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DETECTION OF THE CONTAMINATION OF AFLATOXIN BY USING HPLC AND SEVERAL CONCENTRATIONS OF HEAVY METALS BY USING ATOMIC ABSORPTION SPECTROPHOTOMETERS (AAS) IN BOTH BARED AND PACKED SPICES (IRAQ AND TURKEY)

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Abstract

Twenty two samples from different types of bared and packed spices were collected from Iraqi and Turkish markets. The samples included (11 samples of bared spices, 11 samples of packed spices). All the samples were collected from local markets of Iraqi (Baghdad city) and Turkish (Istanbul city). The investigation of Lead content in bared and packed spices revealed that the value 1.2 ppm was scored in Turkish bared red pepper as a highest value but the lowest 0.3 ppm was observed in Turkish bared sumac sample. The highest value 2.1 ppm Nickel was found in Iraqi packed red pepper sample and the lowest value 0.2 ppm were found in bared Turkish sumac. Zinc higher value was investigated was 78 ppm in Iraqi bared black pepper, while the lowest mean value 15 ppm found in packed Iraqi black pepper sample. The highest value of Iron concentration in spices was 1350 ppm recorded in Iraqi bared black pepper, but the lowest value 25 ppm in bared Turkish sumac. Regarding Cadmium content in spices, the results have found that the highest value 0.06 ppm was detected in Iraqi bared black pepper sample while the lowest value was in two sample 0.01 ppm of Turkish bared black and red pepper. For the Copper content was included the highest value 7 ppm in Turkish black pepper sample and the lowest value 2.5 ppm was found in bared Turkish sumac. The highest value of Aluminum concentration in spices was recorded 12.2 ppm in Iraqi packed red pepper, but the lowest value 3.0 ppm was in packed Iraqi curry. It was found that Iraqi bared and packed Cinnamomum had aflatoxin B_1 11.896, 250.081 ppm, but Iraqi bared and packed turmeric had aflatoxin B_2 with concentrations 44.555, 7989.4 ppm, also, packed Turkish black pepper and sumac had aflatoxin G_1 1.350, 2.355 ppm. The Aflatoxin G_2 with concentrations 58.143, 66.239, 80.213, 9.194, 93.485 and 5.057 ppm were detected in Iraqi packed black pepper, Iraqi bared and packed cumin, Iraqi bared curry and Turkish bared sumac, respectively.

Key words : Aflatoxin, HPLC, spices.

