Risk of Exposure to aflatoxin B_1, ochratoxin A, and fumonisin B_1 from spices used routinely in Lebanese cooking

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Short version of title: spices in Lebanese dishes

Abstract

Spices are susceptible to mycotoxin contamination which can cause gastrointestinal and adverse central nervous symptoms in humans, which highlights the importance of assessing the risk of their consumption on a daily basis. The aim of this study was to assess the risk of mycotoxin intake from spices in routinely prepared Lebanese dishes.

150 households were interviewed about their usage of 27 type of spices and 6 routinely prepared Lebanese dishes. Results showed a high variability in consumption levels. Among the investigated dishes, the minimum number of spices that were consumed in a dish was 13 while the maximum was 18. The mean intake of one spice ranged from 0.26 g/portion observed for cloves to 5.37 g/portion for cinnamon, with its intake per portion more than 1 g in 2/3 of dishes. 20% of portion sizes of coriander, cinnamon and fennel, had an intake exceeding 5 g/portion. Ochratoxin A (OTA) Probable Daily Intake (PDI) had a mean of 0.11 ng/kg-bw/day. Mean PDI of fumonisin B_1 (FB_1) was 79.3 ng/kg-bw/day. Aflatoxin B_1 (AFB_1) PDI had a mean of 1.55 ng/kg-bw/day. The Margin of Exposure (MOE) of AFB_1 ranged from 108.1 to 4444.4. The present study showed that the risk of AFB_1 from spices is a matter of concern while the risk of OTA and FB_1 is limited with the exception of FB_1 from garlic and onion.

Keywords: aflatoxin B_1, ochratoxin A, fumonisin B_1, mycotoxins, spices, dishes, Lebanon, risk assessment

1. Introduction

Spices are used throughout the world as flavoring agents for food and beverages and medicinal purposes (Schweiggert et al., 2007). The global market for seasoning, spices and herbs is likely to exceed US$ 6.5 billion in the near future, according to the International Trade Centre. China is the main world-wide spices supplier (Intelligence CBI, 2015).

Recently, the assessment dietary intake of spices is gaining interest because of their high phytochemicals content, these chemicals are recognized to promote health benefits, prevent chronic diseases and demonstrate antibacterial activities (Kaefer and Milner, 2011; Saleh et al., 2018). Alongside these advantages, spices may be contaminated which represents a health hazard for the consumers; therefore, assessing the intake of spices and potential contaminants is crucial to inform risk assessments for estimating contaminant consumption (Siruguri and Bhat, 2015).

Contaminants on spices are varied and include pesticide residues, heavy metals, food allergens, food additives, and mycotoxins that are produced by mycotoxigenic fungi (EFSA, 2006; Assunção, 2017;; El Darra et al., 2019; EFSA, 2020).

Climate conditions have strong effect on the growth of mycotoxigenic fungi as well as mycotoxin production and accumulation (Schweiggert et al., 2007). A water activity $\geq$ 0.8, a moisture content $\geq$ 16% and a temperature ranging between 25 and 30°C are the optimal conditions for mycotoxin production (Thanushree et al., 2019).

Humans and animals are mainly exposed to mycotoxins by consumption of contaminated food and feed, respectively. The occurrence and dietary exposure to mycotoxins worldwide was evaluated in the past (FAO/WHOA, 2001; FAO/WHO, 2018).

