processes

MDPI

Article

Fungal Isolation, Detection, and Quantification of Aflatoxins in Nuts Sold in the Lebanese Market

Heba Hellany [1,*], Jean Claude Assaf [2,*], Joseph Matta [3,4] and Mahmoud I. Khalil [1,5]

[1] Department of Biological Sciences, Faculty of Science, Beirut Arab University, Beirut P.O. Box 11-5020, Lebanon; m.khalil@bau.edu.lb or mahmoud_ibrahim@alexu.edu.eg
[2] Department of Chemical Engineering, Faculty of Engineering, University of Balamand, P.O. Box 100, Tripoli 1300, Lebanon
[3] Department of Nutrition, Faculty of Pharmacy, Saint Joseph University of Beirut, Medical Sciences Campus, Damascus Road, Beirut P.O. Box 11-5076, Lebanon; chem@iri.org.lb
[4] Industrial Research Institute, Lebanese University Campus, Hadath Baabda, Beirut, Lebanon
[5] Molecular Biology Unit, Department of Zoology, Faculty of Science, Alexandria University, Alexandria 21568, Egypt
* Correspondence: heba.hellany@outlook.com (H.H.); jeanclaude.assaf@balamand.edu.lb (J.C.A.)

Abstract: This study examines the prevalence of aflatoxin contamination in 160 nut samples, both shelled and unshelled (including pistachios, peanuts, and walnuts), from the Lebanese market, focusing on their fungal contamination and specific toxigenic strains. Aflatoxin B1 (AFB1), known for its potent carcinogenic and immunosuppressive properties, was detected in various samples. Moisture content analysis showed that unshelled nuts often exceeded maximum moisture limits more frequently than shelled nuts, with levels ranging from 1.9 to 9.5%. The predominant fungal genus identified through cultivation on potato dextrose agar (PDA) plates was *Aspergillus*. In total, 55% of samples were contaminated with *A. flavus* and 45% with *A. niger*. All toxigenic strains isolated were identified as *Aspergillus flavus*. The aflatoxins, particularly AFB1, were quantified using the enzyme-linked immunosorbent assay (ELISA) and reversed-phase high-performance liquid chromatography (HPLC), revealing contamination in 43.8% of the samples, with concentrations ranging from 0.4 to 25 µg/kg. Some samples notably exceeded the established maximum tolerable limits (MTLs) for AFB1, set between 2 and 8 µg/kg. Shelled pistachios showed the highest contamination rate at 52% and were the most frequent to surpass the MTL of 8 µg/kg for pistachios, whereas walnuts displayed the lowest contamination levels, with only 15.4% exceeding the MTL for aflatoxins.

Keywords: aflatoxins; Lebanese nuts; toxigenic fungi; ELISA; HPLC

Citation: Hellany, H.; Assaf, J.C.; Matta, J.; Khalil, M.I. Fungal Isolation, Detection, and Quantification of Aflatoxins in Nuts Sold in the Lebanese Market. *Processes* **2024**, *12*, 1018. https://doi.org/10.3390/pr12051018

Academic Editor: Jer-An Lin

Received: 8 April 2024
Revised: 28 April 2024
Accepted: 30 April 2024
Published: 17 May 2024

1. Introduction

Aflatoxins are secondary metabolites formed by various types of filamentous fungi, including *Aspergillus flavus* and *A. parasiticus*, commonly discovered as pollutants in numerous agricultural products such as grains, oil-bearing seeds, tree nuts, and spices [1,2]. It is widely recognized that warm and moist climates facilitate the spread of molds that produce aflatoxins, posing a significant risk, particularly in tropical regions [2]. However, the prevalence of contamination is often the result of a mix of weather conditions, environmental influences, and poor farming techniques, such as improper collection and storage of produce [2]. The aflatoxin (AF) category comprises over 20 identified metabolites, with the most significant ones being the naturally occurring types like B1, B2, G1, and G2 [1]. Aflatoxin B1 (AFB1) is identified as the most common and most potent toxin within the group of aflatoxins. Its carcinogenic properties and immunosuppressive effects have been extensively documented in various animals including rats, poultry, cattle, and trout. The degree of its impact varies according to the species, gender, and age of animals [2]. Aflatoxins pose significant health risks across various stages of human life. Aflatoxins can penetrate

the placenta and negatively impact the growth and development of a fetus, leading to major fetus problems, including low birthweight and preterm birth. In addition, exposure to aflatoxin during early childhood has been significantly linked to impairments in growth, development, and immunosuppression in early adulthood [3]. Furthermore, the aflatoxins are genotoxic in the bone marrow and spermatocyte cells, having cytotoxic effects on kidney and liver cells, such as NA synthesis, chromosome segregation, and progression through mitosis impaired by aflatoxins [4]. Aflatoxins also result in protein synthesis impairment, effects on the metabolism of micronutrients, and an increase in immunosuppression. Exposure to aflatoxins in humans has been linked to death; hepatocellular carcinoma; and liver cancer (hepatocellular carcinoma), especially in hepatitis-B-positive individuals [5]. Additionally, it has been documented that aflatoxins can lead to occupational cancers of the lung and skin through inhalation and direct contact, respectively [6].

AFB1 is identified as a potent hepatocarcinogen and a genotoxic agent, and it has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) [7,8]. Each year, approximately 25% of the global food crops, including nuts, are affected by contamination, resulting in losses in both the agricultural and industrial sectors [7]. Nuts including peanuts, almonds, cashews, and pistachios are at high risk of aflatoxin contamination due to their high fat content and various factors, such as pre-and post-harvest conditions, storage conditions, and geographical location [7]. Aflatoxins (AFs) and ochratoxin A (OTA) are common mycotoxins found in nuts [7]. The Rapid Alert System for Food and Feed (RASFF) report highlighted that, from 2010 to 2019, nearly 99% of mycotoxin notifications reported in the U.S. were associated with aflatoxin (AF) contamination, predominantly impacting almonds, pistachios, and peanuts [9].

In the Mediterranean area, nuts are popular and are often consumed due to their health benefits, such as being a good source of beneficial fats (monounsaturated and polyunsaturated), fibers, proteins, vitamins (E and K), and minerals (magnesium and potassium) [10,11].

