



## DETECTION OF MYCOTOXINS IN SUNFLOWER SEED INTENDED OR EDIBLE FOR HUMAN CONSUMPTION FLOWER

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### ABSTRACT

Mycotoxins are a family of biological compounds produced by a group of fungi have the ability to produce secondary metabolic compounds (S.M) when its growth on its environment, and the fact that this metabolic output biological active compounds, additionally they are non-toxic antigen meaning absence her molecular structure of components that drive the living body to form antibodies, most toxic to humans, animals, plants and microorganisms Hence, this study aimed to poll about indications 50 pollution sample of sunflower seeds prepared for human consumption by these toxins through Elisa technology assessment. The results showed the absence of all samples from the Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1), Aflatoxin G2 (AFG2) and Ochratoxins. found Aflatoxin B1 (AFB1) by 100% concentration 5.3– 6.1  $\mu\text{g}/\text{kg}$  and 7.1 – 8.7  $\mu\text{g}/\text{kg}$  in Sun flower seeds unroasted and unsalted but unenvelope bags and sunflower roasted several flavors unenvelope respectively, while the focus in both sunflower seeds salted toasted envelope with bags and sunflower seeds salted toasted envelope bags 2.3-3.4  $\mu\text{g}/\text{kg}$  and 1.7-2.8  $\mu\text{g}/\text{kg}$  and concentration 40% and 30% respectively, and less focus has reached 0.2-0.8  $\mu\text{g}/\text{kg}$  in roasted salted sunflower seeds core envelope bags by 20%, and a sample control of mycotoxins mentioned which has been assessed in this study.

**KEY WORD:** sunflower seed, mycotoxins, ELISA.

### INTRODUCTION

Agricultural crops products Polluted with spores and konidat of fungus and fungal spinning parts and that of the surrounding environment during various stages of production are fungi of determinants of productivity of agricultural crops from its presence in the field and to a more serious during storage, causing major health and economic losses, especially in countries that lack good storage conditions making them unfit for human consumption by altering the nutritional value <sup>[1]</sup> and divided the fungi that infect crops into three sections according to time discharge the poison through the stages of production and circulation of foodstuffs<sup>[2]</sup>:

1. Fungi that infect in the field and include some types of *Fusarium spp.*, *Alternaria spp.*, *Cladsporium spp.*, *Helminthosporium spp.*
2. Storage Fungi that are active in the crops after harvest and include sometypes of *Aspergillus spp.*, *Penicillium spp.*
3. Rot fungi which cause damage to crops such as *Chaetomium*, *Fusarium*, *Sordaria etc.*

It proved that most fungi producing Mycotoxins are a type of storage fungi and rot fungi few capable of producing toxins, which reach the human food directly or indirectly, as well as being targeted poisons, meaning that each Member specialized fungal toxin or more from that score the liver Hepatoxins and heart infect Cardiotoxins, including nerve not hit Neurotoxins. etc, and aflatoxin, one of these toxins, which are derivatives of the compound Difurano coumarine and with low molecular weights, and

a group of close relationship with each represent a secondary metabolic products carcinogen produced by some fungal strains belonging to *Aspergillus flavus*, *Aspergillus parasiticus*, *aspergillus nomius*<sup>[3]</sup>. it has been observed that a very large interest aflatoxin in different parts of the world Since its discovery in the early sixties of the last century and up to the present time because of their carcinogenic and toxic to humans and animals in General, and in recent years he discovered about 20 types of aflatoxin which four main types (AFB1 AFB2, AFG1, AFG2) because of their prominent role in inhibiting human immune mutant embryos and genetic mutations events and birth defects, also from acute and chronic Toxicology urging the formation of tumors and cancer <sup>[4]</sup>. and the most widely informed poison is B1 which causes cancer of the liver cells in humans and many animal species, it has been observed that the amount of 2.2 mg of this poison enough destruction of the liver <sup>[4,5,6]</sup> and noted the Food and Agriculture Organization of the United Nations (FAO) of the United Nations should not consider them food products and poultry feed and cattle blackberries and the risk of mycotoxin contamination estimated at 25%, mycotoxin vary in toxicity, for example less toxic AFG1 of AFB1 and AFB2 but more toxic than <sup>[7]</sup>, and Sun flower plant among the crops that are affected by these toxins risk before and after harvest<sup>[8]</sup>, around summer crop back to the vehicle family compositae english named sunflower and scientific *Helianthus annuus L.* Sun flower seed is the main crop task in Iraq, because of assorted food uses, including extracting oil for oily items into raw material in the manufacture of vegetable oils, and a source of vegetable proteins possess intrinsic

