Investigation of fungal contamination in some types of chips in the market in Karbala

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Abstract. Different types of chips samples sell were collected from some markets in Karbala city, which its made in Jordan (Chipsico and Alsaada), Saudia (Liz, Gilts, Doritos), Iran (Bovac, G Toz and Macho) and Iraq (Hala, Dalia, Al-Aseel). The Iranian Chips were the most contaminated, where Iraqi and Jordanian Chips comes in the second, The results of isolating fungi study in chips types, showed diversity in numbers and kinds of fungi. The genus Aspergillus is a fungus predominate on all isolates and followed by Penicillium. Also different genera including Fusarium, Alternaria, Curvularia and Rhizopus have been isolated. It is mentioned that these fungi are dangerous because they produce mycotoxins, especially aflatoxin and ochratoxin. It was noted that, the Saudi products are free from contamination. Ammonia test and Fluorescence was used to identify the aflatoxin-producing isolates and it appeared that isolates of Aspergillus had great potential to produce these toxins.

Keywords. Chips, aflatoxin, Aspergillus, Penicillium.

1. Introduction

Fungi are eukaryotic organisms that present in all parts of the earth and are characterized by their great variability in terms of color, size and shape. Fungi it’s very important in our daily life as they are a source of food, antibiotics and some important compounds such as proteins, vitamins, organic acids and others [1]. But it may be harmful, as it spoilage foodstuffs, especially those stored and contaminates them with their lethal toxins. Mycotoxins are among the most dangerous known contaminants at the present time, especially aflatoxins, ochratoxin, ergot, and others [2]. Fungi can reach to foodstuffs in a variety of ways. For example, the foodstuff from which these products are made may be originally contaminated and contamination is transmitted to the manufacturing factory. It has not been sterilized, or contamination comes from the environment. Polluted air and water are an important source of contamination, or it may come from hands workers in these factories, especially if they did not observe the health conditions, such as wearing special clothes, wearing gloves and masks, or the pollution may arise from the materials used in the packaging [4]. What exacerbates and increases the problem is the incorrect storage and transportation conditions. Storage for a long time with suitable humidity helps the growth and prosperity of fungi. These fungi begin to secrete their toxins on the food, among the fungi and yeasts known to be a cause of food spoilage, such as Aspergillus and Penicillium, which were considered the source of toxins and organic acids. Rhizopus
is considered a widespread fungus and is a likely pollutant of foodstuffs, whether it open or packed, as it is characterized by fast growth and resistance to difficult conditions. One of the contaminated yeasts of foodstuffs is *Candida albicans*, a widespread yeast that has been isolated from various sources such as milk, fruits, vegetables and others. Food products are considered very suitable medium for growth of fungi because they contain sugars, proteins and salts supporting the requirements of growth, they are either made from grains such as wheat and barley grains as in nestles or from agricultural crops such as potatoes, peppers and strawberries as in chips, ice cream add to it other additives, flavors, preservatives, salts, spices and other substances that add desired taste to the food product [5]. Chips are among the most famous and most popular food demand for it by children and even adults as they are a fast and light meal and do not need preparation and cheap price and there are different forms, types and flavors, some of which are made from potatoes, grains, crops and fruits, and there is no house is almost empty without this food product. Therefore, it is necessary to avoid contaminated types and not consume them, as they may be the cause of many health problems, including toxins, fungal spores, yeast cells and bacteria [6]. The local markets in Karbala city are filled with various local and imported food products and the demand for which has increased recently, these include canned food, chips, juices and other. There has been a great openness in the field of manufacturing and importing these foodstuffs and in some cases the absence of control and follow-up on these products which causes their entry into the markets causing contaminated it with fungi [3]. The aim of the study was to investigate the fungi transmitted by chips and to know which companies and brands are best regarding fungi contamination and comparing local origin with imported ones.

2. Materials and Methods

2.1. Sample collection

The current study included the collection of different samples of chips that existing in the markets of Karbala city randomly, which included all the prevailing brands in the markets from local and imported origins, which are Jordanian (Chipsico and Alsaada), Saudian (Liz, Gillts, Doritos), Iranian (Bovac, G Toz and Macho) and Iraqi (Hala, Dalia, Al-Aseel), samples were transported as quickly as possible to the laboratory for cultivation on the prepared culture medium.