The global occurrence and high toxicity of mycotoxins have attracted the attention of many national and international organizations. The US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have established numerous rules and guidelines to address the issue of mycotoxins in food, including nuts, and feed. For instance, the European Union (EU) has set a maximum level of 15 μg/kg for total aflatoxins in nut samples, whereas the FDA has set a limit of 20 μg/kg for total aflatoxins in different nut samples, including peanuts and pistachios [7]. In Lebanon, the Lebanese Standards Institution (LIBNOR) adheres to European regulations by setting the maximum tolerance levels (MTLs) for AFB1 in nuts as 2 μg/kg for peanuts and walnuts, and 8 μg/kg for pistachios, aligning with the European guidelines of 2–8 μg/kg [12,13].

In Lebanon, there has been an annual rise in the number of liver cancer and kidney disease cases. According to a 2024 GLOBCAN report from the International Agency for Research on Cancer (IARC), the incidence rates of liver and kidney cancer in Lebanon were 0.71 and 2.4 cases per 100,000 individuals, affecting individuals of all ages and genders [14]. In addition, in Lebanon, nuts are highly favored and widely used in cooking, leading to their frequent importation, thus meeting market demands [15]. The majority of nuts available in the Lebanese market are imported, with total imports valued at EUR 96,080 in 2015, encompassing a variety of nuts such as walnuts, almonds, cashew nuts, and hazelnuts. The principal countries supplying these nuts to Lebanon include Iran, Turkey, Saudi Arabia, the USA, and Ukraine [16].

However, the climatic conditions in Lebanon, marked by high humidity and temperatures, create perfect conditions for the development of mold in nuts. This leads to an increased risk of aflatoxin exposure among the Lebanese population due to the presence of aflatoxins in nuts [17]. Thus, it is essential to check food products present in the Lebanese market for contamination and assess their health impacts. Several studies have evaluated the occurrence of mycotoxins in different commodities in Lebanon, such as wheat and wheat products [18], tea [19], rice [20], baby formulae [21], and spices [22].

Therefore, this study aimed to screen for fungal contamination in nuts available in the Lebanese market and to identify the toxigenic fungi found in the samples. Additionally, this research involved an initial evaluation of aflatoxins in nuts using the ELISA, followed by quantification using high-performance liquid chromatography (HPLC).

2. Materials and Methods

2.1. Chemicals

Standards of total aflatoxins and AFB1 were purchased from Supelco (Bellefonte, PA, USA). The acetonitrile and methanol solutions were obtained from Sigma-Aldrich (Steinheim, Germany) and HPLC-grade water was prepared at the Industrial Research Institute (IRI). For the clean-up step, immunoaffinity columns (IACs) were used and purchased from Meizheng (PerkinElmer, Beijing, China). All remaining chemicals and reagents were of analytical grade.

2.2. Sampling of Different Types of Lebanese Nuts

A total of 160 nut samples were collected from supermarkets and stores across different regions of Lebanon, including pistachios with shells (28), pistachios without shells (26), peanuts with shells (27), peanuts without shells (28), walnuts with shells (26), and walnuts without shells (25). The nut samples were collected over one year during the period from June 2022 to June 2023, from three Lebanese governorates: Beirut, Mount Lebanon, and Beqaa. The samples were sourced from large supermarkets and small stores across the three regions in Lebanon. Using the method of sampling, the homogenized and representative samples were prepared, according to the official method proposed by the Association of Official Agricultural Chemists (AOAC) [23], to minimize the size of the sample and ensure thorough mixing. This process aimed to achieve an even distribution of any contaminated parts. For each nut sample, the content was blended and thoroughly mixed to produce a 100 g sample for further testing. All samples were kept in sealed polyethylene bags at a temperature of −20 °C until the time of analysis.

2.3. Determination of Moisture Content

The method from AOAC (2000) was employed to determine the moisture content of the samples [23]. In summary, 5 g of the sample was placed in a dish that had been previously weighed, and it was then oven-dried at 105 °C for a duration of 4 h. Following drying, the samples were left to cool until they reached a stable weight. This experiment was conducted in triplicate. The calculation of moisture content was based on the weight loss, and this was represented as a percentage of the initial weight [24], as demonstrated below:

$$\% \text{ moisture content} = \frac{W2 - W3}{W2 - W1} \times 100$$

where W1 is the weight of an empty Petri dish; W2 is the weight of the sample and the Petri dish before drying; and W3 is the weight of the sample and the Petri dish after drying.

The percentage of shelled and unshelled nuts exceeding the maximum moisture content limits was calculated for all nut types. The maximum moisture content levels for nuts are 8% for unshelled peanuts and 6% for both unshelled pistachios and unshelled walnuts [25,26].

2.4. Isolation and Identification of Mycoflora

The method used for isolating fungi from Lebanese nuts, as outlined by Adetunji et al. [27], involved the direct seed plating technique. In this process, three to four seeds from the nut samples were sterilized using a 2% (v/v) sodium hypochlorite solution inside a sterile conical flask for one minute. Subsequently, the seeds were washed with sterile distilled water three times, each for two minutes, to remove any residual chemical agent effects. Next, four halved cotyledons from the nuts were placed at equal distances on potato dextrose agar (PDA) Petri dishes mixed with 0.01% (w/v) chloramphenicol to inhibit

bacterial growth. This procedure was repeated three times, and the plates were then incubated in the dark at 25 °C for seven days. Following incubation, each distinct fungal colony was transferred to new sterile PDA plates. The fungal isolates were identified based on a comparison of their protein profiles with those available in the database using the matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) technique (Biomérieux, Craponne, France).

The frequency of isolation for each species was determined following the method outlined by Adetunji et al. [27]:

$$\text{Frequency } (\%) = \frac{\text{Number of samples contaminated with a species or genus}}{\text{Total number of samples}} \times 100$$

2.5. Isolation and Detection of Toxigenic Fungal Contaminants

To isolate toxigenic *Aspergillus* species from nuts, an isolation method described by Ozay et al. [28] was followed. The kernels were first surface-sterilized by soaking them in 70% ethanol for 2 min, followed by a 2 min immersion in 0.4% chlorine solution. Subsequently, these kernels were directly plated onto *Aspergillus flavus* and *parasiticus* agar (AFPA) medium [29]. The plates were supplemented with chloramphenicol (100 mg) to inhibit bacterial growth. The plates were then incubated at a temperature of 25 °C for a period of 3 to 5 days. The presence of black pigmentation served as the primary screening criteria for the identification of toxigenic *Aspergillus* species. Following this initial identification, microscopic characterization and the MALDI-TOF technique were employed for a more precise identification. Isolations from nut samples were repeated three times for each sample.