properties free baller because of their content of amino acid and seeds eaten after roasting and salting by human<sup>[9]</sup>, only items un oil concentrated its importance in human before consumption took a expanding plants specializing in roasting and packaging of seeds of this crop and so this expansion consumption and increased demand boosted imports especially from neighboring countries because of poor crop production in Iraq<sup>[10]</sup>, in Iraq and some Arabic countries preferred to use this product if the seed pulp like Sesame mixture with bread<sup>[11]</sup>, sunflower varieties can be divided into three groups (oilseeds, seeds and roasting seeds for application types, and is a compromise between the two previous sets and can use to extract oil from them or roasting) Given the growing importance of this food product and increase demand for the preface It features as the appropriate price, accessibility, and diversity of sizes and flavors added to it, and the process of preparation and prepare and toast it wanted a big requirement, and direct relationship to human health. This study was conducted to assess the level of mycotoxin contamination of this product, by estimating depending on the technique Enzyme-Linked Immuno Sorbent Assay (ELISA), as modern technology is its speed and high accuracy in giving results, and the possibility of analyzing a large number of samples, according to<sup>[12]</sup> that this technique works on the quantitative and qualitative appreciation of the very small amounts of antigens or antibodies in standard minute holes dishes called standard Microtitre Plates and minute sheets can detect 5ng of Antigen or antibody to each ml of the sample with this technology.

#### MATERIALS AND METHODS

Sun flower seed of single and wholesale stalls at local markets deployed in Baghdad to Karada area, jamila, alshorja and Kadhimiya wildly with three replicates per sample weighing 250 g each, as follows:

Ten samples of sunflower seeds are unenvelope bags intended primarily for human consumption but free salt and by products in a variety of sizes and designations (such quality food products and plants used for markets after salting and roasting). The other 10 samples also unwrapped but salted and toasted, 10 samples of this product baked leavened (soaked in brine, plus several flavors) and laminated, modeling included ten samples envelope in bags and weights of different salted and toaster, as well as 10 other crust-free samples (core seed) packed with bags and different weights also, finally, comparative forms (control) obtained from seed inspection and certification service one of the formations and the Ministry of Agriculture certified free of biological and chemical pollutants. All the samples to the lab after placing them in bags of polyethylene and sentence stands, and conducted quantitative and qualitative assessment of mycotoxins (AFB1, AFB2, AFG1, AFG2 and Ochratoxins were performed according to (AOAC)2005<sup>[13]</sup> using diagnostic kit (Neogen's Extraction Kits) and ELISA BioTek type device America origin. Carried out in two stages: the first is the fungal toxin extraction stage and performed the following steps:

Clean private non-coated samples analysis of impurities and exotic materials, grinding process were conducted for all samples through a laboratory mill soft image, to get

smooth powder and then put these models separately in tiny clean made of polyethylene after weight 25g of, taking into account clean the mill well between each meal to get rid of the remnants of the previous model, then added 25 ml of 70% methanol composed of methanol and deionized water by 70:30. mix well to obtain a homogeneous mixture and leave for three minutes, and was nominated by syringe filter membrane filters to get rid of leftover deposit and get leaky content on fungal toxin, with the second phase included the transfer of 100 µl of Conjugate link material to small-sized grooves added to 100 µl of the leaky cauldron and move three times to ensure the homogeneity of each, and this step was transferring 100 µl to another grooves inside container Antibody. And left two minutes, then washed with a special lotion to remove articles that are not linked, then the drying operation poured down and absorbing paper, Absorbent paper after finishing this stage added article basically Substrate (color material) by 100 µl and leave for 3 minutes so that color (usually blue) in the grooves Which changes depending on the concentrations of these toxins, then add then 100 µl solution stop interaction on the kit that is imported for each hole. Transferred to your ELISA Reader optical density is read at wavelength 460 nm concentrations are expressed in (µg/kg).