Introduction

Spices are considered non-perishable products under normal conditions of storage and some of those have antimicrobial and antioxidant properties that allows them to assist in food and self-stability (Ascenção and Filho, 2013). On the other hand, most of these products can act as a major source of microbial contamination, as well as are susceptible to fungal spoilage, mainly by the genera Aspergillus and Penicillium, if any failures occur during their processing (Schreiber *et al.*, 2014). Growth of fungi in spices can promote changes in sensory properties of spices, leading to the depreciation of its market value, and also the concern related to the possibility of mycotoxin formation, due the toxic, carcinogenic, mutagenic and immunosuppressive properties of these fungal compounds (Hocking, 2009). The spices come from various parts of plants such as bark, buds, flowers, fruits, leaves, rhizomes and stigmas. The majority come from tropical countries and is used to flavoring, coloring or preserving food and beverages (FAO, 2005). In tropical climate is common the occurrence of high temperature, humidity and rainfall rates. These climate parameters are suitable for intense microbial growth, especially of fungi. This group of microorganism is widely distributed in soil, organic matter and water and can easily contaminate the spices (Pitt and Hocking, 2009). There are more and more indications that primary liver carcinoma and other serious diseases may be induced by consuming food or using raw materials for food processing contaminated with fungi or mycotoxins, Aflatoxins, ochratoxin A and sterigmatocystin proved resistant to heat and have an ability to accumulate in the organism (Galvano *et al.,* 2005). Even products stored at low temperatures are vulnerable to some fungi (Durakovic´ *et al.,* 1989). Mycotoxins are natural food and feed contaminants, mainly produced by moulds of the genera Aspergillus, Penicillium and Fusarium. Mycotoxins, especially aflatoxins (AFs) and ochratoxin (Zinedine *et al.,* 2006). The exposure of human being to mycotoxins is a life threatening problem, especially in developing Countries where hot and humid climate favours fungal growth and where the food storage conditions are not adequate (Wild and Gong, 2009). Four naturally occurring AFs were identified B1, B2, G1 and G2 with AFB1 being the most common and toxic was classified as human carcinogen (group 1) by the International Agency for Research on Cancer (IARC) (1993). European Commission Regulations set maximum limits for AFB1 and total aflatoxins in some spices (*Caspicum* spp., *Piper* spp., Myristica fragrans, Zingiber officinale, Curcuma longa and mixtures of spices containing one or more of these spices) of 5 µg/kg and 10 µg/kg, respectively (European Commission, 2015). Foods of plant origin can be contaminated by different environmental pollutants, such as heavy metals, coming from anthropogenic activities and accumulating in plant through air, water and soil (Naccari *et al.,* 2015). The most toxic elements are heavy metals especially arsenic As, cadmium Cd, mercury Hg, and lead Pb, which have no biological roles in any living species, and may induce a wide range of toxic effects and developmental abnormalities, especially in children and embryos, have been linked to Alzheimer's and Parkinson's diseases, autism, and cancer. For these reasons, the presence of heavy metals in agricultural products, including dried spices should be strictly controlled (Donggyu *et al.*, 2015). Heavy metals can also accumulate in the soil at toxic levels as a result of long-term application of untreated wastewaters. Soils irrigated by wastewater accumulate heavy metals such as Cr, Zn, Pb, Cd, Ni, etc in surface soil. When the capacity of the soil to retain heavy metals is reduced due to repeated application of wastewater, heavy metals leach into ground water or soil solution available for plant uptake. For the metals derived from anthropogenic sources, this can strongly influence their speciation and hence bioavailability. However, the heavy metal content in plants can also be affected by other factors such as the application of fertilizers, sewage sludge or irrigation with wastewater (Frost and Ketchum, 2000). Plants are the main link in the transfer of heavy metals from the contaminated soil to humans. Heavy metals have a tendency to accumulate in the food chain. Heavy metals have low excretion rates through the kidney which could result in damaging effects on humans even at very low concentrations. Metals such as zinc, copper, iron, manganese, and chromium are essential nutrients; they are important for the physiological and biological functions of the human body. However, an increase in their intake above certain permissible limits can become toxic (Korfali *et al.,* 2013).

Materials and Methods

Heavy metals concentration measurement

Atomic Absorption Spectrophotometers (AAS) Sample analysis

Determination of heavy metal concentrations, a wet digestion of the dried samples was done according to the method described by Jones and Case (1990) Using conc. H_2SO_4 and 30% H_2O_2 mixture. To a 0.5 g of dry-ground sample placed in 100-ml beaker, was added 3.5 mL of 30 % H_2O_2. The content of the beaker was heated to 100°C, and the temperature was gradually increased to 250°C, and left at this temperature for 30 min. The beaker was cooled and another 1 ml of 30 % H_2O_2 was added to the digestion mixture and the contents were reheated again. The digestion process was repeated more than one time until clear solution was obtained. The clear solution was transferred into 50-ml volumetric flask, and completed to the mark with double distilled deionized water. A blank digestion solution was made for comparison. A standard solution for each element under investigation was prepared and used for calibration. Metal measurement was performed with a Perkin-Elmer model 2380 Atomic Absorption Spectrometer, double beam and deuterium background correction.

Measurement of Aflatoxin in studied samples

High Performance Liquid Chromatography (HPLC)

Analysis of Chemicals

All the chemicals of analytical grade used in the present study were purchased from BDH (Poole, England), Merck (Darmstadt, Germany) and Sigma Chemicals (ST. Louis, USA). Standards of AflatoxinB1 (2.02 µg / ml), Aflatoxin B2 (0.500 µg / ml) Aflatoxin G1 (2.01 µg/ ml) and Aflatoxin G2 (0.500 µg\ml) were purchased from Biopure (Tecknopark Tullin, Austria). Standard stock solutions of AFB1, AFB2, AFG1 and AFG2 of concentrations 1 µg/ ml each were prepared by diluting in benzene/ acetonitrile (98:2; v/v). These stock solutions were then stored at 4°C in refrigerator, wrapped in aluminum foil because Aflatoxins gradually breakdown under UV light.

Determination of Total Aflatoxins

Aflatoxins were determined according to standard method of Association of Official Analytical Chemists (AOAC), by thin layer chromatography Briefly (A.O. A.C., 2000).

