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# Susceptibility of Ajwa dates (*Phoenix dactylifera*) to aflatoxin contamination based on liquid chromatography combined with electrospray ionisation-triple quadrupole tandem-mass spectrometry (LC-ESI-MS/MS)

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**Abstract**

Dates are a staple food across the world because of their religious, cultural, and nutritional importance. There are many different varieties of dates, one of which is the Ajwa variety. There have been no reports on the contamination of Ajwa dates by aflatoxins (AFs). Therefore, the present work was conducted to investigate the incidence of AFs in Ajwa dates for the first time. Samples (100) were analysed for AFs using liquid chromatography combined with electrospray ionisation-triple quadrupole tandem-mass spectrometry (LC-ESI-MS/MS). The method was validated and regarded as reliable due to good linearity ($R^2$ > 0.99), satisfactory recovery (61.4 - 105.7%), precision (RSDs ≤ 12.29%), and sensitivity (LOD in the range of 0.042 - 0.013 µg/kg; LOQ in the range of 0.125 - 0.038 µg/kg). Surprisingly, no aflatoxins were detected, which might indicate that this popular type of date is not likely to pose potential health risks, though further research is required.

**Abbreviation**

*A. flavus*, *Aspergillus flavus*; *A. niger*, *Aspergillus niger*; AFs, aflatoxins; $AFB_1$, aflatoxin $B_1$; $AFB_2$, aflatoxin $B_2$; $AFG_1$, aflatoxin $G_1$; $AFG_2$, aflatoxin $G_2$; LC-ESI-MS/MS, liquid chromatography combined with electrospray ionisation-triple quadrupole tandem-mass spectrometry; MRM, multiple reaction monitoring; Q1 Mass, the first quadrupole; Q3 Mass, the third quadrupole; DP, declustering potential; CE, collision energy; CXP, collision cell exit potential; EP, entrance potential; LOD, limit of detection; LOQ, limit of quantification; RSDs, the relative standard deviations.

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## Introduction

The date palm (*Phoenix dactylifera* L.) is one of the oldest recorded fruit trees in the world that goes back 7,000 years (Hegazy and El Sayed, 2014). Dates are cultivated in regions of North Africa, South Asia, and Southwest Asia, especially in the Arab regions (Al-Farsi and Lee, 2008; Khalid *et al*., 2017). Based on the database of the Food and Agriculture Organization of the United Nations (FAO, 2021), the worldwide demand for dates has increased significantly. In 2010, the total global import of dates was 612,273 tonnes, and this value has increased gradually to reach 1,266,781 tonnes in 2019, reflecting the current high consumption of date fruits worldwide.

Saudi Arabia is the leading producer of dates (FAO, 2021), and grows approximately 400 date fruit varieties (Al Hazzani *et al*., 2014). The most common and expensive variety is the Ajwa date, which is cultivated in the holy city of Al-Madinah Al-Munawwarah. Ajwa dates have been a staple food for millions of people all over the world (Khalid *et al*., 2017), and are consumed in large amounts, particularly in Islamic countries (Zhang *et al*., 2013). Ajwa dates have religious value as well as cultural importance for Muslims, and are usually consumed for breaking daylong fasts (Ragab *et al*., 2013). Further, Ajwa dates provide a wide range of essential nutrients and health benefits (Khalid *et al*., 2016; 2017). The main component of Ajwa dates is carbohydrates (70 - 80%), but it also provides

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proteins, fats, potassium, dietary fibres, and bioactive compounds (Gasim, 1994; Assirey, 2015).