Within the 13 mycotoxins regulated in foodstuffs and beverages in Europe, only aflatoxins (AFs) and OTA are regulated in some spices (EC, 165/2010; EC, 1137/2015). Several studies have assessed the occurrence of AFs and/or OTA in spices and herbs (Martins et al., 2001; Fazekas et al., 2005; Kabak and Dobson 2017; Ali and Watt, 2019), whereas recent studies investigated multiple mycotoxins thaks to the availability of LC-MS/MS instrumentations capable to detect and measure several mycotoxins in one run (Reinholds et al., 2016, 2017; Gambacorta et al., 2018; El Darra et al., 2019; Iha and Trucksess, 2019). Gambacorta *et al*., (2018) assessed the occurrence of 17 mycotoxins in sweet pepper (*Capsicum annuum*) produced in Italy. Among 45 samples of fresh and processed forms of sweet pepper, they found 51% of samples were contaminated by OTA with a mean of 29.5 µg/kg and 31% by AFs with a mean of 12.8 µg/kg; most of samples were contaminated by fumonisin although at low levels (9-140 µg/kg). This study confirmed the susceptibility of peppers to mycotoxin contamination (Gambacorta et al., 2018). The study of Wang et al., (2019) confirmed the susceptibility of peppers to mycotoxin contamination. Furthermore, diffusions of some mycotoxins from lesions into the surrounding sound tissues were observed in sweet peppers, implying migration from colonized to non-colonized parts of pepper tissues, which implied the risk of mycotoxin contamination in non-infected parts of food product. In a recent study conducted in Lebanon on 132 samples of marketed spices (94) and herbs (38), spices had greater contamination. In particular, aflatoxins were detected in 19% of spices and 8% of herbs, while OTA was detected in 30% of spices and 11% of herbs, while FB_1 was detected in 60% of spices and 55% of herbs. Moreover, 78 and 10% of positive samples were contaminated by AFs and OTA, respectively, at levels exceeding the European limits (El Darra et al., 2019).

The maximum limits of aflatoxin B_1 (AFB_1) and total aflatoxins have been set in Europe for some spices (*Capsicum spp., Piper spp.*, *Myristica fragrans, Zingiber officinale*, *Curcuma longa* and mixtures of spices containing one or more of these spices) at 5 µg/kg and 10 µg/kg, respectively (European Commission, 2010). The limits of OTA in these spices ranged between 15 and 20 µg/kg (European Commission, 2015). A Tolerable Daily Intake (TDI) of 17 ng/kg-b.w/d for OTA and 1000 ng/kg-bw/d for FB_1 were established by EFSA (EFSA, 2006, 2018). The Margin of Exposure (MOE) approach is currently used for the safety assessment of aflatoxins exposure (EFSA, 2020). Measuring the spices intake and an individual's body weight to assess the contaminant's risk is considered crucial for risk assessment purpose (Siruguri and Bhat, 2015).

To our knowledge, no study has assessed consumption of spices from Lebanese dishes and the subsequent risk of mycotoxin contamination to the community. The purpose of this study is 1) to assess the intake of spices from routinely prepared Lebanese dishes and 2) to measure the Probable Daily Intake (PDI) of AFB_1, OTA and FB_1 from contaminated spices used in the Lebanese population and to assess the relevant dietary risk. For OTA and FB_1 their TDI values were considered whereas for AFB_1 the MOE approach was considered by using the Bench Mark Dose Lower Confidence level (BMDL).

2. Materials and methods

The methodology used in this paper to assess the intake of spices from routinely prepared dishes in Lebanon is developed from the work of Siruguri and Bhat (2015).

2.1. Selection of spices and standardization of household (HH) measures.

A total of 27 common types of spices, including five mixtures of different spices, commonly used in Lebanese cuisine were selected to assess total dietary intake. Table 1 presents the scientific and local names of selected spices evaluated in this study, which comprises 5 spices mixtures. Calculating the exact spices intake required to divide mixtures weights based on the percentages of their ingredients depending on standardised recipes. To measure the weight of the spices, a standard digital measuring spoon (Numed digispoon) was used by the interviewer to calibrate and quantify the spices used by the participating households; this was simple and accurate to use (300 ± 0.1 g). The weights obtained by the weighing spoon, were verified with a professional sensitive electrical balance (Numed Professional extremely high precision balance, 300± 0.001g).

2.2. Spice intake Questionnaire

A detailed questionnaire was prepared to assess the consumption of spices at both household and individual levels for healthy adults (Appendix 1). Dietary life style, socioeconomic characteristics and eligibility criteria were collected among the adult participants selected in this study of 150 families. Questionnaires were completed via face to face interviews with the person responsible for cooking in the family. The usage of 27 common types of spices and spices mixtures (Table 1) was investigated in 6 kinds of commonly prepared dishes: Chicken rice, Kebbeh, Moghrabieh, Mloukhieh, Siyadieh, and Dawood basha. Detailed information were collected including the type of spices used, the frequency of usage and usage amount, the quantity of each spice used in all of these dishes, and the food portion sizes of each eligible family member for each dish. To measure the portion sizes, standardized portioning cups were used (Numed).

