Isolation and Identification of Fungi Contaminating Potato Chips Intended for Children's Consumption and Assessing their Toxins



Hadeel A. AL-Ameri* and Nadeem A. Ramadan

Department of Biology, Faculty of Sciences, Mosul University-Iraq

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Abstract: The study aims to investigate the presence of fungi and their toxins in different samples of potato chips imported from different origins. Fifteen chips' samples were collected from the local markets of Mosul city/Iraq which included various global origins with several flavors of pepper, paprika, hot spices, and cheese. It appears that all potato chips' samples were contaminated with fungi and mycotoxins. It was evident that *Penicillium* spp. were the most predominant fungi followed by Aspergillus spp. and Rhodotorella spp. came third, while Geotrichumspp and yeasts came in fourth. Potato chips from the brand Pringles was contaminated with *Penicillium* spp., and Geotrichum spp. at a percentage of 50, 30% respectively. The Hum Hum brand samples were contaminated with A.terrus and Penicillium spp. at 40, 30% respectively. Dream brand samples were contaminated with A.jamanicum., and Penicillium., at percentages of 30, 60% respectively. Lays 1 with tomato ketchup samples were contaminated with four genera: Penicillium spp., Mucor spp., Rhodotorellaspp., and yeast with percentages of 40, 20, 10, and 10% respectively. Lays2 with French cheese variety was contaminated with the same fungi of lays2 type but with the addition of Aspergilli (A. versicolor and A. niger), which were 60 and 30% respectively. The Patos brand potato chips were contaminated with two Aspergilli (A. astus and A. jamanicum), at 30 and 40% respectively. Zearalenone was found to be the highest contaminant (13.81ppm) of mycotoxins followed by aflatoxins (0.26ppm). Ochratoxin was the least contaminant (0.16ppm) in the analyzed potato chips. It can be concluded that all tested potato chips' samples showed the presence of fungi and mycotoxins. However, all mycotoxins (aflatoxin, ochratoxin, and zearalenone) in the food commodities were within the permissible limits intended for human consumption.

Keywords: Contaminated Chips, Mycotoxins, Aflatoxins, Zearalenone, Ochratoxin.

INTRODUCTION

Food contamination is a major problem, and contaminants vary with different food commodities. Fungi are regarded as one of the most persistent food contaminants, having the ability to produce toxins known as mycotoxin under favorable conditions. These toxins may lead to serious diseases called mycotoxicosis (Manjula et al., 2016). More than 300 fungal toxins have been discovered that are produced by different fungal species (Tairo et al., 2008). The most significant fungi agriculturally are *Aspergillus, Penicillium*, and Fusarium, which can produce toxins

such as aflatoxin, ochratoxin, zearalenone, and trichothecenes (Martins et al., 2001; Milani et al., 2013). The preservation of processed foods from fungal contamination may be achieved by the incorporation of food additives (Jonathan et al., 2012).

Food additives, such as antifungal agents, are chemicals that are usually added to processed foods in order to preserve them from undesirable changes in their color, flavor, or nutritional values as a result of fungal growth (Manjula et al., 2016).

Antifungal agents are usually prepared from

natural sources, plants, or produced chemically (Mmasa et al., 2012). Preferably, they are prepared from natural sources including, spices and flavor materials.

Aflatoxins are secondary metabolites produced by strains of *Aspergillusflavus*, *A. parasiticus*, and *A. nomius*. These metabolites are furocoumarins, including AFB1, AFB2, AFG1, and AFG2 (Amri & Lenoi, 2016). Aflatoxins are fully soluble in solvents such as methanol and chloroform. The presence of a lactone ring in aflatoxin molecules makes them more susceptible to hydrolysis by alkaline solutions (Azizi et al., 2012; Bankole & Adebanjo, 2003).

In humans and animals, aflatoxins affect different cell wall tissues. inhibit DNA function in synthesis, and act as immunosuppressive agents. Aflatoxins are a serious fungal toxin responsible for the contamination of food commodities and the emergence of liver cancer in humans which poses a significant threat to human health (Croft et al., 1986; Wyllie Morehouse, 1978).

The World Health Organization (1979) has identified the permissible limits of aflatoxin in adult foods to be not more than 20 ppb and 0.5 ppb in milk, whereas it should be 20 ppb in feeds. However for baby food, there are no limits allowed Smith and Moss, 1985 U.S. Ochratoxins are a group of mycotoxins produced by several species of *Aspergillus*, especially *A. ochraceous* and some species of *Penicillium* (Gnonlonfin et al., 2012).