2.2. Culture media

Three culture media were used as the following:

2.2.1. *Potato Dextrose Agar (PDA)*

Use for growth of sample in food and dairy samples [7].

2.2.2. *Cocconut Extract Agar (CEA)*

Use the medium for Detection of aflatoxins [8].

2.2.3. *Yeast Extract Agar*

It is highly nutritive medium recommended for plate count of microorganisms [9].

2.3. Fungi isolation

Small pieces of each brand of sample were taken with forceps and placed in Petri dishes, and with three replicates per sample, the dishes were transferred to the incubator at 28 degrees while group of
control, and all dishes were incubated for 7 days with daily examination until appearance of growth [9].

2.4. Purification and Diagnosis

The growing cultures were purified by re-cultivation them on (PDA) medium until obtaining pure cultures. The cultures were examined for the purpose of diagnosis. The diagnosis was according to the shapes, color, nature of the cultures growth, edges and height, as well as examining the cultures under a microscope to observe the hyphae and the structural and reproductive structures of the fungi under study and were compared with the classification keys of fungi to determine fungal species [9].

2.5. Detection of aflatoxins

To detect the ability of isolated fungi to produce mycotoxins, the following two methods were used:

2.5.1. Ammonia solution

For this method a single colony is grown in the middle of a petri dish containing a medium like PDA. Turn over the plate and one or two drop of concentrated ammonium hydroxide solution is placed inside lid. The undersides colonies that produce aflatoxin quickly turn plum to red after the bottom of the Petri dish are inverted over the lid containing ammonium -hydroxide. Basically, if there is no change in color of colonies that’s means do not produce of aflatoxin. Note the greatest color change in the colonies grown on the yeast extract - sucrose and coconut media, a less intense color change in PDA , and the slightest change in color in the media of glucose salts - minerals, all of which prefer the production of aflatoxin [10].

2.5.2. Fluorescence

The isolates of fungi were grown on PDA medium by placing 3(replicates) for each fungal isolate after which it was incubated at a temperature of 25 °C ± 2 °C for a period a week later, a dish was selected for each isolation, and the cultured medium was cut on it by isolating the fungus with a sterile knife in the form of small pieces. Then the pieces were transferred with a sterile needle to an electric mixer containing 50 ml of chloroform and the mixture was mixed for 3 minutes and the mixture was filtered by filter paper. The filtrate sample was taken and put in a clean and sterile beaker and placed in an electric oven at a temperature of 60 ° C, where the amount was concentrated to approximately (1) ml only. The presence of aflatoxin B1 was detected, then it was examined under ultraviolet rays with a wavelength of 360 nanometers. The presence of aflatoxin B1 was detected by matching transfer coefficient (Rf) and fluorescence color of the extracts content of aflatoxins with Standard Substance Dorner and AFB1 [11].

3. Results and Discussion

The results of isolation in Table (1) existence variations in the numbers and genera of isolated fungi. The isolated fungi predominate the fungus Aspergillus, followed by Penicillium fungus. Also Fusarium, Alternaria, Curvularia and Monillia fungi have been isolated. It is mentioned that these fungi are dangerous because they are producing toxins, especially the Aflatoxins and ochratoxins [12]. Aspergillus is one of the moulds found all over the world. Its ability to contaminate food and animal feed is widespread under favourable environmental conditions. It is a large proportion of all molds found in industrial foods. It has a special importance as spoilage organisms of food. The changes due to spoilage caused by the Aspergillus species can be sensorial, nutritional and qualitative nature such as discoloration, pigmentation and moldy development of odors and destinations. Many species grow in very low water activity and are found attacking different foods and producing mycotoxins.
Aspergillus niger is distributed worldwide on a large variety of substrates, which are the most common types of Aspergillus responsible for post-harvest decay [14]. Penicillium is another group of molds with a high appearance. The occurrence of Penicillium in some samples during this investigation is consistent with the. He reported that there is a high percentage of Penicillium types of fodder obtained from the farm with the Penicillium citrinum with the highest incidence. Many types of Penicillium can also produce a wide range of toxic compounds such as Citrine and Citroveridin [15]. [16] noted that the presence in food products of some of these molds, especially Aspergillus flavus and Aspergillus niger, is highly undesirable. Some of it reported to have public health significance because of the production of mycotoxins, which have implication on consumers’ health and food shelf-life decreasing. This is especially prevalent in developing countries, Aspergillus flavus and Aspergillus parasiticus are mainly responsible for mycotoxin production [17].