2.3. Type of dishes routinely prepared in the HHs

The six dishes chosen in this study have different methods of preparation by each HH, each with their own preference for the type and amount of spices used in the food's preparation. Chicken rice and Mloukhieh are dishes requiring boiled chicken, and mainly includes cinnamon, cardamom seeds and cloves. The rice is cooked with the boiled water from cooking the chicken. The Mloukhieh is the Arabic name of Corchorus olitorius leaves. Siyadieh, a dish based on rice is cooked with burned onions to give its special taste and topped with broiled fishes. Moghrabieh is a Lebanese couscous that is made with wheat semolina dough pearls, chick peas and onions. Dawood basha is a meatball stew that can include several types of vegetable in a spicy tomato sauce, the meatballs are called kafta that are made using beef, onions, herbs and middle eastern spices. Kebbeh is composed of bulgur, minced beef meat, onions, basil and spices in a form of dough. It can be stuffed with a mix of spices and cooked beef and pine seeds and is either grilled or fried.

2.4. Assessment of intake of spices

Using SPSS package (IBM®, version 24), the quantities of spices consumed were calculated and expressed as Mean ± Standard Deviation (SD), Median, 90th and 97.5th percentile values, and ranges (minimum and maximum).

To number of portion of spice in a dish and the mean g intake was calculated based on the total quantity of spices added to the entire quantity of each prepared dish (measured in grams), and based on eligible members' portion sizes from each dish; the total quantity of spices was divided and spices' portion sizes were calculated.

2.5. Dietary Exposure to Aflatoxin B_1 and Ochratoxin A contamination in spices

The dietary exposure to AFB_1, OTA and FB_1 through contaminated spices was calculated by multiplying the mean AFB_1, OTA and FB_1 contamination levels in spices commercialized in Lebanon, previously reported by (El Darra et al., 2019), with the intake of spices measured in the present study, then dividing by the average body weight (72.54 kg for the Lebanese population). The equation is as follows (Ali and Watt, 2019):

ng AFB_1 or OTA or FB_1 /kg-bw/day = mean AFB_1 or OTA or FB_1 contamination level in positive spices (ng/g) x daily amount consumed (g/day)/body weight (kg-bw).

2.6. Margin of exposure (MOE) calculation for AFB_1 in spices consumed in Lebanon

Based on studies in animals (Wogan et al., 1974), the EFSA Panel on Contaminants in the Food Chain (CONTAM) selected a benchmark dose lower confidence limit ($BMDL_{10}$) of 0.4 µg/kg-bw/day for the incidence of hepatocellular carcinoma in male rats following AFB_1 exposure to be used in a MOE approach (EFSA, 2020). MOE represents the margin between a dose and an exposure causing cancer in animals or humans with an estimated human exposure to the substance and was calculated as follows:

$$MOE = BMDL_{10}\ (\mu g/kg\text{-}bw/day) /\ \text{Exposure data}\ (\mu g/kg\text{-}bw/day).$$

The $BMDL_{10}$ of 0.4 μg/kg-bw/day was considered to be the most suitable for providing a point of departure for calculating the MOE (EFSA, 2020).

3. Results and discussion

3.1. Type and frequency of spices used and consumed

The distribution of house hold members is shown in Figure 1, which gives the number of adults and individuals living at home.