Date palm fruits serve as an ideal substrate for fungal invasion and spoilage, including species of *Aspergillus*, which can occur at various stages of ripening on trees as well as during storage. The key reason for this is the suitability of the composition of dates for fungal growth (Al Hazzani *et al*., 2014). The presence of fungi not only causes spoilage of dates but can also lead to health-related issues and life-threatening clinical conditions (Tournas *et al*., 2015) if mycotoxins are also produced. In particular, aflatoxins, the most common mycotoxin, in dates have been associated with *A. flavus* and *A. niger*, which are considered the most significant mycotoxigenic fungi responsible for aflatoxin contamination (Gherbawy *et al*., 2012). The invasion of these species of date fruits has been documented (Emam *et al*., 1994; Aidoo *et al*., 1996; Ahmed *et al*., 1997; Ragab *et al*., 2001; Heshmati *et al*., 2017). Furthermore, several surveys conducted in the Mediterranean countries found that the aflatoxigenic aspergilli, particularly *A. flavus* and *A. niger*, are the more frequently detected mycotoxigenic fungi in dried dates, apricots, and prunes (Zohri and Abdel-Gawad, 1993; Aziz and Moussa, 2002; Heperkan, 2006). Moreover, *A. flavus* was also the main cause of aflatoxin contaminants in dried dates and prunes (Ozer *et al*., 2012)

However, there is insufficient knowledge and a lack of published data regarding aflatoxin contamination of date palm fruits (Iqbal *et al*., 2014; Almaghrabi, 2022), Specifically, there is no published data reporting the occurrence of aflatoxin contamination in Ajwa dates. The studies of Al Hazzani *et al.* (2014) and Al-Mutarrafi *et al.* (2019) were designed to evaluate the fungal and bacterial loads in dried fruits (such as Sukkari, Mabroon, Amber, Ajwa, Rabeae, Mashrooq, Al Segae, Khodary, and Khalas), and the results confirmed *Aspergillus* spp. invasion in Ajwa dates, particularly aflatoxigenic aspergilli. The studies did not, however, investigate aflatoxin presence. In this context, with human exposure to aflatoxins being a potential health risk due to the well-documented carcinogenicity, mutagenicity, teratogenicity, and hepatotoxicity of aflatoxins (Eaton and Gallagher, 1994), investigating the safety status of date consumption is crucial. This is especially important since dates are usually consumed raw as a final product without processing. Therefore, the present work was conducted to determine, for the first time, the incidence and contamination level of aflatoxins in Ajwa dates.

## Materials and methods

### *Chemicals and reagents*

LC-grade solvents such as methanol and acetonitrile, and aflatoxin standards were purchased from Sigma-Aldrich Chemical Co. Ltd. (Sigma Aldrich, Saint Louis, MO, USA). The Millipore-Q Water Purification System (Billerica, MA 01821, USA) was used throughout all experiments. All other reagents were of the highest analytical grade.

### *Instrumentation*

The analysis of aflatoxins was performed using liquid chromatography-electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS) with an Exion LC AC system for separation and an AB SCIEX Triple Quad 5500+ MS/MS system equipped with electrospray ionisation (ESI) for detection (AB Sciex, Framingham, Massachusetts, USA). The instrument data were collected and processed using SCIEX OS 1.6.10 software.

The separation of the target analytes was performed with an Agilent Zorbax Eclipse Plus $C_{18}$ Column (4.6 × 100 mm, 1.8 µm) (Agilent, Santa Clara, CA, USA). The mobile phases consisted of two eluents, both containing 10 mM ammonium formate; eluent A was 0.1% formic acid in water, and eluent B was 0.1% formic acid in methanol. The mobile phase gradient was programmed as follows: 10 - 30% B from 0.0 - 2.0 min, 30 - 100% B from 2.0 - 11.0 min, 100% B from 11.0 - 11.5 min, 100 - 10% B from 11.5 - 12.0 min, and 10% B from 12.0 - 15.0 min. The flow rate was 0.6 mL/min, and the injection volume was 10 µL.

For MS/MS analysis, positive multiple reaction monitoring mode (+MRM) was applied with the following parameters = curtain gas: 20 psi; collision gas: 9 psi; ion source temperature: 600°C; ion spray voltage: 5,500 V; ion source gas 1 (nebuliser gas): 60 psi; and ion source gas 2 (drying gas): 60 psi. The other performance parameters of the analytical method are presented in Table 1.