2.6. ELISA Analysis

The nut samples were tested for total aflatoxins (AFs: AFB1, B2, G1, and G2) using the 8031 Veratox Direct Aflatoxin Test Kit (Neogen, Lexington, KY, USA). The Microplate Reader (ELx 800, Bio-Tek, Shoreline, WA, USA) measured the absorbance of the wells at 650 nm. The process involved blending nut samples with 70% methanol at high speeds. The mixture was then filtered through F42 filter paper, and the resulting filtrate was prepared for ELISA testing. Controls (0, 1, 2, 4, and 8 ppb) and sample aliquots of 100 µL were placed into the mixing well, where 100 µL of conjugate had already been added. After mixing, 100 µL from the mixed solution was transferred into the antibody-coated wells and incubated at room temperature for 10 min. Following incubation, the wells were washed five times with a washing solution (distilled water). After drying the wells, 100 µL of substrate was added to each well and incubated for an additional 10 min. The process was completed by adding 100 µL of stop solution to each well. A calibration curve for the standard solutions of aflatoxins was established, and the absorbance of the wells was measured at 650 nm. The limit of detection (LOD) for the ELISA kit employed in the study was determined to be 0.5 ppb.

2.7. HPLC Determination of AFB1

Following the ELISA screening, HPLC analysis was carried out on positive samples. To summarize, a 25 g portion of the sample was blended with 5 g of sodium chloride (NaCl) and a mixture of methanol–water (3:7, v/v). The resulting mixture was then filtered through F24 filter paper. Subsequently, 10 mL of this filtrate was slowly passed through a Meizheng immunoaffinity column (PerkinElmer, China) at a flow rate of 1–2 drops per second and rinsed twice with 10 mL of water at the same rate. The final eluate was used for HPLC analysis.

HPLC analysis was conducted using a JASCO HPLC system (JASCO Corp., Tokyo, Japan) equipped with a fluorescence detector Prominence RF-20Axs (Shimadzu, Kyoto, Japan). Chromatographic separation occurred on a C-18 column (4.6 mm internal diameter × 250 mm, 5 µm particle size, Supelco Discovery) with a water–methanol–acetonitrile

(30:10:10, $v/v/v$) mobile phase at a flow rate of 1 mL/min. The fluorescence detector had an excitation wavelength of 365 nm and an emission wavelength of 435 nm.

2.8. Validation of HPLC

The linearity was determined and a calibration curve was created with AFB1 standards across 5 varying concentrations, ranging from 0 to 10 µg/kg to calculate the coefficient of determination (R^2). The detection limit (LOD) and quantification limit (LOQ) were assessed based on signal-to-noise ratios of 3:1 for LOD and 6:1 for LOQ, respectively [30,31]. The accuracy of the HPLC was confirmed through recovery tests, which involved spiking AFB1 concentrations of 2, 5, and 10 µg/kg into three types of nuts prior to extraction [30]. Additionally, the precision of the HPLC method for detecting aflatoxin B1 (AFB1) was evaluated by preparing samples spiked with AFB1 at concentrations of 0.5, 2.5, and 10 µg/kg, followed by conducting ten injections per concentration in a single session. Precision was assessed by calculating the relative standard deviation (RSD) of the mean [32].

2.9. Estimation of Daily Intake of AFB1

The average daily nut consumption in Lebanon was established through a list which was created using food consumption data from a survey of individual food intake among an adult population in Lebanon [33]. The daily intake of nuts in Lebanese adult population was estimated to be 6 g/day and an average body weight of 72.8 kg was utilized, reflecting the average weight of participants in the food consumption survey [33]. The mean for the total group of pistachios, peanuts, and walnuts was approximately 5.32 in the current study. The estimation of daily intake of AFB1 from nuts consumption in Lebanon can then be estimated by multiplying the detected average AFB1 concentration in the nut samples by the daily consumption of nuts, determined from the food frequency questionnaire [34], as follows:

$$\mathrm{EDI} = \frac{\mathrm{Di} \times \mathrm{Mi}}{\mathrm{W}}$$

where EDI is the estimation of daily dietary AFB1 intake (µg/kg b.w./day), Di is the daily consumption of nuts in an age group (g/day), Mi is the mean level of AB1 in nuts (µg/kg), and W is the weight (kg).

2.10. Statistical Analysis

IBM® SPSS® 2022 software was used for the calculation of means, ranges, and standard errors of the mean (SEMs), and it was also employed to conduct independent sample *t*-tests to identify significant differences with a *p*-value threshold of ≤ 0.05.

3. Results

3.1. Moisture Content Levels

The moisture content of Lebanese nuts collected is shown in Table 1. The moisture contents of pistachios ranged from 2.7 ± 0.11 to 9 ± 0.14%, from 4.9 ± 0.08 to 9.5 ± 0.11% for peanuts, and from 1.9 ± 0.08 to 7.7 ± 0.08% for walnuts. The percentage of samples exceeding the maximum moisture content limits (8% for peanuts, and 6% for both pistachios and walnuts) was observed to be higher in nuts without shells than in those with shells. In total, 50% of unshelled pistachios, 44% of unshelled peanuts, and 36% of unshelled walnuts were above the recommended limits. However, only 28.5% of shelled pistachios, 22% of shelled peanuts, and 11.5% of shelled walnuts exceeded the maximum limits.

Table 1. The moisture content in shelled and unshelled Lebanese nuts.

Lebanese Nuts	Moisture Content Range (%)	
	With Shells	Without Shells
Pistachios	(2.7 ± 0.11)–(7.2 ± 0.14)	(2.7 ± 0.12)–(9.0 ± 0.14)
Peanuts	(4.9 ± 0.08)–(8.9 ± 0.11)	(5.3 ± 0.14)–(9.5 ± 0.11)
Walnuts	(1.9 ± 0.08)–(7.6 ± 0.13)	(3.1 ± 0.12)–(7.7 ± 0.08)

Data are represented as range ± SEM.

3.2. Mycoflora Presence

In total, 80% of the Lebanese nut samples tested on PDA plates were contaminated with fungi. Four fungal species were identified in Lebanese nuts (Table 2). The most common fungi present in all types of nuts included *Rhizopus arrhizus*, *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus*. The most prevalent fungi isolated were *Aspergillus flavus* and *Aspergillus niger*, with 61.2% and 56.5% occurrence rates, respectively. Statistical analysis was performed, and the calculated *p*-values were $\leq$0.05, indicating significant differences between the groups in terms of fungal contamination rates.

Table 2. The distribution of fungal contaminants in Lebanese nuts.