From 150 HHs in total, over 50% of them used the 20 spices of those listed in Table 1: cumin, black pepper, coriander seeds, turmeric, cardamom seeds, allspice, white pepper, paprika, red chili, caraway seeds, cinnamon, sumac, ginger, cloves, nutmeg seeds, seven spices, anise, chicken spices, and rice spices. Table 2 presents the frequency of spice consumption by the households. While assessing the most used spices, over 75% of the total HHs used 9 spices: cardamom (76%), turmeric (78%), coriander (80%), black pepper (80%), sumac (90%), seven spices (90%), cumin (96%), and cinnamon (98%) when the two latter were recorded the most used and consumed spices . The consumed spices include those present in food dishes and drinks such as anise and ginger. As regards usage frequencies, the 2 most consumed spices on a daily basis are 7 spices mixture (38% of HHs) containing: cinnamon, cloves, nutmeg, black pepper, allspice, ginger and mahlab (mahlab was not assessed in this study) and cinnamon (30% of HHs). The most consumed spices on a weekly basis are cinnamon (28.6%), cumin (20.6%) and sumac (18%). While the most consumed spices on a monthly basis are turmeric (30.6%), kebbeh spices (15.3%), then both cumin and coriander (=9.3%). For occasional usage (e.g. 3 times per year or for specific events), the most consumed spice is caraway (20%).

Measuring the intake of spices based on individual levels could be more difficult than that of other food items because the quantities are small and are found in every meal and snack. Consequently, their consumption needs to be studied in general, in particular dishes, and per portion size to measure the whole quantity consumed. We believe that this is a suitable method that permits the amount of mycotoxins consumed to be calculated and allows spices to be classified appropriately in terms of danger to human health.

After interviewing 150 HHs many declared that they did not use a certain type of spice but it was found that this spice was contained in the spice mixture which was consumed. For example, only 86 HHs declared using allspice whilst 136 HHs declared using a seven spices mixture in which 50% of the content was allspice; 57 of these HHs used it on a daily basis. Regardless of this misconception and as we have observed, the majority of households used coriander, cardamom seeds, turmeric, black pepper, cumin, cinnamon, sumac and seven spices, in which the last 6 spices were used more frequently than the other spices either on a daily or weekly basis.

The usage of some spices referred to certain dishes, and thus their usage frequency depends on the frequency of preparing and consuming these dishes. For example, moghrabieh spices is used only in Moghrabieh dish and kebbeh spices for Kebbeh dish (Table 2). In total, as single spices, whole or ground, the usage of 13 type of spices was the minimum number of spices in a single dish among the frequently prepared dishes investigated, while 18 spices was the maximum number of spices used. The Lebanese population, whether in urban or rural areas, consume a lot of spices in their routinely prepared dishes potentially exposing them to probably higher than safe levels of mycotoxins. A previous study published in India showed that the minimum number of spices used in one dish is 5 while the maximum was 11 types (Siruguri and Bhat, 2015). Although India is considered the highest spice consuming country in the world (Ferrucci et al., 2011), our data demonstrate that spice consumption in Lebanon is much higher. We can explain the high spice usage, because each investigated mixture contains at least 6 types of spices such as seven spices and can reach 17 spices per mixture. For example, rice spices contains 11 studied spices plus other ingredients and some other mixtures can be a mixture of more than one spice mixture.

From the HH survey, 69% of the main cooks were employed; this limits their time for food preparation and cooking and leads to them using time saving methods such as using spice mixes (seven spices mixture mainly), which leads to the high consumption of allspice, black pepper, ginger, cloves, nutmeg and cinnamon as one mixture (at least 90.6% of the population). It was observed that cumin not only was highly used but also has been recorded as one of the highest used spices in both Lebanon (145/150) and India (99/100) (Siruguri and Bhat, 2015), see Table 2. Cumin has been reported to benefit gastrointestinal disorders (Mnif and Aifa, 2015) and may be a reason that can explain its high consumption by the Lebanese population as a hot drink or included in food preparation. 98 HHs said they use cumin in Kebbeh which is the highest from our list of dishes. HHs use it separately rather than as an ingredient for chicken spices (4%) or rice spices (6%) in the Chicken rice dish.

