
<17	21	6.43 ^a	1.62	3.58	9.61	<i>p</i> = 0.002 <i>F</i> = 7.188
17.01–24.99	15	5.33 ^{a,b}	1.10	3.91	7.37	
>25	21	4.78 ^b	1.42	2.82	9.44	
Total	57*	5.53	1.57	2.82	9.61	

*Three of the samples were excluded from the analysis because their peanut ratios were not specified on the packaging. ^{a, b}The difference between values marked by different letters is statistically significant.

TABLE 5: Risk of the AFB1 level exceeding the legal limit according to the ingredients of peanut protein bars.

Ingredients	<i>B</i>	RR (95% CI)	<i>p</i>
Sweeteners	−0.582	0.56 (0.058–5.402)	0.615
Honey	3.670	39.24 (0.55–2802.983)	0.092
Nuts other than peanuts	−1.298	0.273 (0.021–3.524)	0.320
Cereals and cereal products	2.983	19.75 (1.947–200.354)	0.012
Coconut	3.009	20.26 (1.176–349.009)	0.038
Milk and dairy products	2.381	10.81 (1.283–91.184)	0.029
Cacao	1.212	3.36 (0.647–17.465)	0.149
Fruit concentrates	2.609	13.56 (0.876–210.033)	0.062
Dried fruits**	−3.953	0.02 (0.001–0.444)	0.014
Dried dates	−0.671	0.51 (0.066–3.982)	0.522

*Cereal and cereal products: puffed rice, oats, oat bran, barley malt. **Dried fruits other than dates. *B* = beta; RR = relative risk.

(0.11–174 ppb) and that 83.3% of the hulled peanut samples had AFB1 contamination [21]. In a study conducted in the Ivory Coast in 2018, it was reported that all examined peanut butter samples had AFB1 contamination; 99.0% of the samples had AFB1 levels above 2 ppb, which is the EU limit, and concentrations reached a maximum of 4535 ppb [22]. In another study carried out in Ghana, it was found that peanuts sold in markets could contain AFB1 levels reaching 337 ppb [23]. The presence of such high and varying amounts of AFs in food items is the result of differences in the growing and storage conditions and agricultural practices suitable for the development of *A. flavus* fungus. However, the fact that many products being sold to consumers exceed the legal limits for AFB1 shows that AF monitoring and supervision should be carried out more strictly.

In the present study, we expected to see an increase in AFB1 levels with increasing percentages of peanut contents of the snack bars. However, we obtained the opposite result. The average AFB1 level (6.43 ± 1.62 ppb) of samples with less than 17% peanut contents ($n = 21$) was found to be significantly higher ($p < 0.05$) than that of samples with more than 25% peanut contents (4.79 ± 1.42 ppb). Other ingredients contained in the samples and the processes applied to the peanuts in the production of the snack bars might have led to this finding. For instance, it has been stated that applying heat treatment to peanuts at 150°C for 30 min can decrease

the AFB1 concentration by 30–45% [24]. It is possible that the samples containing higher amounts of peanuts were viewed as more risky, and more sensitive approaches were applied in the production processes. In a study conducted in Brazil, it was found that the AFB1 contents of peanut samples provided for snack consumption were significantly lower compared to those of foods containing peanuts. In the analysis, 8.6% of the peanut samples and 20.9% of the samples of foods containing peanuts were found to have AFB1 levels of >20.00 ppb. These results were interpreted as revealing that peanuts added to products in the form of mixtures are of lower quality [25]. A similar situation might have happened with the products containing lower amounts of peanuts in the present study. In another study, it was found that the AFB1 contents of stored peanut samples varied widely in the range of 7.00–116.00 ppb. It was also seen that the AFB1 concentrations of peanuts that are not stored in suitable conditions can reach 3276 ppb [26]. In brief, many variables such as the place where the peanut bar is produced, the quality of the peanut samples, the growing and storage conditions, and the production processes of the products can affect the AFB1 content of the final product.

According to the results obtained in the present study, the inclusion of cereal products (RR: 19.75, 95% CI: 1.95–200.35), coconut (RR: 20.26, 95% CI: 1.17–349.00), and dairy products (RR: 10.82, 95% CI: 1.28–91.18) in peanut snack bars can significantly increase the risk of AFB1 exceeding the legal limits ($p < 0.05$). In a study that examined 48 samples from 10 different parts of Ghana, it was found that the amount of AFB1 in snacks that only contained cereals (1.00–11.70 ppb) was significantly lower than that of snacks that contained both cereals and peanuts (1.00–796.00 ppb). The presence of peanuts, as a dried legume with the highest susceptibility to AF contamination, was a contributing factor to these results. In addition, it was highlighted in that study that low-quality peanuts were possibly used in some snacks in the form of a mixture. Additionally, many other factors were said to be influential in the wide range of AFB1 contents of samples with peanuts, such as the quality of the peanuts, cereals included in the product ingredients, and storage time and conditions. In a study of cereal-based foods, it was found that more than 50% of the considered samples contained AFB1 amounts exceeding the legal limit (>2 ppb), with AFB1 concentrations ranging from 0.18 to 23.27 ppb [27]. In particular, when the samples containing mixed cereals were examined, it was seen that 93% of them contained AFB1, and the average AFB1 concentration was 4.63 ppb [27]. In another previous study, the AFB1 contamination rates were found to be 96% and 57%, respectively, for corn and rice samples, and the rates of samples exceeding the limit of the European Union (2 ppb) were 58% and 24%, respectively. Moreover, maximum concentrations were found to be 80 ppb for corn and 14 ppb for rice, which were much higher compared to the values obtained for peanut butter [22].