They are colorless, crystalline in texture, with blue fluorescence usually emitted from them when exposed to ultraviolet rays. These toxins dissolve in acidic solutions, with moderate solubility in methanol and chloroform. OTAs have the ability to bind to serum albumin. OTAs could cause intestinal ulcers, affect the liver and reduce its efficiency, and affect kidneys, and cause intestinal

disorders. Zaralennon is another mycotoxin and is one of the phenolic compounds with estrogenic characteristics (Savino et al., 2007). It is a crystalline compound, white in color, and glows with blue-green fluorescence usually emitted from this toxin when exposed to wide UV long-waves. Its discovery was in 1916 when symptoms of vomiting and poisoning were described in humans due to the consumption of bread made of wheat infected by *Fusarium*, (especially *F. graminearum*) (Bullerman & Bianchini, 2007).

This study aimed to identify, isolate, and investigate the presence of fungi in different samples of potato chips imported from different origins, then estimate some mycotoxins in these products.

MATERIALS AND METHODS

Sample collection: Fifteen samples of potato chips were collected from the local markets of Mosul city, which included various global origins with different flavors (pepper, paprika, hot spices, and cheese: Table 1).

Isolation of fungi: One gram from every of potato chips' sample was grounded and transferred to glass bottles containing 9 ml of sterilized distilled water, mixed with a stirrer, and left to settle down. Subsequently, 1 ml of the mixture was streaked on three plates of PDA for each Sample. Plates were incubated at 28°C for one to two weeks. Plates were then examined for the developing fungal colonies and diagnosed according to the approved diagnostic keys (de Hoog & Guarro, 1995; Pitt & Hocking, 2007)

Diagnosis of *Aspergillus* **species**: Diagnosis was based on the growth on different media and under different temperatures according to (Pitt & Hocking, 2007) by using three types of media for diagnosis, Czapek Yeast-extract Agar (CYA), Malt Extract Agar (MEA), and Glycerol (25%) Nitrate agar (G25N)

Table (1) Types of Potato Chips, Country of origin and flavor additive.

Sample No.	Flavor additive	Manufacturing Country	Product name
1	Hot&Spicy	Belgium	Pringles
2	HotPepper	Syria	HumHum
3	Ketchup	Iran	MazMaz
4	HotFlavor	Turkey	Patos
5	HotPepper	SaudiArabia	Dream
6	HotFlavor	Turkey	Cipso
7	Paprika	Jordan	SnackMix
8	HotPepper	Turkey	Doritos
9	Cheese And Onion	Kuwait	Fico Fresh
10	Tomato ketchup	SaudiArabia	Lays1
11	French Cheese	SaudiArabia	Lays2
12	Cheese	Turkey	Patos
13	Ketchup	Syria	Lux
14	Cheese	Syria	Boshr
15	Garnish	Syria	Mamito

Isolates of the Aspergillus species were diagnosed depending on the morphological cultural characteristics and its image under the microscope by plating them on PDA and incubating at 28 ° C for seven days. Culturing was done by using a sharp cork borer to transfer part of the edge of the colony under septic conditions and placing them in the center of three agars mentioned above, with three replicates of each fungal isolate. Agars were incubated in an inverted state under three temperatures, 5° C, 25 ° C, and 37 ° C for seven days. Species of the Aspergillus genus were diagnosed through their form of growth, colony colour, and colony diameter depending on the taxonomic key of the Aspergillus species (Pitt & Hocking, 2007).

Mycotoxins analyses of potato chips: Before opening potato chip packages, sterilization of outer covering was done with 70% alcohol. Twenty grams of potato chips samples were grounded, placed in plastic bags, and subjected to extraction for mycotoxins analyses. Aflatoxin, ochratoxin, and zearalenone, were analysed using enzyme-linked immunosorbent assay (ELISA) at the veterinary laboratory in the province of Erbil.

Method of mycotoxins extraction:

Five grams of grounded potato chips samples were transferred to conical flasks. To these samples, 25 ml of 70% methanol was added when aflatoxin and zearalenone were extracted, and 50% was used when ochratoxin was extracted. Samples were shaken for three minutes then filtered using Whatman No.1 filter papers. 5 ml of the filtrate was transferred to a test tube. However, in the case of zearalenone, 1 ml of the filtrate was diluted four times with sterile distilled water (1:4). All samples were placed in the refrigerator until analysis.