### *Preparation of standard solutions*

Briefly, 100 µL of aflatoxin standard with a concentration of 1 µg/mL ($AFB_1$ and $AFG_1$) and 0.3 µg/mL ($AFB_2$ and $AFG_2$) was allowed to dry in 10 mL volumetric flasks. Then, 10 mL of methanol was

**Table 1.** MS/MS parameters for positive MRM transition.

| | Q1 Mass (Da) | Q3 Mass (Da) | DP (Volts) | CE (Volts) | CXP (Volts) | EP (Volts) |
|---|---|---|---|---|---|---|
| $AFB_1$ | 313.040 | 285.000 | 116.000 | 33.000 | 16.000 | 10 |
| | 313.040 | 241.100 | 116.000 | 51.000 | 14.000 | 10 |
| $AFG_1$ | 329.017 | 243.000 | 136.000 | 37.000 | 14.000 | 10 |
| | 329.017 | 311.000 | 136.000 | 31.000 | 18.000 | 10 |
| $AFB_2$ | 315.200 | 287.100 | 91.000 | 39.000 | 14.000 | 10 |
| | 315.200 | 259.100 | 91.000 | 45.000 | 22.000 | 10 |
| $AFG_2$ | 331.200 | 313.000 | 131.000 | 36.000 | 14.000 | 10 |
| | 331.200 | 245.000 | 131.000 | 49.000 | 18.000 | 10 |

added. The mixture was mixed well by vortexing for 1 min. The final concentrations were 10 ng/mL ($AFB_1$ and $AFG_1$) and 3 ng/mL ($AFB_2$ and $AFG_2$). From these stock solutions, serial dilutions were prepared in methanol to prepare different concentrations of $AFB_1$ and $AFG_1$ (0.125, 0.25, 0.5, 1, 2, and 4 ng/mL), and $AFB_2$ and $AFG_2$ (0.0375, 0.075, 0.15, 0.3, 0.6, and 1.2 ng/mL). The stock solutions were stored at -20°C, and allowed to reach room temperature before use. The serial dilutions were prepared immediately prior to the experiments.

*Plant material*

The Ajwa date fruit belongs to the Arecaceae family, which is also known as the palm family in the monocot order Arecales. The genus *Phoenix* contains 14 species including *P. dactylifera* (Hussain *et al*., 2014; APG, 2016). Ajwa dates are visually distinctive: being an elongated ovoid shape around 2 to 2.5 cm in length, black, and wrinkled (Gasim, 1994; Elsharawy *et al*., 2019).

*Sampling*

Briefly, a total of 100 Ajwa date samples were randomly collected from different markets in Jeddah, Saudi Arabia from June to November 2020. The samples had been grown and cultivated from the native Ajwa date palm-producing area of Al-Madinah Al-Munawwarah, Saudi Arabia. From each source, a sample size of at least 1 kg was purchased. The sampling procedure was as described previously by Masood *et al*. (2015) and Han *et al*. (2016). Each sample was pitted and cut into small pieces and finely ground, then packed in polyethylene bags, and stored at -18°C until analysis.

*Sample preparation*

The extraction of the samples was done following Martos *et al.* (2010) and Han *et al.* (2016) with slight modifications. Briefly, from each homogenised date sample, 2 g was weighed into a 50 mL polypropylene centrifuge tube, and extracted with 8 mL of extraction solution using acetonitrile:water (80:20, v:v). The mixture was shaken for 20 min and centrifuged for 5 min at 4,000 rpm at 10°C, followed by filtration through a 0.45 µm PTFE filter. Finally, 0.5 mL of the filtrate was diluted (1:1, v:v) in 5 mM ammonium acetate before injection into the LC-MS/MS system.

