INVESTIGATION ABOUT THE PRESENCE OF OCHRATOXIN A IN SOME DRIED FRUITS IN MOSUL MARKETS

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ABSTRACT

Mycotoxin Ochratoxin is one of the main toxins produced by fungi that causes many health problems. The investigation was carried out for the presence of the Ochratoxin in three types of dried fruits, which are apricots, raisins and currants, which were collected from the markets of the city of Mosul, the left side, the right side, the district of Tal Afar and the Al-Ayadiyah district. In the period between October and January. With 15 samples for each type of dried fruits. Samples were analyzed using enzyme linked immunosorbent assay (ELISA) technique. And as we know that the normal ratio of mycotoxin in dried fruits is 10 ng/ml, the mean of the apricot samples was 4.644 ng/ml, the mean of the raisins samples was 3.391 ng/ml and the mean of currants samples was 2.263 ng/ml. And the samples that exceeded the normal range were one sample of apricots, which was 11.631 ng/ml, And one sample of raisins, which was 10.105 ng/ml. As for the rest of the samples, they were all within the normal range.

Keywords: Dried fruits, ELISA, Mosul, Ochratoxin A.

Introduction

Dried fruits are important food material around the world which is concentrated form of fruits, recommended daily in order to gain Phytochemicals and antioxidants (Chang et al., 2016). Dried fruits took a part of human life since prehistoric times which is rich of live promoting bioactive compounds (Alasalvar et al., 2020). Dried fruits can be prepared by our hands easily and can be bought everywhere in earth, in 2018, the most consumption was in middle east 28%, Europe 27%, Asia 24%, North America 13% and 8% for other regions (Rybicka et
Unfortunately, these important food materials can be contaminated by different microorganisms including fungi (Abbas et al., 2019). Contamination of food and feed with fungi and mycotoxins is a problem that threatens many developing countries, especially those that lack good food storage conditions and are a source of great concern, which prompted these countries (Makun et al., 2010). Contamination with fungi can lead to produce mycotoxins, for example, aflatoxins, ochratoxins, patulin, and ergot alkaloids causing diseases and health problems. Some of the mycotoxins are carcinogenic agents (Karaca et al., 2010). As for ochratoxin A, it is produced by several types of Aspergillus and Penicillium, and it is a common mycotoxin that pollutes food. Contamination of food commodities such as cereals, grain products, coffee seeds, dried vine fruits, wine, grape juice, spices, and licorice occurs worldwide. Ochratoxin A is formed during crop storage and is known to cause a number of toxic effects in animal species. The most sensitive and obvious effect is kidney damage, but the toxin can also affect the development of fetuses and the immune system. There is clear evidence of nephrotoxicity and kidney cancer in animals as a result of exposure to Och. On the contrary, this association has not been completely clear in humans, yet the effect on the kidneys has been proven (WHO, 2018). Ochratoxins are secondary metabolites that are produced by 3 main species of molds: Aspergillus ochraceus, Penicillium verrucosum, Aspergillus niger, and especially A. carbonarius. Ochratoxins A and B are the only types of ochratoxin that can be found in food and feed. Ochratoxin A is a chlorinated derivative isocoumarin with an amide ring linked to phenylalanine (Aish et al., 2004). Ochratoxin A (OTA) is among the most frequent contaminants in dried fruits like raisin and apricot (Wei et al., 2017). Ochratoxin A is the most studied mycotoxin due to its toxicological significance in human and animal diets. OTA is known as nephrotoxic, teratogenic, immunotoxic, and carcinogenic effects in animals. The International Agency for Research on Cancer (IARC) classified OTA 2B carcinogen due to toxicity on rats (European commission, 2010). Crops contaminated with mycotoxins reflect a loss of income for agricultural producers. The strategies that prevent the entry of these metabolites in the food chain protect public health and reduce economic losses caused by contaminated agricultural products (Astoreca et al., 2010). Therefore, the presence of OTA mycotoxin was estimated to reveal the real situation of contamination in Mosul markets.
Methods and Materials

Sampling: 15 samples were selected from each of the dried fruits of apricots, currants and raisins, which are the most contaminated with fungi after culturing 35 samples of each type from the markets of the city of Mosul, the left side, the right side, the district of Tal Afar and the Al-Ayadiyah district. In the period between October and January, Ochratoxin A was detected using a special kit obtained from the company ElabScience-USA.

Reagents: Methanol, Sodium bicarbonate.

Preparing for the experiment: All samples and reagents were re-tempered to room temperature before use, The ELISA device was turned on in order to heat it up, and the experiment parameter was adjusted.