3.2. Type of spices used by the HHs in routinely prepared dishes.

Table 3 presents the type of spices used by the HHs in routinely prepared dishes. When we observed the types of spices added to chosen dishes, Chicken rice was the dish that contained the highest number of spices (18 studied spices). Depending on the type of dish, the types of spices vary, some families also use numerous mixtures in one dish. For example with the Chicken rice dish, the boiled water contains specific spices used in the rice preparation. Other types of spices added to the rice could be commercially blended or used separately by the HH. Even with these variations, cinnamon is used by the HHs in most of the dishes, and it is most common among all dishes. Black pepper, cloves, ginger, allspice and nutmeg are commonly used in all of the dishes. The results of a similar study conducted in India (Siruguri and Bhat,

2015) do not totally agree with our findings for cinnamon, black pepper and cloves since there they were used mostly in rice dishes. Furthermore, due to cultural factors and eating habits, the most commonly used spice in India was chillies. White pepper is common also in Lebanon but the lower number of HHs who use it, indicate that it is less significant, plus chicken spice mixtures are a source of white pepper and it is not added intentionally for many HHs (Table 3).

3.3. Spice intake based on portion size of spices consumed

3.3.1. Spice intake based on portion size of spices consumed from all dishes

The total number of portion sizes calculated for all the spices from the 6 dishes consumed by the 150 HHs was 14725. Based on adult member's portion sizes, the most consumed spices (>10%) were cinnamon (14.2%), black pepper (12.4%), allspice (11.6%), cloves (11.5%), nutmeg (11.1%), and ginger (10.1%). The quantity consumed was evaluated by calculating the mean intake (g), the median, 90th and 97.5th percentiles, as shown in Table 4. The mean intake of allspice, black pepper, turmeric, cumin, coriander, cardamom, paprika, red chili, caraway, fennel, anise, and cinnamon was >1 g/portion size and had the highest mean intake (5.37 g/portion) and maximum consumption of 42.7 g/portion. A mean intake <1 g/portion size was observed in the remaining spices including cloves which recorded the lowest mean intake of 0.26 g/portion. Looking into the median values, half of the spices had a value <1 g/portion and the other half had a value >1 g/portion. Again cinnamon recorded the highest value in both the 90th and 97.5th percentiles, 20.01 and 30.18 g/portion, respectively, whereas cloves recorded the lowest (0.48 and 0.70 g/portion, respectively) where a respective of 14 and 17 types of spices have a 90th and 97.5th percentiles above 1 g/portion.

The variability of spice intake depends on various factors including the differences between the family's preferences, number of adults in each family, and the adult portion size. Due to the lack of literature in this area, it was difficult to make comparisons. Calculating the adult consumption of spices and relevant mycotoxin levels provides a clear picture to estimate the mycotoxins ingested. The total number of portion sizes considered in the present study (14725) were obviously higher than that reported in India (1905) or in Norway (Siruguri and Bhat, 2015) where the Norwegian number was not noted. The number of adult members in each Indian household was not reported and there are a number of factors that could affect the portion sizes as well as their quantity. Assuming a hypothesis that the Indian people tend to use spices in sufficient quantity but with low variability in the same dish, in this study more dishes (6 compared to 4 in India) were chosen, and also more interviewed HHs (150, 100), more single spices (22, 15), and about 70% of the HHs were composed of more than 2 adults (figure 1) which make our population large regardless of the excluded members due to unmatched eligibility criteria.

The mean intake of spices ranged from 0.14 to 3 g/portion in India (Siruguri and Bhat, 2015), and from 0.5 to 1.3 g/portion in Norway (Carlsen et al., 2011). In contrast, the mean intakes in Lebanon in this study ranged from 0.09 to 5.37 g/portion. One of the factors that affected the portion size of the spices was the quantity consumed of each dish by each adult. Regarding cinnamon, the maximum level (42.7 g/portion) found in our study was extremely high compared to other spices in our study and indeed in the other studies (Carlsen et al., 2011; Siruguri and Bhat, 2015). As reported above, we calculated the spices as a whole and in their ground form and it depended on their use in the 6 dishes. Thus, this high level is considered normal when at least 4 out of the 6 dishes could contain cinnamon in its 2 forms. The mean intake of cinnamon in Lebanon, Norway and India were in 5.37, 1.3, 0.77 g/portion respectively.