*Method validation*

The method was validated in terms of linearity, sensitivity, accuracy, and precision, as well as for the absence of matrix effects. Linearity was investigated by constructing a calibration curve of six points for the targeted analytes in triplicate. In particular, linearity ranged from 0.125 - 4 ng/mL for $AFB_1$ and $AFG_1$, and 0.0375 - 1.2 ng/mL for $AFB_2$ and $AFG_2$. The sensitivity of the method was estimated by determining the limit of detection (LOD) and limit of quantification (LOQ). The LOD was calculated based on a signal-to-noise ratio (S/N) of 3, while LOQ has a ratio of 10. The accuracy of the method was determined by using two different fortification levels in triplicate applied to the standard solution: 0.250 and 1 ng/mL for $AFB_1$ and $AFG_1$, 0.075 and 0.3 ng/mL for $AFB_2$ and $AFG_2$, respectively. The relative standard deviations (RSDs) on the same day were used to determine the intraday precision, while the values after one week were used for interday precision. The matrix effects for the four targeted

analytes in blank Ajwa date samples were also investigated in triplicate by spiking 2 g of Ajwa dates with 200 µL of aflatoxin standard (10 ng/mL $AFB_1/AFG_1$ and 3 ng/mL $AFB_2/AFG_2$), and then the samples were allowed to dry before the extraction procedures; the final concentrations were calculated to be 1 ng/g $AFB_1/AFG_1$ and 0.3 ng/g $AFB_2/AFG_2$ (0.125 ng/mL $AFB_1$ and $AFG_1$; 0.0375 ng/mL $AFB_2$ and $AFG_2$). Two different concentrations were also prepared by spiking the Ajwa samples with 10 and 50 µL of aflatoxin standard (1 µg/mL $AFB_1/AFG_1$ and 0.3 µg/mL $AFB_2/AFG_2$). After the extraction procedures, the final concentrations were 5 ng/g $AFB_1/AFG_1$ and 1.6 ng/g $AFB_2/AFG_2$ (0.625 ng/mL $AFB_1$ and $AFG_1$; 0.1875 ng/mL $AFB_2$ and $AFG_2$); 25 ng/g $AFB_1/AFG_1$ and 8.3 ng/g $AFB_2/AFG_2$ (3.125 ng/mL $AFB_1$ and $AFG_1$; 0.9375 ng/mL $AFB_2$ and $AFG_2$).

## Results and discussion

In the present work, a sensitive LC-ESI-MS/MS method was validated and applied to investigate aflatoxin contamination in Ajwa date samples. Currently, the LC-MS/MS technique is one of the most common methods of analysis for aflatoxins, enabling an unambiguous determination of different types of aflatoxins in a single run (Azaiez *et al*., 2014; 2015; Han *et al*., 2016; Abdallah *et al*., 2018; Quaglia *et al*., 2020). The results show that good linearity was achieved with correlation coefficients ($R^2$) higher than 0.99 for the four targeted analytes (Table 2). The LC/ESI (+)/MS/MS chromatograms of the individual retention times of the lowest and highest concentration of AFs standard ($AFG_1$, $AFB_1$, $AFG_2$, and $AFB_2$) are shown in Figure 1.

The sensitivity of the analytical method was evaluated, and the LOD and LOQ achieved were 0.042 and 0.125 µg/kg for $AFB_1$ and $AFG_1$, while the levels were 0.013 and 0.038 µg/kg for $AFB_2$ and $AFG_2$, respectively. The values of the method sensitivity were significantly below the legal limits for aflatoxins legislated by the Gulf Standardisation Organization (GSO) and European Union (EU) member states. The maximum acceptable GSO and EU levels are 10 µg/kg for total aflatoxins in dried fruit intended for further processing, and 4 µg/kg for dried fruit intended for direct human consumption (EU, 2010; GSO, 2019). The EU set a very low limit of acceptable levels of $AFB_1$ in particular because it is the most toxic among all analogues of aflatoxins. Specifically, the EU set a level of 5 µg/kg of $AFB_1$ for dried fruit intended for further processing, with an even lower level of 2 µg/kg for dried fruit intended for direct human consumption (EU, 2010). In addition, the LOD and LOQ of the current method were also below the legal limit set by the FDA in the USA at a level of 20 µg/kg for total aflatoxins in a foodstuff (FDA, 2018). Regarding repeatability of the LC-MS/MS procedure, the RSDs for intraday and interday precision were in the range of 1.93 - 12.29% and 0.60 - 4.86%, respectively. The performance characteristics of the analytical method are presented in Table 2. With regard to the recoveries of aflatoxins in spiking samples, the values obtained were in the range of 61.4 - 105.7%, with RSDs varying from 0.61 - 8.59% (Table 3).