Fifty grams from each sample spices powder was extracted with 250 ml acetone/water (85:15 v/v) using blender for 3minutes and filtered through whatman filter paper No 4. A 150 ml of filtrate was collected in 400 ml beaker. Then 170 ml of 0.02 N sodium hydroxide and 30ml ferric chloride along with about 3 gm basic copper carbonate added to the filtrate in 400 ml beaker, mixed well and added to the mixture in 600 ml beaker. This solution mixture was filtered and transferred 150 ml to 500 ml separating funnel. This 150 ml of 0.03 % sulphuric acid was added and extracted twicely with 10 ml of chloroform. Lower chloroform extract layer was transferred to another separating funnel and 100 ml of 0.02 M potassium hydroxide was added, swirled gently for 30 seconds and left it for layer separation. Chloroform extract layer was collected in a vial. Of this 8 ml was evaporated to dryness at 45°C under gentle stream of nitrogen on a heating block. The residue was dissolved in 200 µl benzene/ acetonitrile (98:2 v/v) and subjected to thin-layer chromatography. Final identification and quantification of total Aflatoxin were performed by one-dimensional TLC on pre coated silica gel plates (Merck, Germany). The plates were developed in a saturated chamber with chloroform/xylene/acetone (60:30:10; v/v/v). The samples spots were observed under long wave ultraviolet light (λ=365 nm) and determined by visual comparison with Aflatoxins standard spots. Confirmation of the identity of Aflatoxins was carried out with the spray of 50 % sulphuric acid and using the Trifluoroacetic acid (TFA) reaction (Scott,1984).

Results and Discussion

Heavy metals in bared spices

Heavy metals in packed spices

Highest Pb value 1.2 ppm recorded in bared Turkish red pepper, while the lowest value 0.2 ppm found in bared Turkish sumac, but the highest Pb value 1.1 ppm recorded in Turkish sumac, while, the lowest value 0.3ppm was recorded in Turkish black and red pepper The recorded concentrations of Lead in bared and packed spices is less than FAO/WHO maximum permissible limit of lead (10 $mg.kg^{-1}$) consumed medicinal herbs was (WHO and FAO,2007).

The highest value of Ni in bared spices 2 ppm was detected in bared Iraqi black pepper, while the lowest value 0.7ppm that found in bared Turkish sumac, but the highest value in packed spices was 2.1 in packed Iraqi red pepper while the lowest was 0.8ppm in packed Turkish sumac, Nickel is toxic metal in low concentration, the accepted value in food 0.10 ppm which set by FAO/WHO (1996). While, the results show all the spices bared and packed recorded above the standard limit of WHO (1996).

The higher value of Zn 78ppm was recorded in bared Iraqi black pepper, whereas the lowest value in bared spices that recorded in bared Turkish black pepper was 16.5ppm. Also, the higher value 70ppm was recorded in packed Iraqi cumin while the lowest value 15ppm was determined in packed Iraqi black pepper, According WHO and FAO (2005) which proposed that the acceptable value of zinc concentration in food was 50 ppm, so the results were recorded in this study with acceptable value, except the bared Iraqi red pepper, bared Iraqi cumin and packed Iraqi cumin samples (78,70,70 ppm), respectively. highest value of Fe 1350ppm was recorded in bared Iraqi black pepper, whereas the lowest value 25 ppm was recorded

Heavy metals in spices

S.No.	Common name	Scientific name	Zn ppm	Fe ppm	Cu ppm	Ni ppm	Pb ppm	Al ppm	Cd ppm
1.	Black pepper	*Piper nigrum*	15.0	50	5.7	1.7	0.3	8.5	0.02
2.	Red pepper	*Capsicum*	38.0	105	6.9	2.1	0.9	12.2	0.03
3.	Cumin	*Cuminum cyminum*	70.0	190	5.7	1.6	0.8	7.0	0.04
4.	Turmeric	*Curcuma longa*	*27.0*	110	3.3	1.5	0.8	7.0	0.05
5.	Curry	*Murraya koenigii*	33.0	103	4.5	1.3	0.7	3.0	0.02
6.	Cinnamomum	*Cinnamomum verum*	30.0	950	3.8	1.3	0.8	7.0	0.02
7.	Ginger	*Zingiber offcinale*	31.0	85	3.8	1.1	0.6	6.0	0.02
8.	Sumac	*Rhus coriaria*	25.3	50	2.9	0.9	0.5	5.1	0.02
9.	Turkish sumac	*Rhus coriaria*	20.0	58	2.3	0.8	0.3	4.7	0.02
10.	Turkish black pepper	*Piper nigrum*	23.5	450	6.9	1.6	1.1	12.0	0.02
11.	Turkish red pepper	*Capsicum*	33.2	85	5.0	1.2	0.3	4.5	0.02