Coconut powder and coconut oil are also classified as risky foods in terms of AF contamination, and in the present study, it was found that the risk of AFB1 in peanut bars with coconut was significantly high. In a previous study conducted in Turkey,

coconut powder, black pepper, red pepper flakes, and powdered pepper were examined in terms of the AF risk, and it was concluded that the best substrate for AF-producing fungi to reproduce was coconut powder. Tropical regions with high moisture and temperatures where coconuts grow also provide an ideal environment for aflatoxigenic fungi to reproduce [28]. These AFs can be transferred to cooking oils. In another study, 37.5% of 32 coconut oil samples were found to contain AF above 1 ppb, and this value reached a maximum of 60.92 ppb. On the other hand, in samples of other oils (palm olein, sunflower oil, sesame oil, olive oil, and corn oil), no AFs were found [29].

Another factor contributing to the high AFB1 contents of the peanut bars sampled in our study was found to be dairy products. This result is surprising because, generally, the AF type commonly encountered in compositions containing dairy products is AFM1. The AFB1 in the fodder of cows, sheep, and goats is absorbed from the gastrointestinal canal and transferred to the liver via the portal vein, and then, it is converted into AFM1 with the catalysis of p450 enzymes. In instances where animals are exposed to more AFB1 than they can metabolize, both toxins may appear in their milk [30]. In one study, AFB1 levels were found to be 0.7–1.5 ppb in 42% of pasteurized milk samples and 13% of UHT milks [31]. Even though these amounts are far below the legal limits, considering that the samples examined in this study had AFB1 of 9.61 ppm at most, these samples might provide significant contributions to the overall AFB1 value. This finding might have occurred as a result of a common interaction between AFB1 and other ingredients found in peanut bars that contain milk. Low sample numbers unfortunately create restrictions regarding the certainty and generalizability of the results.

In all stores selling peanut bars, regardless of the type of store, it was found in the present study that more than 50% of all obtained samples had AFB1 of ≥ 5 ppb. This rate was highest in the stores selling sports products at a level of 83.3%, although this finding was not statistically significant ($p > 0.05$). This raises the question of whether the storage and/or selling conditions in these stores are suitable for food items or not. Chen et al. found that there were no statistically significant differences between products from different stores in terms of amounts of AFB1, but Elshafie et al. observed that the incidence of AFB1 was lower in retail stores samples than in samples prepared by street sellers [32, 33].

When the placement of the products within the stores was examined, products sold at the cash register had the lowest AFB1 values (4.81 ± 1.35 ppb) and products sold on the bottom shelves of their respective aisles had the highest (6.2 ± 1.04 ppb) ($p > 0.05$). This might be because products placed at the cash register are sold more frequently and product circulation is more rapid; therefore, the storage period in the store is shorter. Similarly, Mutegi et al. revealed that increased storage periods of products correlated with increased levels of AFB1 [34]. However, in our study, when the remaining shelf-lives of samples with AFB1 levels of < 5 ppb and ≥ 5 ppb were examined, the averages were found to be very close and the standard deviations were very wide; therefore, it was not possible to come to a concrete conclusion.

5. Conclusion

In this pilot study, it was determined that most peanut snack bars purchased from various stores in Ankara (Turkey) were contaminated with AFB1 and a significant portion of these bars exceeded the legal limit for AFB1. Furthermore, the AFB1 levels of these products did not significantly differ according to environmental conditions of sale. Therefore, it was concluded that not permitting the sale of peanut snack bars with AFB1 levels higher than the legal limit is the most significant preventive action to be taken. In this regard, clarifying the criteria in the current legislation in both Turkey and the world for the frequency of sampling and the analysis of products with low volumes available for retail sale, such as peanut snack bars, would be an effective approach together with the frequent performance of the necessary inspections. Considering that these products can produce AFB1 during their shelf-life, establishing the frequency of inspections and formalizing that frequency in law will be helpful for avoiding the occurrence of such undesired compounds. In addition, it may be recommended that the AFB1 concentration of samples containing peanut-cereal-coconut and dairy products together, which are found to have a higher risk in terms of AFB1 compared to other components, should be closely monitored. However, further studies with larger numbers of samples and more sensitive analytical methods are required to provide further evidence.

Data Availability

The data supporting the conclusions of the article are fully described within the article and the database is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors designed and drafted the work or revised it critically for important intellectual content and approved the final content of this article.

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