**Table 2.** Performance characteristics of analytical method including linearity, sensitivity, accuracy, and precision.

| | Slope | Intercept | $R^2$ | Linear range (ng/mL) | LODs (µg/kg) | LOQs (µg/kg) | Fortified levels (µg/kg) | Repeatability (%RSD) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Intraday | Interday |
| $AFB_1$ | 971341 | 76031 | 0.9995 | 0.125 - 4 | 0.042 | 0.125 | 0.250 | 4.46 | 1.69 |
| | | | | | | | 1 | 6.98 | 4.26 |
| $AFG_1$ | 789869 | 60513 | 0.9996 | 0.125 - 4 | 0.042 | 0.125 | 0.250 | 3.12 | 0.60 |
| | | | | | | | 1 | 5.62 | 4.78 |
| $AFB_2$ | 559893 | 14664 | 0.9992 | 0.0375 - 1.2 | 0.013 | 0.038 | 0.075 | 7.42 | 3.28 |
| | | | | | | | 0.3 | 12.29 | 4.86 |
| $AFG_2$ | 501588 | 10748 | 0.9996 | 0.0375 - 1.2 | 0.013 | 0.038 | 0.075 | 1.93 | 3.35 |
| | | | | | | | 0.3 | 4.28 | 2.18 |

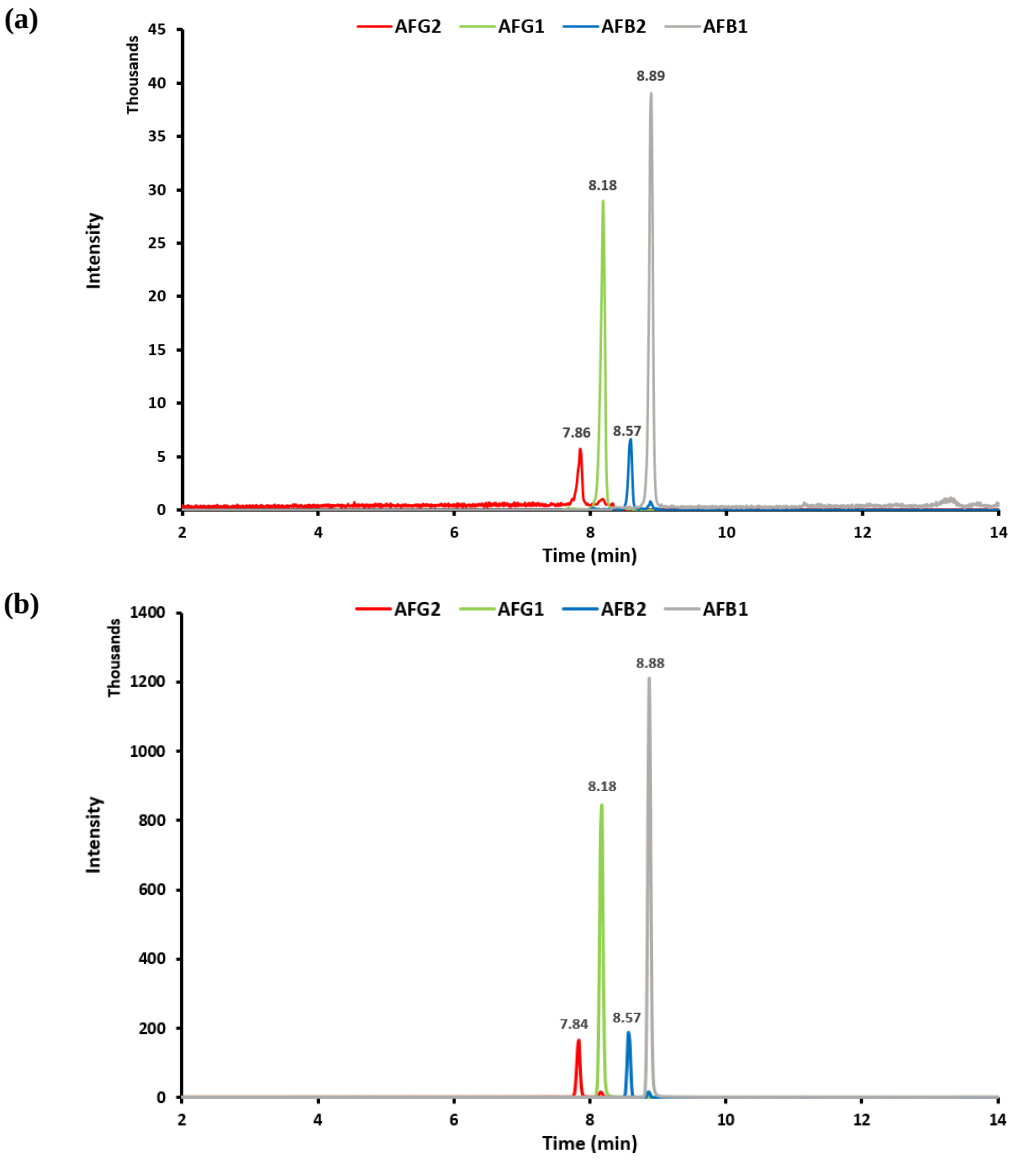