Black pepper is one of the most highly consumed spices in many countries. Due to its presence in various Lebanese mixtures, including the seven spices mixture being the most famous (90.6% of HHs), it represents 12.2% of the total number of portions in the present study. The mean intake of black pepper from all dishes (1.07 g/portion) was higher than the Indian result (0.97 g/portion), however, the mean intake of this spice in individual dish such as Chicken rice (0.94 g/portion) was lower when compared to similar Curry Indian dish (1.27 g/portion). We should note that Lebanon suffers from ground spice adulterations, e.g. cumin is replaced by coriander, especially because the ground form of spices is widely used by the population (Osman et al., 2019; QUALEB, 2015). Therefore, the dishes need higher quantity of spices to reach the desired taste.

Table 5 shows the level of spice intake with respect to the portion size consumed. The percentage of total number of portions of each spice was classified into 3 levels of spice intake: less than 1 g, between 1 and 5 g, and above 5 g. More than ¾ of the number of portions of red chili, garlic powder, onion powder, ginger, cloves and nutmeg, used less than1 g. Less than 1 g was also observed for black pepper, paprika, and white pepper with a percentage of number of portions ranging between 50% and 74%. The level of intake between 1 and 5 g was observed in cardamom, caraway, and anise in more than ¾ of the portions. However, coriander, cinnamon, and fennel recorded a level of intake that exceeded 5 g in more than 20% of their portions. The level of spices consumed per portion varies for each spice. More than half of allspice portion sizes ranged between 1 and 5 g. Not only is there a misconception about the mixture's ingredients, as previously discussed, but they consumed this spice in remarkable amounts without knowing. There was a similar consumption of nutmeg between Lebanon and India, but by comparing both studies turmeric (1-5 g), coriander, cinnamon and caraway (>5 g) were higher in Lebanon.

Identifying the reasons for these differences in consumption is likely spice dependent. Turmeric is one of the spices that gives a special yellow color to rice in addition to its special taste and benefits. Despite the high usage of this condiment in both India and Lebanon, the cause differs. Based on several studies, the high level of turmeric consumption is normal in India (Siruguri and Bhat, 2015), while in Lebanon, business may play a role in adulterating the spice which drives the person cooking to add more of it to the dish to reach the expected taste. Adulteration could be a chemical dye that resembles turmeric's colour, or it could be a biological adulterants such as starch from cheaper sources (Sasikumar et al., 2016). These kinds of adulterations were found in spices originating from India, Honduras, Indonesia, Jamaica and China, and it is likely to be found in Lebanon as well.

3.3.2. Spice intake based on portion size of spice consumed from individual dishes

According to the total number of portion sizes for each dish, Chicken rice recorded the highest number of portions (Table 6) at 3810. The remaining dishes are reported in descending order: Dawood basha, Mloukhieh, Kebbeh, Moghrabieh, then Siyadieh. The mean intake of cinnamon was the highest among the consumed spices in Mloukhieh, Kebbeh, and Chicken rice; the mean intake of caraway, cardamom, and allspice were the highest in Moghrabieh, Siyadieh, and Dawood basha respectively. In the Chicken rice dish, 21.39 g of cinnamon was recorded as the highest 90th percentile in all dishes, while a 0.06 g of fennel was recorded as the lowest 90th percentile level in Moghrabieh. The spices that had a median intake >1 g were different within the examined dishes. In Mloukhieh, allspice, coriander, cardamom, and cinnamon had an intake level >1 g, in Kebbeh only the levels of cardamom and cinnamon from the previous listed spices exceeded 1 g, paprika and turmeric were new spices that exceeded 1 g level. Cinnamon was the spice with a median intake >1 g in 2/3 of the dishes. The total mean intake of spices ranged between 12.37 g in Dawood basha and 29.88 g in Mloukhieh. In Siyadieh, Chicken rice, Kebbeh, and Moghrabieh the total mean intake of spices were 25.21, 23.84, 18.42 and 16.07 g, respectively (Table 6).