1- Note before starting the experiment: The materials and equipment used in the experiment were clean, single-use pipettes were used to avoid side contamination during the experiment.

2- Preparation of solutions: The solutions were prepared depending on the number of samples selected for the experiment.

- Solution (1): Methanol 70%. Methanol (V): Deionized water (V) = 7:3
- Solution (2): 0.1 of NaHCO₃. 4.2 g of NaHCO₃ was dissolved in 500 ml of deionized water.
- Solution (3): Washing buffer: Concentrated washing buffer was diluted x 20 with deionized water: concentrated washing buffer (V): deionized water (V) = 1:19.

3- Preparing samples before starting the experiment:
   a- The samples were ground with a hand grinder and then placed in a homogenizer for complete mixing.
   b- 2 g of each crushed sample were weighed and then placed in a 50 ml centrifuge tube, and 10 ml of 70% methanol (Solution No. 1) were added to it. The tubes were shaken manually for 5 minutes, then entered the centrifuge at 4000 cycles per minute for 10 minutes at room temperature.
   c- 1 ml of filtrate was taken, and 1 ml of NaHCO₃ (Solution No. 2) was added to it and stirred to mix completely.
   D- 50 μL of each sample was taken for examination and analysis.
Examination: All reagents and samples were returned to room temperature prior to use. All reagents were thoroughly mixed by gently rotating and stirring before sucking. Foam was avoided. The Elisa plate must be at a temperature of 2-8°C before preparing it and during use, and therefore it was placed in the refrigerator until use.

1- Numbering: Standards and samples were numbered in order, then recorded in the standard register attached with the kits, in accordance with the order of the samples in the ELISA plate.

2- Addition: 50 μL of each of the standards and samples were added in the order numbered above in step #1 to etch the ELISA plate, then 50 μL of anti-body solution was added to each hole. Homogenize well, and incubated in the shade at 37°C for 30 minutes.

3- Washing: The cap was carefully removed, then the liquid was removed. Immediately, 300 μL of washing buffer was added (Solution No. 3) to each pit for washing from the washing buffer store. The process was repeated five times, with an interval of 30 seconds for each time. The plate was inverted onto a thick filter paper to dry it and get rid of some of the existing bubbles.

4- HRP conjugate: 100 μL of HRP conjugate was added to each hole, and incubated in the shade at 37°C for 30 minutes.

5- Washing: step no.3 repeated with washing device.

6- Color change: 50 μL of reagent A was added to each hole, then 50 μL of reagent B was added, stirred gently for 5 seconds to mix well, and incubated in the shade at 37°C for 15 minutes.

7- Stop reaction: 50 μL of stop solution was added to each pit, gently stirred to mix well.

8- Optical Density (OD) measurement: The optical density was measured at a wavelength of 450 nm with a micro-plate reader. This step was carried out immediately after the completion of the stop reaction step (ElabScience Ochratoxin ALeaflet).

Results and Discussion: 105 samples of dried fruits (35 samples of raisins, 35 samples of currants and 35 of Apricots) cultured on PDA media for 7 days and 28
C° (Saadullah, A., & K Abdullah, S., 2014) to discover the most polluted samples with Aspergillus sp. and Penicillium sp. which are most productive to mycotoxin Ochratoxin A (Wei et al., 2017). As we know that the normal range of Ochratoxin A in dried fruits is 10 ng/ml (Chebil S. et al., 2020). In view of this, 45 samples were selected, which are the most contaminated with fungi to test the presence of ochratoxin in them.

All 45 samples were positive to OchratoxinA in different concentrations (shown in table 1), the highest value was in one of Apricot samples 11.631 ng/ml then while the lowest value was also in Apricots 0.061 ng/ml. Statistical analysis of the descriptive type was used to clarify:

- The mean of Raisins was 3.391 ng/ml, the mean of Apricots was 4.644 ng/ml and the mean of Currants was 2.263 ng/ml.

- The median of Raisins was 10.105, the Apricots was 8.897 and the currants was 1.009.

The results were recorded and depicted. A standard curve was created by plotting the absorbance ratio for each standard on the y-axis versus the logarithmic concentration on the x-axis to draw a semi-logarithmic plot. The average absorbance value of the sample was added to the standard curve to get the corresponding concentration (Figure 1). (ElabScience OchratoxinA Leaflet).