Fungi Isolate	Number of Samples Infected	Frequency of Isolation (%)
A. flavus	84	61.2
A. niger	73	56.5
R. arrhizus	55	42.6
C. albicans	38	30

3.3. Incidence of Toxigenic Fungal Contaminants

Differentiating between toxigenic and atoxigenic fungi was achieved using AFPA plates, with toxigenic fungi displaying an orange reverse color and atoxigenic fungi exhibiting a black reverse color. The subsequent culturing of the positively identified isolates on PDA plates confirmed that they all belonged to the *Aspergillus flavus* species, with no detection of *Aspergillus parasiticus* among the isolates. Analyzing the distribution of toxigenic *A. flavus* across various types of Lebanese nuts revealed its presence in 38% of the samples. However, the pistachio samples had the highest incidence of toxigenic *A. flavus* contamination, affecting 40% of the samples.

3.4. ELISA Analysis

In this study, the initial screening of contaminated samples with total aflatoxins was conducted using the ELISA method, which is effective for efficiently assessing contaminants in a large volume of samples and in a shorter time. The results indicated that out of 160 samples, 70 samples of both shelled and unshelled nuts (43.8%) exhibited contamination levels exceeding the MTL for total aflatoxins (15 µg/kg) [7] and they were selected as possible positive samples. Pistachio samples were identified as the most contaminated samples (52%), while the lowest levels of contamination were detected in peanuts and walnuts.

3.5. HPLC Analysis

For the further verification and quantification of AFB1 levels, HPLC was utilized. Table 3 provides a summary of AFB1 findings in samples of Lebanese nuts. Out of 70 positive samples analyzed, 14.3% exceeded the MTL for AFB1 in nuts (2 µg/kg for peanuts and 8 µg/kg for both pistachios and walnuts), and all these samples were unshelled. No shelled nuts surpassed the MTL. The highest concentration of AFB1 was detected in an unshelled pistachio sample, followed by an unshelled peanut sample, with concentrations of 25 µg/kg and 16 µg/kg, respectively. The mean ± SEM levels of AFB1 in Lebanese nut samples ranged from 1.08 ± 0.06 to 9.4 ± 1.04 µg/kg. In addition, *p*-values were calculated for each group of nuts using the Mann–Whitney Test.

Table 3. Contamination levels of AFB1 in shelled and unshelled Lebanese nuts.

Sample	N	Positive Samples	% Exceeding MTLs	Range (μg/kg)	Mean ± SEM (μg/kg)	*p*-Values
Peanuts (shelled)	27	9	0%	4.0–9.1	1.08 ± 0.06	<0.001
Peanuts (unshelled)	28	12	33.3%	3.8–25.0	4.70 ± 0.95	<0.001
Pistachios (shelled)	28	13	0%	4.6–10.0	5.24 ± 0.17	<0.001
Pistachios (unshelled)	26	14	38.5%	3.0–12.0	9.40 ± 1.04	<0.001
Walnuts (shelled)	26	8	0%	0.4–16.0	4.40 ± 0.16	<0.001
Walnuts (unshelled)	25	14	15.4%	0.9–9.0	7.25 ± 0.36	<0.001

3.6. Validation of HPLC

A calibration curve for AFB1 standards was constructed, and the results indicated that the AFB1 concentrations were proportional to the corresponding areas. The coefficient of determination was determined to be 0.820. Method performance parameters, such as the LOD and LOQ, were calculated from the calibration curve and found to be 0.245 μg/kg and 0.817 μg/kg, respectively. The precision of the HPLC was evaluated and it was within an acceptable range, with relative standard deviations (RSDs) ranging from 0.2 to 1.7%. In addition, the recovery rates obtained for the selected matrices ranged from 89 to 95%.

3.7. Relation between Moisture Content and AFB1 Levels in Nut Samples

According to the Pearson correlation (r = 1), the significance (Sig.) was less than 0.05, indicating a significant correlation between moisture content and AFB1 levels, as measured by HPLC. In other words, as the moisture content in the nut samples increases, the AFB1 level also increases. This pattern was observed with the highest AFB1 level found in a pistachio sample (19.1 μg/kg), where the moisture content was also the highest. A similar observation was made for the peanut sample.

3.8. Daily Intake of AFB1

The EDI of AFB1 in Lebanese nuts was calculated. Using an average body weight of 72.8 kg, the estimated daily intake of AFB1 in nuts was found to be equal to 0.43 μg/kg b.w./day.

4. Discussion

The moisture contents of pistachio samples ranged from 2.7 ± 0.11 to 9 ± 0.14%. These results exceeded the findings reported by Mohammadi-Moghaddam et al. [35], who found moisture levels ranging from 0.05 ± 0.02% to 2.19 ± 0.04%. In addition, the moisture content in the tested peanut and walnut samples fell within the ranges of 4.9 ± 0.08% to 9.5 ± 0.11% and 1.9 ± 0.08% to 7.7 ± 0.08%. These findings were found to be lower than the moisture content reported by Oyedele et al. for groundnut samples [36], which varied from 2.2 to 19.7%. It was observed that a significant portion of the unshelled nut samples exceeded the recommended moisture levels (6% for walnuts and pistachios, and 8% for peanuts). In total, 50% of unshelled pistachios, 44% of unshelled peanuts, and 36% of unshelled walnuts surpassed these levels. This indicates that the unshelled samples have a higher risk of mycoflora contamination and aflatoxin presence, as aflatoxin production is notably influenced by various factors such as moisture content, relative humidity, and ambient temperature [37]. Furthermore, the elevated moisture content observed in nut samples could result from inadequate packaging or the absence of quality packaging materials [37]. As nuts are hygroscopic colloidal materials, the nuts absorb moisture from their surroundings until equilibrium is achieved, as highlighted by Oladapo et al. [38]. Moreover, the moisture content of nuts could be affected by the harvesting methods employed by the farmers [37].