Despite that cloves were consumed as seeds, especially during chicken boiling, and powder as part of several spices blends, the intake remained <1 g per portion for 98% of the investigated portions. A similar result was found in India for cloves but not for cardamom seeds/powder where 93% of Indian portions contain <1 g of cardamom, whereas the majority of Lebanese portion sizes contain 1-5 g of cardamom seeds/powder. Again, cardamom seeds as well as cinnamon sticks are the principal ingredients in the Lebanese chicken boiling step, to give the chicken a non-rancid smell and taste. Cinnamon intake in Lebanon was much higher than in India and comparable with that in Norway but different in sources since confectionary and other bakery products contain cinnamon in Norway (Carlsen et al., 2011; Siruguri and

Bhat, 2015). The higher intake of caraway and coriander in Lebanon, as compared to India, may be due to the dishes chosen in our study since caraway is the basic spice of Moghrabieh (Table 6, Table 3) and coriander is quite often used to prepare Mloukhieh (Table 5). As shown in Table 3, 79 out of 150 HHs use coriander to prepare Mloukhieh, whereas the rest of them use fresh coriander instead of dried and ground coriander. It was assumed that the Indians consume greater quantity of red chili since hot and spicy food is popular in India, which is one of the highest consumers in the world for this spice. (Ferrucci et al., 2011). In fact about 70% of the consumed portions contain 1-5 g of red chili per portion (Siruguri and Bhat, 2015), whereas more than 70% of portions evaluated in our study contain <1 g per portion. The highest mean intake of red chili was observed for the Siyadieh dish which contains 1.73 g/portion which is lower than 4 g/portion in India (Siruguri and Bhat, 2015), despite its lower number of portion sizes compared to those in Mloukhieh, Kebbeh, Chicken rice, and Dawood basha (table 6). Most of the participating families have children that are less likely to consume hot spices (Figure 1), so some adult participants added the hot spices directly onto their own plates.

3.4. Dietary Exposure to AFB_1, OTA and FB_1 from Spices Marketed in Lebanon

To assess the risk of OTA, the TDI value of 17 ng/kg-bw day set by the European Food Safety Authority was used to evaluate the risk of daily exposure of OTA (EFSA, 2006). The results of the spice intake assessed in the present study and the data on the occurrence of OTA in spices marketed in Lebanon (El Darra et al., 2019) have been used to calculate the probable daily intake (PDI) of this mycotoxin from each spice (Table 7). The results of calculated mean PDI of OTA from each contaminated spice ranged from 0.03 ng/kg-bw/day for black pepper to 0.23 ng/kg-bw/day for nutmeg whereas the overall mean PDI, calculated from the 7 contaminated spices, was 0.11 ng/kg-bw day. These values were far below the TDI of this mycotoxin and represented 0.2-1.4% of TDI (Table 7). In fact, the mean level of OTA in positive samples was 9.13 µg/kg, which is below the European limits in spices, 15-20 µg/kg (European Commission, 2006). Nutmeg is used in 5 out of the 6 dishes considered in this study and contained the highest level of OTA (34 µg/kg) in agreement with Reinholds et al., (2017) who identified this spice as the only one containing a level of OTA (14 µg/kg) close to the maximum permitted level in Latvia. However, the consumption of Nutmeg resulted in a PDI of 0.23 ng/kg/bw/day which is only 1.4% of TDI of OTA. Nutmeg belonged to the spices that were 8% of dietary intake of OTA in EU (Miraglia and Brera 2002). The calculated PDI of AFB_1 from each positive spices ranged between 0.09-3.7 ng/kg-bw/day whereas the overall mean PDI, calculated from positive samples, was 1.55 ng/kg-bw/day. Since IARC classified Aflatoxins as group 1 carcinogens (IARC, 2016), their dietary exposure should be reduced as much as possible. Since any level of exposure can be unsafe (Luzardo et al., 2016), the calculated levels of MOE confirmed this statement. Table 7 shows the MOEs derived from the calculated PDI of AFB_1. In general, MOE of 10,000 or higher, is considered of a low risk from a public health point of view and is reasonably considered as a low priority for risk management actions, based on a benchmark dose lower confidence limit from an animal carcinogenicity study (Constable and Barlow, 2009). Values of MOE were <10,000 for the 8 spices found contaminated by AFB_1 and commonly used in the preparation of the 6 dishes considered in this study (Table 7). MOE values ranged from 108.1 for coriander to 444.4 for cardamom. Only positive samples were used to calculate MOEs therefore this approach may have given an overestimation of the risk. The limited number of samples tested for each kind of spice is another drawback of the present study. Further studies are necessary to check the occurrence of AFB_1 in a conspicuous number of samples for each of the 18 spices used in the 6 dishes commonly prepared in Lebanese households. Once robust data on AFB_1 occurrence will be available, the mean intake of each spice reported in the present study can be used for accurate MOEs estimations. A recent study conducted in Malaysia found that the probable daily intake of aflatoxins had an overall mean of 0.59 ng/kg-bw/day and a range of 0.21 to 1.32 ng/kg-bw/day (Ali and Watt, 2019). Their minimum is higher than ours (0.09 ng/kg-bw/day) while their maximum is less than ours (3.7 ng/kg-bw/day). The overall mean PDI found in the Malaysian study (0.59 ng/kg-bw/day) is less than our (1.55 ng/kg-bw/day), while their overall mean MOE of 520 was lower than our (1038) because Malaysia is among the top 3 countries reported to have high spice consumption i.e. 13 g/person/day (Ali and Watt 2019). EFSA established a TDI for FB_1 of