ELISA technique was used for samples analyses using Neogene Kits and as follows: 100 µl of samples and control were pipetted in the red plate wells followed by 100 µl of Enzyme conjugate, mixed two to three times for homogenization. Mixtures were transferred to white wells in another plate and left for 10-20 seconds at room temperature. A plate was washed with distilled water five times. Then 100 µl of the substrate were added and mixed for 10-20 seconds and left for 3 minutes at room temperature. 100µl of stop solution was finally added. Results were obtained using Neogen Vertex software Vera tax ELISA Reader.

RESULTS

As illustrated in Table 2, it appears that all potato chips' samples were contaminated with fungi and mycotoxins. Pringles potato chips brand was contaminated with the following fungi; A. niger, A. astus, Penicillium spp., Geotrichum spp., and Chaetomium spp., at a percentage of 20, 30, 50, 30, and 10% respectively. Hum Hum potato chips were contaminated with three fungi: A. terrus, A. niger, and Penicillium spp. at 40, 20, and 30% respectively. Three fungi contaminated the MazMaz brand samples and included Penicilliumspp., Geotrichumspp, and Rhodotorellaspp. in descending percentages of 30, 20, and 10% respectively. Potato chips were also contaminated with three fungi; Penicilliumspp., Geotrichum spp. and Mucor spp., at 40, 20, and 10% respectively.

The Dream brand of potato chips was contaminated with A. jamanicum., Penicillium, Geotrichum spp., and Chaetomium spp. at percentages of 30, 60, 30, and 10% respectively. The same fungi were isolated from the Dream and Cipso brands except for Aspergillus, which was A. flavus, and these fungi were in the following percentages 50, 70, 40, and 10% in the same order respectively. Two species of Aspergilli, A. flavusand A. parasiticus were isolated from Snack Mix potato chips at a percentage of 80 and 60%, in addition to Penicillium spp. and Geotrichum spp. at a rate of 60 and 10% respectively.

The same Aspergillus spp. of Snack Mix was also contaminating samples from Doritos potato chips in addition to A. niger at a rate of 70, 70, and 30%. Penicillium was also isolated from the chips brand at a percentage of 20%. Five fungi were isolated from Fico Fresh potato chips brand including A. niger, A.astus, Penicillium spp., Rhizopus spp., and Trichodermaspp, at a rate of 30, 40, 40, 10, and 20% respectively. Lays with tomato ketchup was contaminated with three fungi namely Penicillium spp., Mucor spp., Rhodotorellaspp., and yeast with percentages of 40, 20, 10, and 10% respectively (Table 2).

Lays2 with French cheese potato chips were found to be contaminated with the same fungi of the previous Lays type, but with the addition of Aspergilli (A. versicolor and A. niger), which were 60 and 30% respectively. The Patos brand chips were contaminated with two Aspergilli (A. astus and A. jamanicum), at 30 and 40% respectively, and 20% for Penicillium, Rhodotorella spp. and yeasts.

The Lux type of potato chips was also contaminated with two Aspergilli (*A. astus and A. niger*) at 20 and 10%, with *Penicilli-um*10%, and Yeasts 10% (Table 2).

Table (2) Percentage of fungi isolated from chips.

Product No.	Product name	Fungi	% of Isolation
1	Pringles	Aspergillusniger	20
		A.astus	30
		Penicillium spp.	50
		Geotrichum spp.	30
		Chaetomium spp.	10
2	Hum Hum	A. terrus	40
		Aspergillusniger	20
		Penicillium spp.	30
3	MazMaz	Penicillium spp.	30
		Geotrichum spp.	20
		Rhodotorella spp.	10
4	Patos	Penicillium spp.	40
		Geotrichum spp.	20
		Mucor spp.	10
5	Dream	A.Jamanicum	30
		Penicillium spp.	60
		Geotrichum spp.	30
		Chaetomium spp.	10
6	Cipso	A.flavus	50
v	1	Penicillium spp.	70
		Geotrichum spp.	40
		Chaetomium spp.	10
7	Snack Mix	A.flavus	80
,		A.parasiticus	60
		Penicillium spp.	60
		Geotrichum spp.	10
8	Doritos	A.flavus	70
		A.parasiticus	70
		Aspergillusniger	30
		Penicillium spp.	20
9	Fico Fresh	A.astus	30
	110011000	Aspergillusniger	40
		Penicillium spp.	40