S.No.	Common name	Scientific name	Zn ppm	Fe ppm	Cu ppm	Ni ppm	Pb ppm	Al ppm	Cd ppm
1.	Black pepper	*Piper nigrum*	78.0	1350	6.3	2.0	1.0	5.2	0.06
2.	Red pepper	*Capsicum*	30.0	970	3.8	1.3	0.8	7.0	0.02
3.	Cumin	*Cuminum cyminum*	70.0	173	6.3	1.9	0.7	6.5	0.05
4.	Turmeric	*Curcuma longa*	28.0	117	3.3	1.4	0.9	3.5	0.04
5.	Curry	*Murraya koenigii*	33.5	250	4.5	1.4	0.5	8.8	0.03
6.	Cinnamomum	*Cinnamomum verum*	33.0	198	4.5	1.4	0.7	11.1	0.02
7.	Ginger	*Zingiber offcinale*	32.2	93	3.8	1.0	0.4	5.5	0.02
8.	Sumac	*Rhus coriaria*	21.0	35	3.0	0.8	0.3	4.7	0.02
9.	Turkish sumac	*Rhus coriaria*	22.5	25	2.5	0.7	0.2	4.0	0.02
10.	Turkish black pepper	*Piper nigrum*	16.5	40	7.0	1.5	0.3	4.0	0.01
11.	Turkish red pepper	*Capsicum*	20.0	450	6.3	1.4	1.2	11.0	0.01

in bared Turkish sumac. On other hand the Fe concentration in packed spices mentioned that the highest value was 950ppm in packed Iraqi Cinnamomum but the lowest value had been 50ppm in packed Iraqi black pepper, According to FAO/WHO (2005) the acceptable value of iron was 300 ppm, so most of bared and packed spices have the concentration of Fe was less than the acceptable value of iron standard, except the bared Iraqi black pepper, bared Iraqi red pepper, bared Turkish red pepper, packed Iraqi cinnamomum and Turkish packed red pepper were 1350,970,450,950 and 450ppm, respectively.

Cd content in bared spices be with highest value 0.06ppm in Iraqi black pepper, whereas the lowest value 0.01 ppm in Turkish red pepper, but the Cd concentration in packed spices, the highest value which detected 0.05ppm in Iraqi turmeric while the lowest value was 0.01ppm in Iraqi Ginger, FAO/WHO (2007) levels of cadmium exceeding 0.3 $mg.kg^{-1}$, the permissible limit set by for medicinal herbs and plants. All the results of Cd in bared and packed spice were below the acceptable level value of WHO and FAO 0.3ppm.

Regarding Cu concentration ensures that, the highest value in bared spices was 7.0 ppm was detected in bared Turkish black pepper, while the lowest value was 2.5ppm which that found in bared Turkish sumac. About the packed spices it was noticed that the highest values (6.9) ppm was recorded in Turkish black pepper but 2.3 ppm was the lowest values of copper found in Turkish sumac, World Health Organization (2005) has given the maximum permissible limits of copper is 50 ppm, All bared and packed spices which studied were below the limit value.

Highest mean Al value in bared spices was in 11.1 ppm was recorded in bared Iraqi Cinnamomum, while the lowest mean value 3.5 ppm was found in bared Iraqi turmeric. On other hand the highest Al value in packed spices was 12.2ppm in packed Iraqi red pepper, while the lowest was 3.0 ppm in packed Iraqi curry.

Heavy metal contents in spices and medicinal plants depend on climatic factors, plant species, air pollution, and other environmental factors (Sovljanski *et al.*, 1989). Abou-arab and Abou donia (2000) affirmed that the heavy metals contents in spices varied depending on the country of origin, environmental pollution levels, plant part and technological processes.