1000 ng/kg-bw/day (EFSA, 2018). Table 7 shows the mean FB_1 levels in positive samples of spices as well as the PDI values and the % of TDI of FB_1 for each spice. The results of calculated mean PDI of FB_1 from positive spices ranged between 0.2-331.7 ng/kg-bw/day whereas the overall mean PDI was 79.3 ng/kg-bw/day. All PDI values were below the TDI value of this mycotoxin and represented 0.02-33.2% of TDI. In particular, for garlic and onion the PDI values were significant and represented 20.3% and 33.2% of the TDI of FB_1, respectively (Table 7). The very high levels of FB_1 contamination in these two spices (23,338 and 55,959 µg/kg) did not result in higher PDI values thanks to the low mean amount of spices consumed (0.43 and 0.63 g/portion). In previous studies the reported levels of FB_1 in spices ranged from low levels (5-135 µg/kg) (Waskiewicz et al., 2013; Reinholds et al., 2016; Tonti et al., 2017; Gambacorta et al., 2018) to high mean level in 94 spices (6,432 µg/kg), especially in garlic and onion (up to 113,475 µg/kg) (El Darra et al., 2019). However, the dietary intake of FB_1 could not be reported due to the lack of data on spice consumption in this country. In the present study we have calculated the dietary intake of FB_1 from spices consumed in Lebanon and we have demonstrated that the consumption of a single spice (onion) can reach 33.2% of TDI of this mycotoxin.

4. Conclusions

The method used in the present study to estimate spices consumed at individual levels prevent data losses resulted from considering only the general household usage of spices. Studying popular dishes simplifies understanding of spices used and their consumption among the targeted population. Estimating the portion sizes of the spices is considered an important step towards a more accurate mycotoxins risk assessment. Based on the results of the current study, the Lebanese population tend to consume various types of spices in one dish whether in the form of mixtures or as single spice in which some of them were in a noteworthy amounts. Cinnamon (5.37), fennel (3.76), and caraway (3.64) were the 3 highest consumed spices per portion whilst cloves (0.26) and onion powder (0.43) were the 2 lowest consumed spices per portion.

While the level of OTA in consumed spices is low and isn't a matter of concern, the level of AFB_1 is higher and considered risky. Considering that the concern for this mycotoxin originates only for spices, further investigation should be done on the overall daily food intake at the individual level to measure the total aflatoxin intake among the Lebanese population and take serious actions to reduce its risk. Monitoring urinary AFM_1 concentrations in Lebanese population can also be used to provide quantitative information on daily aflatoxin intake at the individual level. Fumonisins should be regulated in some spices such as garlic and onion to protect consumers, especially those consuming high amount of spices highly susceptible to fumonisin contamination.

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