Various fungi were identified in the nut samples collected from three governorates in Lebanon. These results closely match the microbial metabolite profiles obtained from the samples, indicating a broad spectrum of fungal species that colonized the kernels at some point, from the field to storage or the market. The mycoflora were present in every sample of tree and ground nuts immediately after procurement from the market. The prevalence of

A. niger and *A. flavus* species in the nut samples corroborates the findings of previous studies that documented the dominance of these organisms in their nut samples [24,27,39,40]. Additionally, *R. arrhizus* was found in a significant number of the nuts (42.6%). This finding supports the observations of previous researchers who have identified various *Rhizopus* species contaminating nut samples [24,41,42]. However, unlike other researchers [27,40,43], no *Penicillium* species were isolated in the current study. This could be because the presence of *Penicillium* tends to inversely correlate with that of *Aspergillus* [25]. In terms of fungal contamination in nuts and their products, groundnuts and tree nuts such as almonds, pistachios, and walnuts are primarily contaminated by fungi, including *Aspergillus*, *Penicillium*, *Rhizopus*, *Alternaria*, and *Fusarium* [25]. Several factors contribute to the high incidence of fungi in Lebanese nuts. The continuous exposure of nuts to sunlight during the day followed by recondensation at night can lead to an increase in the microbial content in samples. Without the proper control of storage temperatures and humidity, molds capable of producing aflatoxins may rapidly multiply, presenting a significant food safety issue and heightening the risk of aflatoxin production [44]. *Aspergillus* species can flourish under optimal conditions, specifically at temperatures of 27–33 °C, within a pH range of 5–6, and when the water activity is between 0.82–0.99 [44]. The initial contamination of nuts by fungi can take place in the field, with the possibility of further deterioration and mycotoxin production during prolonged storage periods [45].

It is widely recognized that the production of aflatoxins (AFs) is specific to the species [46]. Therefore, studying and identifying the toxigenic species among the nuts sold in the Lebanese market was important. The toxigenic fungus *A. flavus*, known for its role in producing aflatoxins, was the predominant species found in Lebanese nuts, present in 38% of the samples. Notably, *A. parasiticus* was absent in all nut samples that were examined. Similar findings have been reported in previous research studies conducted in the USA, Iran, and Canada [47–49], reinforcing these observations. This pattern may be attributed to the fact that *A. parasiticus* is known for generating substantial quantities of aflatoxins, yet it is commonly found at a low frequency ($\leq$5%) in both food and soil samples [36].

ELISA analysis offers a practical approach for concurrently detecting contaminants in numerous samples efficiently and cost-effectively. However, it lacks the accurate quantification of contaminants due to potential sample matrix effects and the risk of overestimating contaminants at very low concentrations [31]. In addition, there is a risk of false-positive outcomes in ELISA tests because of the cross-reactivity of antibodies [7]. To mitigate this problem, confirming the results through a reliable chromatographic method, HPLC, was performed. In this study, 70 samples were tested using ELISA to find total aflatoxins and selected as potentially positive samples. These samples were further analyzed using HPLC for AFB1 quantification.

Pistachio samples were identified as the most contaminated, where 52% of the total pistachio samples were contaminated by AFB1. The contamination of AFB1 in Lebanese nut samples varied, with an average of 1 to 9.4 µg/kg. However, Raad et al. [33] previously documented AFB1 contamination in Lebanese nuts, where a mixed sample of nuts, seeds, olives, and dried dates exhibited an average AFB1 level of 0.1 µg/kg. Similarly, Soubra et al. [17] investigated aflatoxin contamination in nuts with levels of total aflatoxins varying between 0.5 to 8.0 µg/kg, which is lower than the average reported in the current study. The variations in findings between this investigation and others could result from variations in the timing of sample collection, the methodologies employed, the preparation processes, and the analytical techniques used.

On the other hand, our findings indicated that pistachios were more susceptible to AFB1 contamination compared to other types of nuts analyzed in the study, with 50% of pistachio samples affected. Moreover, pistachios had the highest percentage of samples exceeding the MTL of AFB1 in nuts, with 38.5% of positive samples. This result aligned well with reports of border rejections noted on the RASFF portal for Lebanese imports into the European Union, where pistachio samples were the most frequently rejected nut type due to their high AFB1 content [15]. The levels of AFB1 in pistachios rejected

for imports from Lebanon varied, with an average of 57 to 890 µg/kg [15]. Globally, research studies on nut contamination have also reported the frequent occurrence of AFB1, predominantly in pistachio samples across various regions. For instance, in Italy, pistachios were found to be the most contaminated among various nut samples, with 50% of samples contaminated between 8.2 and 354.5 µg/kg [50]. Similarly, in Morocco, the analysis of different nuts revealed that pistachio samples had the highest rates of contamination for both total aflatoxins and AFB1, with both contaminants found in 45% of the pistachio samples [51]. In Europe, the European Food Safety Authority (EFSA) in 2007 examined the presence of aflatoxins in 20,016 nut samples, including almonds, Brazil nuts, hazelnuts, cashews, pistachios, and peanuts. The findings indicated that Brazil nuts and pistachios had the highest rate of samples testing positive for aflatoxins [52]. The results were in agreement with the above results, underscoring that pistachios are considered a significant source of dietary aflatoxin exposure from tree nuts, contributing to 7–45% of the total AF exposure humans face from all sources [53]. Pistachios are regarded as having the greatest risk of aflatoxin contamination, primarily because their shells split towards the end of the maturation process. This shell serves to shield the kernel of the pistachio, and the splitting renders pistachios vulnerable to mold and insect infestations [53]. The walnuts exhibited the lowest contamination levels, with only 15.4% of samples surpassing the MTL for aflatoxins, a minimal occurrence attributed to the specific antioxidant hydrolysable tannin, present in walnuts, which can inhibit the formation of aflatoxins [30].

Upon comparing shelled and unshelled nuts, it was noted that all samples exhibiting AFB1 contamination levels exceeding the MTL were unshelled nuts. Specifically, 15.4% of unshelled walnuts, 38.5% of unshelled pistachios, and 35% of unshelled peanuts were contaminated with AFB1 levels surpassing the MTL. Notably, the highest AFB1 contamination level was found in an unshelled pistachio sample, reaching 25 µg/kg. These findings align closely with research conducted by Kabak et al. [54] who discovered that unshelled hazelnuts had higher levels of four types of aflatoxins, while no shelled hazelnuts were contaminated by any aflatoxin. This suggests that the shell of various nuts serves as a natural protective barrier, helping to prevent mold growth, fungal infestation, and the subsequent aflatoxin contamination of the kernel [54,55].

The results showed a high EDI for AFB1 (0.43 µg/kg b.w./day), which was higher than the dietary intakes of AFB1 from peanuts in Korea (0.025 ng/kg b.w./day) [56] and the EDI from nuts in China, which varied between 0.0179 and 0.0183 ng/kg b.w./day [57]. This exposes the Lebanese population to significant risk, especially since this EDI pertains only to nuts and not to the total food consumed by the Lebanese population. This raises concerns for the adult Lebanese population. The Lebanese diet, characterized by a rich variety of plant-based foods such as fruits, vegetables, legumes, nuts, and seeds [58], indicates that these foods require risk management measures at the level of aflatoxins. The reduction in risk necessitates a comprehensive system strategy that incorporates specific agricultural practices, the use of biological control techniques, and the improvement of the host plant's resistance. This should be combined with post-harvest technologies, such as proper drying, storage, and the sorting of nut products to remove contamination [59].