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Product No.	Product name	Fungi	% of Isolation
		Rhizopus spp.	10
		Trichoderma spp.	20
10	Lays 1	Penicillium spp.	40
		Mucor spp.	20
		Rhodotorella spp.	10
		Yeasts	10
11	Lays 2	A.versicolor	60
	·	A.niger	30
		Mucor spp.	20
		Penicillium spp.	30
		Rhodotorella spp.	20
12	Patos	A.astus	40
		A.Jamanicum	30
		Penicillium spp.	20
		Rhodotorella spp.	20
		Yeasts	20
13	Lux	A.astus	20
		A.niger	10
		Penicillium spp.	10
		Yeasts	10
14	Boshar	A.candidus	20
		A.niger	10
		Penicillium spp.	20
		Mucor spp.	20
		Rhodotorella spp.	30
		Yeasts	40
15	Mamito	A.parasiticus	20
		Penicillium spp.	30
		Geotrichum spp.	10
		Rhodotorella spp.	20
		Yeasts	30

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Five species of molds and yeasts were contaminating Bushar potato chips brand; A. candidus (20%), A.niger (10%), Penicillium (20%), Mucor spp. (20%), Rhodotorella spp. (30%), and yeast (40%). the Mamito chips were contaminated with four species of fungi and yeast which were, A. parasiticus (20%), Penicillium spp. (30%), Geotrichum spp. (10%), Rhodotorella spp. (20%), and yeasts (30%). From figure (1) it is evident that Penicilliumspp. was the most predominant fungi which contaminated different potato chips' brands (27.45%), followed by Aspergillus spp. (23.52%). In third place was *Rhodotorel*la spp. (13.72%), while in fourth place was Geotrichumspp. and yeasts (9.8%). Chaetomiumspp was in fifth place being 7.84%. Mucorspp and Trichodermaspp. were the least isolated contaminant of different chips' types which accounted for 5.88 and 1.96% respectively.

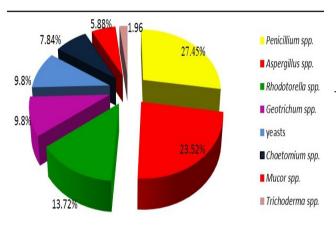


Figure (1): Percentages of different fungi and yeasts isolated from different potato chips' brands.

The amount of mycotoxins in chips' samples was determined in this study. Table (3) shows that all of the chips' types were contaminated with aflatoxin with a percentage ranging from 0.1 to 2.5 ppm. While Patos, Dream, Cipso, Snack Mix, Doritos, Fico Fresh, in addition to Mamito, had no aflatoxin contamination. Percentages of ochratoxin were between 0.1 and 0.5 ppm. While Pringles, Maz Maz, Patos, Doritos, Fico Fresh,

and Lay's 2 had no ochratoxin contamination. Potato chips' types contaminated with zearalenone had percentages ranging from 4.5 to 46.4 ppm. However, MazMaz, Fico Fresh, and Lay's 1 showed no zearalenone contamination. Finally, Boshar potato chips were the most contaminated samples with three mycotoxins: aflatoxin, ochratoxin, and zearalenone, with percentages of 2.6, 0.5, and 33.2 ppm respectively.

It appears that zearalenone was the most abundant contaminant (13.81 ppm) among the three examined mycotoxins (Fig, 2), followed by aflatoxin (0.26ppm), whereas ochratoxin, was the least contaminant (0.16ppm) in the tested potato chips.

Table (3) Occurrences of mycotoxins in potato chips' samples.

		Mycotoxins		
Sample No.	Product	Aflatoxin ppm	Ochratoxin ppm	Zearalenone ppm
1	Pringles	0.3	0.0	6.5
2	Hum Hum	0.3	0.4	4.5
3	MazMaz	0.2	0.0	0.0
4	Patos	0.0	0.0	14.7
5	Dream	0.0	0.4	7.9
6	Cipso	0.0	0.1	10.2
7	Snack Mix	0.0	0.5	11.2
8	Doritos	0.0	0.0	19.9
9	Fico Fresh	0.0	0.0	0.0
10	Lays1	0.1	0.2	0.0
11	Lays2	0.1	0.0	12.1
12	Patos	0.0	0.1	26.4
13	Lux	0.3	0.1	13.2
14	Boshar	2.6	0.5	33.2
15	Mamito	0.0	0.4	46.4
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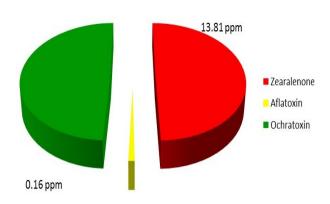


Figure (2): Mycotoxins in analyzed potato chips.