#### RESULTS & DISCUSSION

The results show samples described in table (1) indications of contamination by toxic AFB2, AFG1 and AFG2 and Ochratoxins during the process of estimating concentrations of these toxins, probably due to the preventive measures in place to reduce fungal contamination and prevent the formation of mycotoxins in food products, primary prevention is through control of fungal infections by fungi crop infestation and mycotoxin contamination events, The development of disease-resistant varieties of plant assets, taking into account the natural, biological and chemical factors including moisture content in the crop inventory, and the storage temperature, ventilation, and strain the mushrooms and the nature of the material<sup>[14]</sup>. Result agreed with a study in Khartoum and Kordofan island regions of Sudan to estimate concentrations of both AFB2, AFG1, AFG2 and Ochratoxins specifically in 56 samples of vegetable oils Derived from vegetable seeds including sunflower, proven free of these toxins<sup>[15]</sup>, the highest standard for the presence of mycotoxins AFB1 in all samples Sun flower seed is salted but the packing and toasted sunflower seed leavened several 100% and flavors hit 6.1-5.3, 7.1-8.7 µg/kg, respectively (table 1). To cause high rates of this fungal toxin concentrations in these nutrients is owning types of mycotoxin-producing fungi species ability to secrete large number of enzymes analyzed for nutrients used in nutrition and growth even in low moisture content as well as the relative density of blackboards produced, and genetic factors related to mushrooms and genetic ineage and its production of these mycotoxins<sup>[16]</sup>, and often these products these poisons fungus through additives (flavorings and fillers), this prove in many studies on scientific sources<sup>[17]</sup>. Returning to the table, 1, note that this fungal toxin concentrations ranged between 2.3-3.4 µg/k. In four samples of seed and non-roasted

salted sunflower envelope bags total 10 and 40%, and in three samples of the total ten love sunflower salted toasted scoopy bags by 30% total concentration 2.8-1.7 µg/kg, and less contaminated samples with this innate poison at the heart of the sun-roasted salted roasted envelope bags in two out of 10 by 20% and the concentration of 0.2 – 0.8 µg/kg I thought, with a sample control of any indication of the presence of the toxins mentioned in the study, and these percentages because of different concentrations are due to fungal strains type available which vary in their ability to produce fungal toxin type and focus<sup>[18]</sup>, there are some factors that facilitate fungal invasion and producing toxins such as mechanical damage, and mixing process (mixing) The old goods contaminated with non-polluting goods and cool product after it is roasted and not to fill it is hot<sup>[19]</sup>, despite the high stability of the toxin aflatoxin toward thermal transactions, but many studies have shown that certain transactions effective thermal these toxins

"crack off"?, this is indicated by a study of researchers<sup>[20]</sup> the relative stability of most traditional food mycotoxins that are average temperature between 80-120°C , But may be present in very small amounts and sometimes gets demerger or fragment at high temperature above 150 °C of boiling temperature and frying, roasting and pasteurization, in another study showed that produce mycotoxins including aflatoxin in all cases don't yield growth of some species and genus ones producing *Aspergillus*, fungal isolation also doesn't mean the presence of toxins and does not produce poison by fungi unless specific environmental conditions as increase humidity with appropriate temperatures<sup>[21]</sup>, looking at the primary study samples from toxin aflatoxins find they are within the permissible limits Iraqi Standard Specification<sup>[22]</sup>.<sup>1</sup>with 10 µg/kg.

**TABLE 1:** Average concentration of mycotoxins (µg/kg) in the examined samples of Sun flower with using Elisa

Sample name	Sample number	Number of samples contaminated with mycotoxins is estimated as percentage (%)					Mycotoxin concentration µg/kg
		AFB1	AFB2	AFG1	AFG2	Ochratoxin	
Seeds of Sun flower (comparison)	1	0	0	0	0	0	0
non roasted salted and un envelope sunflower	10	10(100)	0	0	0	0	5.3 – 6.1
roasted salted and un envelope sunflower	10	4 (40)	0	0	0	0	2.3 - 3.4
Roasted fermentative several flavors un envelope sunflower	10	10(100)	0	0	0	0	7.1 - 8.7
Roasted Salted envelope sunflower	10	3(30)	0	0	0	0	1.7 - 2.8
Roasted Salted envelope core seed sunflower	10	2(20)	0	0	0	0	0.2 - 0.8
Total	50	29(58)	0	0	0	0	

## CONCLUSION

1. Accuracy and degree of fumbling exhibited by Elisa technique in estimating concentrations of these mycotoxins.
2. The main causes of these toxins is the resulting storage in stores may be infected or badly ventilated stores and high humidity.
3. Bad packing during storage
4. Mix the old goods contaminated with non-polluting goods.
5. Lack of rules, wooden lift goods stored on earth.

## RECOMMENDATION

1. Usability techniques compare HPLC and liquid chromatography, LCMS-mass spectrometry to estimate concentrations of these toxins.
2. Make sure the additive free of biological and chemical contaminants testing for mycotoxins before marketing.
3. The packing process and careful selection of good[6]. specification packaging and scalable storage.
4. No excessive intake of flavorings added to a food product because it's a double edged sword.
5. Review of food legislation and health in concentrations of[7]. mycotoxins permitted in food standard specifications for these toxins negative effects on public health.

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