Table 1. Isolated fungi from Some type of chips.

| Origin  | brand      | fungi                  | percentage of appearance |
|---------|------------|------------------------|--------------------------|
| Iranian | Bovac      | Penicillium digitatum  | 9                        |
|         |            | Aspergillus flavus     | 7                        |
|         |            | Curvularia platzi      | 1                        |
|         | G Toze     | Rhizopus stolonifer    | 1                        |
|         | Masho      | Aspergillus flavus     | 6                        |
|         |            | Penicillium digitatum  | 3                        |
|         |            | Fusarium sp.           | 1                        |
|         |            | Alternaria alternata   | 2                        |
|         |            | Total                  | 12                       |
| Jordanian | Chipsico  | Aspergillus niger      | 3                        |
|         | Alsaada    | Penicillium digitatum  | 1                        |
| Saudian | Liz        | -                      | 0                        |
|         | Gilles     | -                      | 0                        |
|         | Doritos    | -                      | 0                        |
| Iraqi   | Hala       | Aspergillus flavus     | 2                        |
|         |            | Penicillium sp.        | 1                        |
|         | Dalia      | Fusarium sp.           | 1                        |
|         |            | Penicillium sp.        | 2                        |
|         | Al-Aseel   | Aspergillus niger      | 6                        |

The Iranian chips types were find the most contaminated samples and Iraqi chips types came second followed by Jordanian chips and finally Saudian chips without any fungal contamination. This may be due to the fact that the Iranian chips remain in the stores for long periods, not to mention the time needed to reach Iraq, as well as the machines used in production from the old generations due to the blockade imposed on the Islamic Republic, while notes that Saudi products are free from contamination. The reason for this may be due to adopting modern methods of production and marketing and benefiting from foreign expertise.
Figure 1. *Curvularia platzi*. 

Figure 2. *Alternaria* sp.

Figure 3. *Penicillium* 

Figure 4. *Aspergillus*.

Figure 5. Aflatoxin production on (CEA). 

Figure 6. Aflatoxin production on (CEA).

Figure 7. Aflatoxin detection under (UV). 

Figure 8. Aflatoxin detection under (UV).
### Table 2. Aflatoxin production by fungal isolates on different media.

| Chips samples | Fungal isolates | Fungal toxins secreted on three media |
|---------------|-----------------|--------------------------------------|
|               |                 | PDA   | CEA    | Yeast  |
| Bovac         | *Penicillium digitatum* | -     | ++     | ++     |
|               | *Aspergillus flavus*   |        | ++     | ++     |
|               | *Curvularia lunata*    | +++    | +      | +++    |
| G Toze        | *Rhizopus stolonifer*  | ++     | +      | -      |
| Masho         | *Aspergillus flavus*   | ++     | +++    | +++    |
|               | *Penicillium digitatum*| -      | ++     | ++     |
|               | *Fusarium* sp.         | +      | +      | -      |
| Chipsico      | *Aspergillus niger*    | -      | +++    | +++    |
| Alsaada       | *Penicillium digitatum*| -      | +      | +      |
| Hala          | *Aspergillus flavus*   | +      | +++    | +++    |
|               | *Penicillium* sp.      | -      | ++     | +      |
| Dalia         | *Fusarium* sp.         | +      | +      | ++     |
|               | *Penicillium* sp.      | -      | +      | ++     |
| Al-Aseel      | *Aspergillus niger*    | -      | ++     | +++    |

+++ fungal production of aflatoxin (very sparkling)
++  fungal production of aflatoxin (medium sparkling)
+   fungal production of aflatoxin (weak sparkling)
- Non-toxic

The Table (3) showed the ability of fungal isolates to produce toxins on three different media (PDA, CEA and Yeast) as the results showed great susceptibility of both *Aspergillus* and *Penicillium* to produce aflatoxin followed by other fungal species, the culture medium yeast was the suitable medium that stimulate fungi to produce toxins followed by CEA medium and finally PDA medium.