DISCUSSION

When different foods are contaminated by various fungi, producing and non-producing mycotoxins depends on the fungi itself, the type of food product, and the surrounding environment (Milani et al., 2013). Despite the difficulty of the final elimination of pollution, molds, and the presence of mycotoxins, hard work is needed to minimize the adverse effects of these toxins by using the best way possible, whether physical or chemical (Do & Choi, 2007).

In the presence of such fungal mycotoxins in different imported brands of potato chips, there is evidence that Iraqi authorities have failed to conduct an inspection and standardization and quality control measures. Fortunately, the contamination of various brands is located within the limits of 20 ppb, based on the rates approved by AOAC. The presence of this low percentage could be due to the physical separation of the good potatoes from those contaminated before the preparation of potato chips. This may remove 40-80% of the aflatoxin that is considered to be the most toxic and potent carcinogen which has been directly correlated to adverse health effects, such as liver cancer. Aflatoxins have acute and chronic toxicity produced mainly by Aspergillus parasiticus and Aspergillus flavus in tropical and subtropical regions (Milani et al., 2013). This may be due simply to the contamination of food additives and pepper, which is often contaminated with *Aspergillus*, or because of the composition of potato chips, consisting of a mixture of corn, rice, flour, and potatoes, as there may be a source of pollution or fungal toxins within these components. Also, heat treatment and autoclave treatment do not completely remove aflatoxin from foods (Thieu et al., 2008), despite the fact that some studies have shown that roasting is a good way to reduce levels of aflatoxin in peanuts.

The significance of Aspergillus fungi in food comes from its toxic, mutagenic, and carcinogenic effects. It has been ranked by the International Agency for Research on Cancer as one of the first-class carcinogens, and there are studies on liver poisoning in Kenya and India, that showed that viral hepatitis could increase the likelihood of cancer due to the presence of ochratoxin residues in samples of potato chips that have been studied and produced by fungi Asperdillus and Penicillium. Fortunately, the level of ochratoxin was within the limits allowed in food (20 ppb) and not more than 5 ppb per kg of body weight per day.

According to data provided by the European Commission, the daily consumption of ochratoxin ranges from 0.02-1.9 ppb /kg body weight /day (Christensen et al., 1977).

It is interesting to note that some types of potato chips contained moderate levels of zearalenonem despite that no *Fusarium* spp. were isolated from the tested chips. Such results may appear confusing, but it could be said that *Fusarium* spp. are the most fungal species present in the processing methods for chips' preparation, while the other species of fungi like *Aspergillus* and *Penicillium* spp. were present during the storage of these food products. The presence of zearalenone residues in potato chips may be due to its high stability against different processing methods, Different concentrations of zearalenone were found in all tested potato chips (both in

temperate areas like Belgium, or subtropical areas such as Saudi Arabia), the worldwide incidences of *Fusariumgraminearum* and zearalenone produced by this fungus have been well documented (Bahrami-Samani et al., 2017).

As potato chips are considered as a light snack the current study's results were near to the average zearalenone level of 20 ppb of breakfast cereals, snack foods, popcorn, and cornmeal, in the U.S.A. (warner). The hypothesis stated that potato chips may be made of a mixture of rice, wheat, corn, and other ingredients, which may explain here the presence of zearalenone, which is frequently found in all major cereal grains worldwide, also, ubiquity of *Fusarium* spores (Nelson et al., 1983).

F.graminearum (the producer of zearalenone) a soil inhabiter, it is also considered as a storage fungus, since growth and toxin production may occur under various storage condition. Corn and wheat are most susceptible to invasion by this fungus (Bahrami-Samani et al., 2017).

Efforts have been made to reduce the level of zearalenone by various chemical, physical, and biological processing methods. In this study, potatoes were subjected to physical methods of heat treatment. The fate of zearalenone depends on its distribution in the food matrix and its chemical properties, such as heat stability (Krnjaja et al., 2013).