It was observed from the results that the values of ochratoxin A in apricots were the highest compared with raisins and currants. Where the storage period was longer and used to eat less than raisins and currants, which exposes them to stay in the shops for a longer times. The most contaminated samples were from (Bab Al-Saray Market) on the right side of Mosul and (Nabi Yunus Market) on the left side, where the shops are in narrow and closed places and are more crowded with people and dried fruits are not covered. It was found that the fungi contaminating it was Aspergillus niger which was filling the dishes. The least contaminated samples were in the Al-Ayadiyah district and the Mohandessin district (the left side of Mosul) where the shops are more spacious and clean and the dried fruits are covered and not exposed to the atmosphere. While the dishes were slightly contaminated with Aspergillus and Penicillium, perhaps 1 or 2 out of 10 cultivated fruits.

The results varied from Selouane et al., (2009), they said that the amount of ochratoxin A in the grapes was less than 4 ng/ml. Ochratoxin was less present at Heshmati et al., (2017) they explained that 45.45%, 22.72%, 45.45%, and 50% of mulberry, date, fig and apricot samples,
respectively. Di sanzo et al. (2018) said that they found Ochratoxin A only in 21 samples of dried figs and this is, of course, less than what we found. The results differed from Iamanaka et al. (2005) who didn't find any contamination in Apricots. Bejaoui et al. (2006) said they found Ochratoxin A (OTA) value in grapes up to 2.8 ng/ml and this is less than from our values. Abdallah et al. (2018) mentioned that they found OTA only in 3 samples of dates and the values ranged from 1.48 to 6.070 ng/ml. Aspegillus niger was the main cause of production of OTA (Abarca et al., 2019) which appeared in more than 75% of the samples after culturing them on PDA medium. In Figure (2) we see the curve is turbulent and there is a clear discrepancy in the values due to the severe contamination of the apricot samples as they are sold mainly in the month of Ramadan and after that remain in the stores for long periods. In Figures (3) and (4) we see the curves are more stable than the mentioned curve. This is due to the fact that raisins and currants are sold on a daily basis and do not remain in stores for long periods.

Table 1 : shown OchratoxinA concentrations in some dried fruits.

<table>
<thead>
<tr>
<th>Apricot samples code*</th>
<th>OchratoxinA value</th>
<th>Raisin samples code*</th>
<th>OchratoxinA value</th>
<th>Currants samples code*</th>
<th>OchratoxinA value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>0.576</td>
<td>R2</td>
<td>1.593</td>
<td>C2</td>
<td>0.922</td>
</tr>
<tr>
<td>A3</td>
<td>0.061</td>
<td>R3</td>
<td>0.887</td>
<td>C3</td>
<td>0.727</td>
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<tr>
<td>A5</td>
<td>9.833</td>
<td>R5</td>
<td>0.674</td>
<td>C5</td>
<td>0.631</td>
</tr>
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<td>A8</td>
<td>4.709</td>
<td>R6</td>
<td>1.454</td>
<td>C6</td>
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<tr>
<td>A9</td>
<td>0.296</td>
<td>R7</td>
<td>1.018</td>
<td>C7</td>
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<td>4.439</td>
<td>C10</td>
<td>2.069</td>
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<tr>
<td>A11</td>
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<td>R11</td>
<td>7.278</td>
<td>C11</td>
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<tr>
<td>A12</td>
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<td>R12</td>
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<td>C12</td>
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<td>R14</td>
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<td>R19</td>
<td>0.809</td>
<td>C19</td>
<td>0.437</td>
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<td>A22</td>
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<td>R22</td>
<td>4.700</td>
<td>C20</td>
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<tr>
<td>A24</td>
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<td>R23</td>
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<td>C22</td>
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<tr>
<td>A25</td>
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<td>R25</td>
<td>3.288</td>
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</tr>
<tr>
<td>A28</td>
<td>0.681</td>
<td>R29</td>
<td>1.993</td>
<td>C25</td>
<td>7.727</td>
</tr>
</tbody>
</table>

Figure (1) : Samples Standard curve.

Figure (2) : OchratoxinA value in Apricots.(Horizontal: No. Of samples, Vertical: OTA value)

Figure (3) : OchratoxinA value in Raisins.
Conclusion

This study shed light on the true values of ochratoxin A in dry fruits in the markets of Mosul and its suburbs. The study showed significant contamination with this toxin. There are samples that crossed the international natural ratios, and there is a discrepancy in the ratios between types of dried fruits. Apricots were more contaminated because they were consumed less and stay in storage for a longer period. Poor storage conditions and lack of attention to hygiene will lead to the accumulation of poison in the stored dried fruits, and consequently, their unfitness for consumption.

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References