5. Conclusions

The analysis of 160 nut samples collected from the Lebanese market revealed significant insights into fungal and aflatoxin contamination. The findings highlighted the predominance of *Aspergillus* species, especially *A. flavus*, as a main contributor to aflatoxin contamination in nuts. Notably, it was found that the unshelled nuts had a greater tendency to exceed the maximum moisture content limits, making them more vulnerable to AFB1 contamination, with pistachio samples being the most affected. A significant portion of tested samples (43.8%) had detectable aflatoxin levels, underscoring the critical need for strict quality control within the nut distribution chain. The contamination of nuts with AFB1 levels ranging from 0.4 to 25 µg/kg underscores the urgent need for continued surveillance regarding aflatoxin content in nuts. Notably, the maximum allowable AFB1

levels in Lebanon were set at 2 µg/kg for walnuts and peanuts, and 8 µg/kg for pistachios. This study provides insights into the potential health risks associated with nut consumption in Lebanon, particularly the concern for an increased risk of liver cancer among consumers. Additional studies and surveillance efforts are essential to assess the prevalence of various mycotoxins in nuts sold in the Lebanese market. Moreover, future studies should explore the local practices of nut storage and distribution, which could significantly impact food safety.

Author Contributions: This work was a collaborative effort among all authors. Conceptualization and design: M.I.K., J.C.A. and H.H.; methodology: H.H. and J.M.; resources: H.H. and J.M.; writing—original draft preparation: H.H.; review and editing: M.I.K. and J.C.A.; main supervision and manuscript finalization: M.I.K.; co-supervision: J.C.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: All data in this review are included within the edited manuscript.

Acknowledgments: We would like to express our gratitude to Dalia El-Badan (Department of Biological Sciences, Faculty of Science, Beirut Arab University, Lebanon) for providing Ferric Ammonium Citrate used in preparing the AFPA media for the detection of toxigenic fungi in our study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Assaf, J.C.; Nahle, S.; Chokr, A.; Louka, N.; Atoui, A.; El Khoury, A. Assorted Methods for Decontamination of Aflatoxin M1 in Milk Using Microbial Adsorbents. *Toxins* **2019**, *11*, 304. [CrossRef]
2. Marchese, S.; Polo, A.; Ariano, A.; Velotto, S.; Costantini, S.; Severino, L. Aflatoxin B1 and M1: Biological Properties and Their Involvement in Cancer Development. *Toxins* **2018**, *10*, 214. [CrossRef] [PubMed]
3. Amir, M.; Shahzad, A.; Faraz, A.; Sajid, M.; Afzal, K.; Naeem, I.; Ismail, A.; Mumtaz, Z. Health effects of aflatoxins in fetus, infants, and children. In *Aflatoxins in Food: A Recent Perspective*; Springer: New York, NY, USA, 2022; pp. 99–122.
4. Deabes, M.M.; Darwish, H.R.; Abdel-Aziz, K.B.; Farag, I.M.; Nada, S.A.; Tawfek, N.S. Protective Effects of *Lactobacillus rhamnosus* GG on Aflatoxins-Induced Toxicities in Male Albino Mice. *J. Environ. Anal. Toxicol.* **2012**, *2*, 2–9. [CrossRef]
5. Wild, C.P.; Gong, Y.Y. Mycotoxins and human disease: A largely ignored global health issue. *Carcinogenesis* **2010**, *31*, 71–82. [CrossRef]
6. Benkerroum, N. Chronic and Acute Toxicities of Aflatoxins: Mechanisms of Action. *Int. J. Environ. Res. Public Health* **2020**, *17*, 423. [CrossRef] [PubMed]
7. Hellany, H.; Assaf, J.C.; El-Badan, D.; Khalil, M. Quantification, Prevalence, and Pretreatment Methods of Mycotoxins in Groundnuts and Tree Nuts: An Update. *Processes* **2023**, *11*, 3428. [CrossRef]
8. Tahavori, A.; Ghaderi, F.; Nemati, M. Determination of aflatoxins in nuts of Tabriz confectionaries by ELISA and HPLC methods. *Adv. Pharm. Bull.* **2012**, *2*, 123.
9. Alshannaq, A.; Yu, J.-H. Analysis of E.U. Rapid Alert System (RASFF) Notifications for Aflatoxins in Exported U.S. Food and Feed Products for 2010–2019. *Toxins* **2021**, *13*, 90. [CrossRef]
10. Ros, E. Contribution of Nuts to the Mediterranean Diet. In *The Mediterranean Diet*; Elsevier: Amsterdam, The Netherlands, 2015; pp. 175–184.
11. De Souza, R.; Schincaglia, R.; Pimentel, G.; Mota, J. Nuts and Human Health Outcomes: A Systematic Review. *Nutrients* **2017**, *9*, 1311. [CrossRef]
12. Daou, R.; Joubrane, K.; Khabbaz, L.R.; Maroun, R.G.; Ismail, A.; El Khoury, A. Aflatoxin B_1 and ochratoxin A in imported and Lebanese wheat and -products. *Food Addit. Contam. Part B* **2021**, *14*, 227–235. [CrossRef]
13. European Commission. Aflatoxins, Guidance Document for Competent Authorities for the Control of Compliance with EU Legislation on Aflatoxins. Available online: https://food.ec.europa.eu/document/download/17a22129-d928-4a82-874a-1b41bc09a422_en?filename=cs_contaminants_catalogue_alfatoxins_guidance-2010_en.pdf (accessed on 1 January 2024).
14. Ferlay, J.; Ervik, M.; Lam, F.; Laversanne, M.; Colombet, M.; Mery, L.; Piñeros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. *Cancer Today—Global Cancer Observatory*; International Agency for Research on Cancer (IARC): Lyon, France, 2018.
15. Daou, R.; Hoteit, M.; Bookari, K.; Joubrane, K.; Khabbaz, L.R.; Ismail, A.; Maroun, R.G.; Khoury, A. el Public health risk associated with the co-occurrence of aflatoxin B1 and ochratoxin A in spices, herbs, and nuts in Lebanon. *Front. Public Health* **2023**, *10*, 1072727. [CrossRef] [PubMed]
16. Leeters, B. *Value Chain Analysis Lebanon Fresh Fruit and Vegetables*; Commissioned by The Centre for the Promotion of Imports from Developing Countries (CBI); The Netherlands Ministry of Foreign Affairs: Hague, The Netherlands, 2018.