**Figure 1. (a)** LC/ESI (+)/MS/MS chromatogram showing individual retention times of the lowest concentration of AFs standard; **(b)** chromatogram showing the highest concentration of AFs standard.

**Table 3.** Recoveries of aflatoxins in dates ($n$ = 3).

| | Fortified level (µg/kg) | Measured level (mean ± SD) (µg/kg) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| $AFB_1$ | 0.125 | 0.08 ± 0.003 | 61.4 | 4.35 |
| | 0.625 | 0.64 ± 0.004 | 102.9 | 0.61 |
| | 3.125 | 3.09 ± 0.050 | 98.9 | 1.61 |
| $AFG_1$ | 0.125 | 0.08 ± 0.005 | 64.2 | 6.22 |
| | 0.625 | 0.66 ± 0.012 | 105.7 | 1.77 |
| | 3.125 | 3.11 ± 0.024 | 99.5 | 0.78 |
| $AFB_2$ | 0.0375 | 0.02 ± 0.002 | 66.4 | 8.59 |
| | 0.1875 | 0.20 ± 0.004 | 104.2 | 2.24 |
| | 0.9375 | 0.94 ± 0.010 | 100.2 | 1.08 |
| $AFG_2$ | 0.0375 | 0.03 ± 0.001 | 73.8 | 3.69 |
| | 0.1875 | 0.20 ± 0.004 | 108 | 2.01 |
| | 0.9375 | 0.95 ± 0.019 | 101.1 | 2.05 |

RSD = relative standard deviation of repeatability.