Only Iraqi bared and packed cinnamomum was contain of Aflatoxin B1 with 250 ppm as higher value and 11.89 ppm as lower value, these results appear to be with higher concentrations which proposed by both Turkish Food Codex 5ppm for Aflatoxin B1 and 4ppm as a permissible value which suggested by Eu and Turkish Food Codex, the maximum limits of Aflatoxin B1 (5 ppm) and total Aflatoxin (10 ppm) set in the Turkish Food Codex. Only Iraqi bared and packed Turmeric was contain of Aflatoxin B2 with 7989.4ppm as higher value and 44.555 ppm as lower value, these results appear to be with higher concentrations which proposed by both Turkish Food Codex 10ppm and 4 ppm as a permissible value which suggested by Eu.Turkish sumac and black pepper were contained 1.350 and 2.355 ppm, respectively, of Alfatoxin G1, all other spices studies (bared and packed) for both Iraqi and Turkish samples were free from G1 Aflatoxin these results appear to be with lower concentrations which proposed by both Turkish Food Codex (10ppm) and (4ppm) as a permissible value which suggested by Eu. The bared spices samples studies, explained that the Cumin, Curry, Turkish Sumac and Turkish red pepper were contains a concentration of Alfatoxin G2 which are as follow, 80.213, 9.194, 93.485 and 5.057ppm, respectively. (Table 3.19), but the packed group, included two contaminated samples with Aflatoxin G2 were black pepper 58.143ppm and Cumin 66.239ppm. While, the bared and packed Iraqi red pepper, packed Iraqi black pepper, bared Iraqi curry, bared Iraqi ginger,

Aflatoxin in spices
Concentration of Aflatoxin in bared spices (ppm)
Concentration of Aflatoxin in packed spices (ppm)

S.No.	Common name	Scientific name	Aflatoxin B1con.PPM	Aflatoxin B2con.PPM	Aflatoxin G1con.PPM	Aflatoxin G2con.PPM
1.	Black pepper	*Piper nigrum*	0	0	0	0
2.	Red pepper	*Capsicum*	0	0	0	0
3.	Cumin	*Cuminum cyminum*	0	0	0	80.213
4.	Turmeric	*Curcuma longa*	0	44.555	0	0
5.	Curry	*Murraya koenigii*	0	0	0	9.194
6.	Cinnamomum	*Cinnamomum verum*	11.896	0	0	0
7.	Ginger	*Zingiber offcinale*	0	0	0	0
8.	Sumac	*Rhus coriaria*	0	0	0	0
9.	Turkish sumac	*Rhus coriaria*	0	0	0	93.485
10.	Turkish black pepper	*Piper nigrum*	0	0	0	0
11.	Turkish red pepper	*Capsicum*	0	0	0	5.057

S.No.	Common name	Scientific name	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2
1.	Black pepper	*Piper nigrum*	0	0	0	58.143
2.	Red pepper	*Capsicum*	0	0	0	0
3.	Cumin	*Cuminum cyminum*	0	0	0	66.239
4.	Turmeric	*Curcuma longa*	0	7989.4	0	0
5.	Curry	*Murraya koenigii*	0	0	0	0
6.	Cinnamomum	*Cinnamomum verum*	250.081	0	0	0
7.	Ginger	*Zingiber offcinale*	0	0	0	0
8.	Sumac	*Rhus coriaria*	0	0	0	0
9.	Turkish sumac	*Rhus coriaria*	0	0	1.350	0
10.	Turkish black pepper	*Piper nigrum*	0	0	2.355	0
11.	Turkish red pepper	*Capsicum*	0	0	0	0

bared Iraqi sumac, bared Turkish red pepper, packed Iraqi ginger, packed Iraqi sumac and packed Turkish black pepper not have Aflatoxins G2. These results appear to be with higher concentrations which proposed by both Turkish Food Codex (10ppm) and (4ppm) as a permissible value which suggested by EU, while the Iraqi bared curry and Turkish bared red pepper are above the permissible value of EU but less permissible value of Turkish Food Codex. (Anonymous, 2002).

The incidence of Aflatoxins in food is relatively high in tropical and subtropical regions, where the temperature and humid weather provides optimal conditions for the growth of the molds (Rustom, 1997). May be related to contaminated through process Therefore, the regular monitoring of these spices will be needed continuously (Sung-Hye *et al.*, 2007). It is know that environmental conditions at the place of fungi growth (temperature, water activities, the matrix composition, moisture content, pH of the media, physical contamination of the substrate, antifungal properties, and other factors) play an important role in mycotoxin accumulation in spices (Pitt and Hocking, 2009).

Conclusion

1. The concentration of Iron was the highest value in both bared and packed spices when compare with other heavy metals
2. Aflatoxin G2 type is most common Aflatoxin appearance in bared and packed Iraqi and Turkish spices but Aflatoxin G1 found only packed Turkish sumac and black pepper, while the Aflatoxin B1 found only in bared and packed Cinnamomum and Aflatoxin B2 found only in bared and packed turmeric.

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