17. Soubra, L.; Sarkis, D.; Hilan, C.; Verger, P. Occurrence of total aflatoxins, ochratoxin A and deoxynivalenol in foodstuffs available on the Lebanese market and their impact on dietary exposure of children and teenagers in Beirut. *Food Addit. Contam. Part A* **2009**, *26*, 189–200. [CrossRef] [PubMed]
18. Elaridi, J.; Yamani, O.; Al Matari, A.; Dakroub, S.; Attieh, Z. Determination of Ochratoxin A (OTA), Ochratoxin B (OTB), T-2, and HT-2 Toxins in Wheat Grains, Wheat Flour, and Bread in Lebanon by LC-MS/MS. *Toxins* **2019**, *11*, 471. [CrossRef] [PubMed]
19. Hassan, H.F.; Tashani, H.; Ballouk, F.; Daou, R.; El Khoury, A.; Abiad, M.G.; AlKhatib, A.; Hassan, M.; El Khatib, S.; Dimassi, H. Aflatoxins and Ochratoxin A in Tea Sold in Lebanon: Effects of Type, Packaging, and Origin. *Int. J. Environ. Res. Public Health* **2023**, *20*, 6556. [CrossRef] [PubMed]
20. Hassan, H.F.; Kordahi, R.; Dimassi, H.; El Khoury, A.; Daou, R.; Alwan, N.; Merhi, S.; Haddad, J.; Karam, L. Aflatoxin B1 in Rice: Effects of Storage Duration, Grain Type and Size, Production Site, and Season. *J. Food Prot.* **2022**, *85*, 938–944. [CrossRef] [PubMed]
21. Elaridi, J.; Dimassi, H.; Hassan, H. Aflatoxin M1 and ochratoxin A in baby formulae marketed in Lebanon: Occurrence and safety evaluation. *Food Control* **2019**, *106*, 106680. [CrossRef]
22. Gambacorta, L.; El Darra, N.; Fakhoury, R.; Logrieco, A.F.; Solfrizzo, M. Incidence and levels of Alternaria mycotoxins in spices and herbs produced worldwide and commercialized in Lebanon. *Food Control* **2019**, *106*, 106724. [CrossRef]
23. Latimer, G.W. (Ed.) Aflatoxins. In *Official Methods of Analysis of AOAC International*; Oxford University Press: New York, NY, USA, 2023.
24. Adetunji, M.C.; Alika, O.P.; Awa, N.P.; Atanda, O.O.; Mwanza, M. Microbiological Quality and Risk Assessment for Aflatoxins in Groundnuts and Roasted Cashew Nuts Meant for Human Consumption. *J. Toxicol.* **2018**, *2018*, 1308748. [CrossRef] [PubMed]
25. Gelderblom, W.C.A.; Shephard, G.S.; Rheeder, J.P.; Sathe, S.K.; Ghiasi, A.; Motarjemi, Y.; Rheeder, J.P. Edible Nuts, Oilseeds and Legumes. In *Food Safety Management*; Elsevier: Amsterdam, The Netherlands, 2014; pp. 301–324.
26. Swathi, Y.; Rajanikanth, P.; Jella, S.N.; Mangala, U.N.; Adithya, G.; Anilkumar, V.; Sudini, H.K. Effect of sub-optimal moisture levels on the quality of groundnut (*Arachis hypogaea* L.) during storage in triple-layer hermetic storage bags. *Front. Sustain. Food Syst.* **2024**, *7*, 1275133. [CrossRef]
27. Adetunji, M.C.; Ngoma, L.; Atanda, O.O.; Mwanza, M. A polyphasic method for the identification of aflatoxigenic *Aspergilla* from cashew nuts. *World J. Microbiol. Biotechnol.* **2019**, *35*, 15. [CrossRef]
28. Ozay, G.; Seyhan, F.; Pembeci, C.; Saklar, S.; Yilmaz, A. Factors influencing fungal and aflatoxin levels in Turkish hazelnuts (*Corylus avellana* L.) during growth, harvest, drying and storage: A 3-year study. *Food Addit. Contam. Part A* **2008**, *25*, 209–218. [CrossRef]
29. Pitt, J.I.; Hocking, A.D.; Glenn, D.R. An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. *J. Appl. Bacteriol.* **1983**, *54*, 109–114. [CrossRef]
30. Leong, Y.-H.; Ismail, N.; Latif, A.A.; Ahmad, R. Aflatoxin occurrence in nuts and commercial nutty products in Malaysia. *Food Control* **2010**, *21*, 334–338. [CrossRef]
31. Chun, H.S.; Kim, H.J.; Ok, H.E.; Hwang, J.-B.; Chung, D.-H. Determination of aflatoxin levels in nuts and their products consumed in South Korea. *Food Chem.* **2007**, *102*, 385–391. [CrossRef]
32. Asghar, M.A.; Iqbal, J.; Ahmed, A.; Khan, M.A.; Shamsuddin, Z.A.; Jamil, K. Development and validation of a high-performance liquid chromatography method with post-column derivatization for the detection of aflatoxins in cereals and grains. *Toxicol. Ind. Health* **2016**, *32*, 1122–1134. [CrossRef]
33. Raad, F.; Nasreddine, L.; Hilan, C.; Bartosik, M.; Parent-Massin, D. Dietary exposure to aflatoxins, ochratoxin A and deoxynivalenol from a total diet study in an adult urban Lebanese population. *Food Chem. Toxicol.* **2014**, *73*, 35–43. [CrossRef]
34. Zhang, W.; Liu, Y.; Liang, B.; Zhang, Y.; Zhong, X.; Luo, X.; Huang, J.; Wang, Y.; Cheng, W.; Chen, K. Probabilistic risk assessment of dietary exposure to aflatoxin B1 in Guangzhou, China. *Sci. Rep.* **2020**, *10*, 7973. [CrossRef]
35. Mohammadi-Moghaddam, T.; Razavi, S.M.A.; Taghizadeh, M.; Pradhan, B.; Sazgarnia, A.; Shaker-Ardekani, A. Hyperspectral imaging as an effective tool for prediction the moisture content and textural characteristics of roasted pistachio kernels. *Food Meas.* **2018**, *12*, 1493–1502. [CrossRef]
36. Oyedele, O.A.; Ezekiel, C.N.; Sulyok, M.; Adetunji, M.C.; Warth, B.; Atanda, O.O.; Krska, R. Mycotoxin risk assessment for consumers of groundnut in domestic markets in Nigeria. *Int. J. Food Microbiol.* **2017**, *251*, 24–32. [CrossRef]
37. Macri, A.M.; Pop, I.; Simeanu, D.; Toma, D.; Sandu, I.; Pavel, L.L.; Mintas, O.S. The Occurrence of Aflatoxins in Nuts and Dry Nuts Packed in Four Different Plastic Packaging from the Romanian Market. *Microorganisms* **2020**, *9*, 61. [CrossRef]
38. Oladapo, A.S.; Abiodun, O.A. Effect of packaging materials on moisture and microbiological quality of roasted cashew nut (*Anarcardium occidentale* L.). *Res. J. Eng. Appl. Sci.* **2014**, *3*, 98–103.
39. Jubeen, F.; Bhatti, I.A.; Maqbool, U.; Mehboob, S. Fungal Incidence, Aflatoxin B_1, Tocopherols and Fatty Acids Dynamics in Ground and Tree Nuts during Storage at Two Moisture Levels. *Int. J. Agric. Biol.* **2012**, *14*, 521–527.
40. Keskin, Z.S.; Gürsoy, N. Investıgatıon of natural mycoflora and aflatoxin formation in hazelnuts and products. *Cumhur. Sci. J.* **2019**, *40*, 967–977. [CrossRef]
41. Tobin-West, M.; Dimkpa, S.; Osakwe, J. Isolation and identification of fungi associated with raw groundnut seeds sold at four major markets in Port Harcourt Metropolis, Rivers State. Biology. *Agric. Healthc.* **2018**, *8*, 29–34.