Based on the method validation, the analytical method used in the present work could be regarded as reliable for the determination of the four targeted analytes ($AFB_1$, $AFG_1$, $AFB_2$, and $AFG_2$) in the investigated Ajwa date samples because of the good linearity ($R^2$ > 0.99), satisfactory recovery (61.4 - 105.7%), precision (RSDs ≤ 12.29%), and sensitivity (LOD in the range of 0.042 - 0.013, and LOQ in the range of 0.125 - 0.038 µg/kg) that were successfully achieved. The relative sensitivity of LC-MS/MS reported in the literature for determination of aflatoxins in date palm fruits has varied. In the study by Azaiez *et al.* (2014), the LOD were 0.08, 0.08, 0.16, and 0.3 µg/kg for $AFB_1$, $AFB_2$, $AFG_1$, and $AFG_2$, respectively, while the LOD reported by Han *et al.* (2016) was 0.1 µg/kg for $AFB_1$ and $AFB_2$, and 0.3 µg/kg for $AFG_1$ and $AFG_2$. In a more recent study conducted in Asyut, Egypt concerning $AFB_1$ and $AFB_2$ contamination in dried dates using LC-MS/MS, the sensitivity of the method was 0.05 and 0.03 µg/kg for $AFB_1$ and $AFB_2$, respectively (Abdallah *et al*., 2018). Next, this reliable analytical method was applied to 100 commercial date palm fruits (Ajwa dates) purchased from local markets in Jeddah, Saudi Arabia. Among all investigated samples, no aflatoxins ($AFB_1$, $AFG_1$, $AFB_2$, and $AFG_2$) were detected in the date samples. The results were consistent with the previous findings of Hegazy and El Sayed (2014), Azaiez *et al*. (2015), Han *et al*. (2016), and Quaglia *et al*. (2020). These studies investigated aflatoxin contamination in relatively small numbers of different date varieties in Egypt (5), Spain (27), China (40), and Italy (20), and all the dates were found to be free from aflatoxin contamination.

Aflatoxin contamination has been investigated and detected in different date varieties (Luttfullah and Hussain, 2011; Ibrahim *et al*., 2013; Iqbal *et al*., 2014; 2018; Masood *et al*., 2015; Azaiez *et al*., 2015; Asghar *et al*., 2017; Heshmati *et al*., 2017; Abdallah *et al*., 2018), but no specific data have been reported on aflatoxin occurrence in Ajwa dates previously. In general, the findings from the literature confirm the susceptibility of date fruits to aflatoxin contamination, and show that the levels of contamination vary worldwide. In Pakistan, different authors have reported total aflatoxins (total AFs) occurrence in different date varieties, with average levels of 2.5 µg/kg (Luttfullah and Hussain, 2011), 4.11 µg/kg (Iqbal *et al.*, 2014), 6.32 µg/kg (Masood *et al*., 2015), 0.24 µg/kg (Asghar *et al*., 2017), and 5.30 µg/kg of total AFS (Iqbal *et al.*, 2018). Azaiez *et al.* (2015) investigated the incidence of aflatoxins in date fruits in Tunis. Aflatoxins were found in 46% of the samples, with an average level of 1.14 µg/kg of $AFB_2$, 1.4 µg/kg of $AFG_1$, and 1.7 µg/kg of $AFG_2$, respectively. Heshmati *et al.* (2017) found that 41% of the investigated Iranian date samples were contaminated with an average level of 2.6 µg/kg of total AFS. In the study of Abdallah *et al.* (2018) from Egypt, out of 20 investigated samples, $AFB_1$ and $AFB_2$ were detected in only one sample at levels of 14.4 and 2.44 µg/kg, respectively. A high level of aflatoxin contamination in dates was reported in samples from Yemen, where the contamination levels ranged from 110 to 180 µg/kg (Alghalibi and Shater, 2004).