### 4. Conclusion

According to this study we conclude that these different types of chips are the same as the foodstuffs be exposed to contamination with various contaminants and therefore have a great impact on the health of human who deals with these foodstuffs, and the possibility of producing various types of toxins, therefore we recommend the need to use modern technologies in the production of foodstuffs such as chips and not to import them from poor and cheap sources.

### 5. References

[1] Al-Khalaf SS 2011 *Study of the toxicological effects of aflatoxin B1 and B2 in some pathological, biochemical and histological parameters of male white rat and ways to reduce their effects* Master Thesis, College of Science, University of Kufa.

[2] Al-Fatlawi IA-W A-R 2014 *A molecular study of some fungi producing aflatoxins isolated from some types of nuts* Master Thesis, College of Science for Girls, University of Babylon.

[3] Qahtan F and Abdullah A 2002 *Detection of Avala B1, B2 toxins, orca A in yellow corn and some of its products* Master Thesis, College of Agriculture, University of Baghdad.
[4] Pitt JI and Hocking AD 2009 *Fungi and food spoilage* 3rd Edition Springer Science and Business Media, London, New York.

[5] Aziz NH and Moussa LAA 2002 Influence gamma radiation on mycotoxin producing molds and mycotoxins in fruits *Food Control* 13 281.

[6] Shaker R J, Thalaj MM and Bedewi AS 2012 Isolation and diagnosis of molds producing mycotoxins from the most consumed foods in Iraqi markets *Tikrit Univ. J.* 13 44.

[7] Majid K A and Ihsan AA 2019 Survey of fungi found in books on the shelves of the libraries of the University of Qadisiyah - Iraq. *IOP Conf. Series: Materials Science and Engineering* 571 012042.

[8] Saito M and Machida S 1999 Arapid identification method for aflatoxin producing strains *A. flavus* and *A. parasiticus* by ammonia vapor. *Mycoscience* 40 205.

[9] Watanabe T 2002 *Pictorial atlas of soil and seed fungi morphologies of cultured fungi and key to species* 2nd edition. CRC press Washington.

[10] Akbas M and Ozdemir M 2006 Effect of different ozone treatments on aflatoxin degradation and hysicochemical properties of pistachios *J. Sci. Food Agric.* 86 2099.

[11] Sobolev VS and Dorner JW 2002 Clean up procedure for determination of aflatoxins in major agricultural commodities by liquid chromatography *J. Assoc. Offic. Analyt. Chem. Int.* 85 642.

[12] Hong LS, Yusof, NIM and Ling H 2010 Determination of aflatoxins B1 and B2 in peanuts and corn based products *Sains Malaysiana* 39 731.

[13] Adejumo TO and Adejoro DO 2014 Incidence of aflatoxins, fumonisins, trichothecenes and ochratoxins in Nigerian foods and possible intervention strategies *Food Sci. Qual. Manag.* 31 127.

[14] Ashiq S 2015 Natural occurrence of mycotoxins in food and feed: Pakistan perspective. *Comprehensive. Rev. Food Sci. Food Safety* 14 159.

[15] Barbosa TS, Pereyra CM, Soleiro CA, Dias EO, Oliveira AA, Keller KM and Rosa CAR 2013 Mycobiota and mycotoxins present in finished fish feeds from farms in the Rio de Janeiro State. *Brazil. Int. Aquat. Res.* 5 1.

[16] Adegoke GO 2004 *Understanding Food Microbiology* 2nd ed.; Shalom Press: Ibadan, Nigeria.

[17] Bankole S, Shollenberg M and Drochner W 2006 Mycotoxins in food systems in Sub Saharan Africa: A review *Mycotoxin Res.* 22 163.