42. Nkwonta, C.G.; Medina, A.; del Carmen Alamar, M.; Terry, L.A. Impact of postharvest processing on the fungal population contaminating African walnut shells (*Tetracarpidium conophorum* Mull. Arg) at different maturity stages and potential mycotoxigenic implications. *Int. J. Food Microbiol.* **2015**, *194*, 15–20. [CrossRef]
43. Chein, S.H.; Sadiq, M.B.; Datta, A.; Anal, A.K. Prevalence and identification of *Aspergillus* and *Penicillium* species isolated from peanut kernels in central Myanmar. *J. Food Saf.* **2019**, *39*, e12686. [CrossRef]
44. Richard, J.L. Some major mycotoxins and their mycotoxicoses—An overview. *Int. J. Food Microbiol.* **2007**, *119*, 3–10. [CrossRef]
45. das Chagas Oliveira, F.F.; Offord, L. Bacterial and yeast counts in Brazilian commodities and spices. *Braz. J. Microbiol.* **2002**, *33*, 145–148.
46. Rodrigues, P.; Venâncio, A.; Lima, N. Mycobiota and mycotoxins of almonds and chestnuts with special reference to aflatoxins. *Food Res. Int.* **2012**, *48*, 76–90. [CrossRef]
47. Bayman, P.; Baker, J.L.; Mahoney, N.E. Aspergillus on tree nuts: Incidence and associations. *Mycopathologia* **2002**, *155*, 161–169. [CrossRef]
48. Fani, S.R.; Moradi, M.; Probst, C.; Zamanizadeh, H.R.; Mirabolfathy, M.; Haidukowski, M.; Logrieco, A.F. A critical evaluation of cultural methods for the identification of atoxigenic Aspergillus flavus isolates for aflatoxin mitigation in pistachio orchards of Iran. *Eur. J. Plant Pathol.* **2014**, *140*, 631–642. [CrossRef]
49. Arrus, K.; Blank, G.; Abramson, D.; Clear, R.; Holley, R.A. Aflatoxin production by Aspergillus flavus in Brazil nuts. *J. Stored Prod. Res.* **2005**, *41*, 513–527. [CrossRef]
50. Diella, G.; Caggiano, G.; Ferrieri, F.; Ventrella, A.; Palma, M.; Napoli, C.; Rutigliano, S.; Lopuzzo, M.; Lovero, G.; Montagna, M. Aflatoxin contamination in nuts marketed in Italy. Preliminary results. *Ann. Ig. Med. Prev. Comunit Comunità* **2018**, *30*, 401–409.
51. Juan, C.; Zinedine, A.; Moltó, J.C.; Idrissi, L.; Mañes, J. Aflatoxins levels in dried fruits and nuts from Rabat-Salé area, Morocco. *Food Control* **2008**, *19*, 849–853. [CrossRef]
52. Cunha, S.C.; Sá, S.V.M.; Fernandes, J.O. Multiple mycotoxin analysis in nut products: Occurrence and risk characterization. *Food Chem. Toxicol.* **2018**, *114*, 260–269. [CrossRef]
53. Soares Mateus, A.R.; Barros, S.; Pena, A.; Sanches Silva, A. Mycotoxins in pistachios (*Pistacia vera* L.): Methods for determination, occurrence, decontamination. *Toxins* **2021**, *13*, 682. [CrossRef]
54. Kabak, B. Aflatoxins in hazelnuts and dried figs: Occurrence and exposure assessment. *Food Chem.* **2016**, *211*, 8–16. [CrossRef]
55. Habibipour, R.; Tamandegani, P.R.; Farmany, A. Monitoring of aflatoxin G1, B1, G2, and B2 occurrence in some samples of walnut. *Environ. Monit. Assess.* **2016**, *188*, 669. [CrossRef]
56. Ok, H.E.; Kim, H.J.; Shim, W.B.; Lee, H.; Bae, D.-H.; Chung, D.-H.; Chun, H.S. Natural occurrence of aflatoxin B1 in marketed foods and risk estimates of dietary exposure in Koreans. *J. Food Prot.* **2007**, *70*, 2824–2828. [CrossRef]
57. Wang, Y.; Nie, J.; Yan, Z.; Li, Z.; Cheng, Y.; Farooq, S. Multi-mycotoxin exposure and risk assessments for Chinese consumption of nuts and dried fruits. *J. Integr. Agric.* **2018**, *17*, 1676–1690. [CrossRef]
58. Hwalla, N.; El Khoury, D.T.D. Lebanese traditional diets and health effects. In *Wild-Type Food in Health Promotion and Disease Prevention: The Columbus Concept*; Humana Press: Totowa, NJ, USA, 2008; pp. 493–498.
59. World Health Organization. *Safety Evaluation of Certain Contaminants in Food: Prepared by the Eighty-Third Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*; World Health Organization: Geneva, Switzerland, 2018.