Despite the widespread and increasing consumption of dates, it was found that aflatoxin contamination in date palm fruits is less frequently investigated in comparison with such contamination in other commodities. However, dates could be an important source of aflatoxin exposure due to the suitability of dates as a medium for fungal invasion and the subsequent aflatoxin production (Ozer *et al*., 2012; Al Hazzani *et al*., 2014). In addition, the direct consumption of dates without further processing could result in direct, undiluted exposure to aflatoxins. It is notable that aflatoxins in Ajwa dates were investigated for the first time in the present work, and it was significant that no aflatoxins were found. This finding was unexpected because considering the composition of Ajwa dates (high moisture and sugar contents in particular), Ajwa dates would be expected to serve as an ideal substrate for fungal invasion (Al Hazzani *et al*., 2014; Al-Mutarrafi *et al*., 2019) and the subsequent aflatoxin contamination. Additionally, the climate conditions of Jeddah, Saudi Arabia provide a good environment for fungal invasion and the formation of aflatoxin since the temperature during the year ranges from 23.5 - 32°C, with a minimum humidity of 50%. Moreover, a high incidence of aflatoxins was reported in the work of Ibrahim *et al.* (2013), where aflatoxins were detected in 80% of different date varieties (Sukkari, Dokeiny, Salg, Razeiz, Sakiee, Khalas, Roshodiah, Nabtat Aly, Khattary, Meinifi, Dooglet, and Helwa) marketed in Riyadh, with an average of 1.39 and 2.01 µg/kg for AFB1 and total AFs, respectively. In contrast to the work of Ibrahim *et al.*

(2013), the findings in the present work revealed for the first time the incidence of aflatoxin contamination in Ajwa dates.

One explanation for not detecting aflatoxins could be due to not analysing a sufficient number of date samples. It is well-known that the distribution of aflatoxins in agricultural products does not follow a normal distribution. However, the present work was designed to generate sufficient sample numbers to overcome the 'sampling' problem. However, it does not seem likely that the absence of positive aflatoxin detection in the samples was due to this cause.

Another factor to consider is that, despite the potential of *Aspergillus* spp. invasion in Ajwa dates, as was confirmed in the study of Al Hazzani *et al.* (2014), the absence of subsequent aflatoxin formation could depend on the particular compositional profile of Ajwa dates. In this regard, Ajwa date fruits are distinguished among all dates varieties because of their unique composition and phytochemical profile, which has been proven to possess valuable strong antioxidant, anti-inflammatory, antimutagenic, hepatoprotective, nephroprotective, and anticancer activities in addition to antimicrobial activities (Zhang *et al*., 2013; Hussain *et al*., 2014; 2019; Khalid *et al*., 2017; Elmaa *et al*., 2018; Alshwyeh, 2020). A recent study by Risan *et al.* (2018) evaluated the efficacy of Ajwa date extracts in reducing aflatoxin $M_1$ produced by *A. flavus*. The results showed that the Ajwa date extract exhibited antifungal activities against *A. flavus* and detoxified $AFM_1$ in milk. The present work highlighted the interest in investigating the antitoxic properties and the potentials of Ajwa dates flesh to degrade and detoxify aflatoxins, namely $AFB_1$, $AFB_2$, $AFG_1$, and $AFG_2$. Consequently, it is a feasible hypothesis that the specific chemical composition of Ajwa dates is able to inhibit aflatoxin biosynthesis, despite the presence of its causal fungi.

## Conclusion

The present work revealed that no aflatoxins, surprisingly, were detected in any of the investigated Ajwa date samples. The present findings are of interest for public health as a whole, and for the Muslim population, in particular, due to their religious practices. Ajwa dates are consumed on a daily basis in Islamic countries because it is believed that Ajwa dates have a wide range of protective effects in traditional medicine, as described in the Islamic literature. Despite the fact that the findings of the present work might suggest that this popular type of dates does not pose potential health risks to individuals, further research is required in order to understand whether analysis of larger sample numbers can confirm the results, or whether inherent properties of Ajwa dates could prevent aflatoxin biosynthesis. The existing method is sensitive, specific, and reproducible by using LC MS/MS for aflatoxin determination. The proposed method demonstrated high sensitivity, accuracy, precision, and simple preparation extraction for aflatoxins.

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