Although all the tested residual mycotoxins (aflatoxin, ochratoxin, and zearalenone) were within the permissible limits of these toxins in the food commodities intended for human consumption, however the new legislation indicates that products intended for human consumption, or as an ingredient in food must comply with a limit of 4 ppb for total aflatoxin, ochratoxin and zearalenone.(Park & Stoloff, 1989).

Prevention of mycotoxins formation is believed to be the best means of managing hazards associated with mycotoxins contamination. In addition, an effective food safety management program must include prevention, setting regulatory limits, the establishment of monitoring programs, control through good agricultural practices, control through processing, decontamination through specific treatments, and consumer and producer education (Park & Stoloff, 1989).

CONCLUSION

Based on the results, it appears that all potato chips' samples were contaminated with fungi and mycotoxins, especially *Penicillium* spp. and *Aspergillus* spp. in addition to some other fungi. The study also concluded that zearalenone was the highest contaminant (13.81 ppm) among the three examined mycotoxins, followed by aflatoxin (0.26ppm). Whereas ochratoxin was the least contaminant (0.16ppm) in the analyzed potato chips.

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عزل وتعريف الفطريات الملوثة لرقائق البطاطس المخصصة للأطفال وتقدير السموم الفطرية الموجودة فيها

هديل احمد العامري ونديم احمد رمضان قسم علوم الحياة، كلية العلوم، جامعة الموصل، العراق

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المستخلص: تهدف الدراسة الحالية إلى التحري عن وجود الفطريات في عينات مختلفة من رقائق البطاطس المستوردة من مناشىء مختلفة. وتضمنت الدراسة جمع 15 عينة من رقائق البطاطس من الأسواق المحلية لمدينة الموصل العراق من مصادر عالمية مختلفة بنكهات مختلفة من الفلفل والبابريكا والتوابل الحارة والجبن. تبين أن عينات رقائق البطاطس جميعها كانت ملوثة بالفطريات والسموم الفطرية، وبينت الدراسة أن أنواع فطر ..Penicillium spp كانت الأكثر انتشارًا ثم أنواع الفطر .Aspergillus spp وفي المرتبة الثالثة أنواع خميرة .Rhodotorella spp ثم جاءت أنواع .Aspergillus spp وبقية الخمائر. رقائق البطاطس من نوع Pringles كان ملوثا بالفطريات.Penicillium spp. ، Penicillium spp. بنسبة 50، 30٪ على التوالي. تلوث عينات رقائق البطاطس Hum Hum بـ A.terrus و 30، 30٪ على التوالي. تلوث عينات رقائق و 30٪ على التوالي. أظهرت عينات رقائق البطاطس من نوع Dream بنسب Penicillium. و 30٪ على التوالي. أظهرت مئوية 30، 60٪ على التوالي. تلوثت عينات رقائق البطاطس Lays1 بنكهة الطماطم بأربعة أجناس هي Penicillium .spp. و Mucor spp. والخميرة بنسب 40 و 20 و 10 / على التوالي. وتلوث رقائق Rhodotorella spp. والخميرة بنسب 40 و 20 و 10 / على التوالي. وتلوث رقائق البطاطس نوع Lays2 بالجبنة الفرنسية بالفطريات نفسها للنوع السابق فضلا عن أجناس A. versicolor و A. Niger بنسبة 60 و 30٪. تلوث رقائق البطاطسPatos باثنين من الفطريات وهي A.astus و بنسبة 30 بنسبة 30 بنسبة 30 و 40٪ على التوالي. أظهرت النتائج وجود السموم الفطرية في عينات رقائق البطاطس وأن سم zearalenone الملوث الأعلى وبنسبة 13.81 جزء في المليون، يليها الأفلاتوكسين (0.26 جزء في المليون)، والأوكراتوكسين كان الاقل نسبة بين العينات (0.16 جزء في المليون). من الدراسة يمكن استتتاج أن عينات الرقائق المدروسة كلها أظهرت وجود الفطريات وبنسب مختلفة ومنها بعض الفطريات المنتجة للسموم الفطرية. كذلك فإن السموم الفطرية جميعها في العينات aflatoxin و ochratoxin كانت ضمن الحدود المسموح بها للاستهلاك البشري.

الكلمات المفتاحية: تلوث رقائق البطاطس، السموم الفطرية، الأفلاتوكسين، زيرالينون، الأوكراتوكسين.

^{*} هديل احمد العامري hadeelahmed.mu@gmail.com قسم علوم الحياة، كلية العلوم، جامعة الموصل، العراق.