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DETECTION OF TOTALAFLATOXINSAND OCHRATOXINA RESIDUES IN SOME MEAT PRODUCTS

By

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ABSTRACT

The food contamination with mycotoxins is a global problem. The most commonly observed mycotoxins are total aflatoxins (AFs) and ochratoxin A (OTA). Intense investigations have been conducted by studying the existence of the various mycotoxins to find out how they affect human food chain. This study was conducted on two hundred and fifty samples of different meat products (minced meat, basterma, kofta, and luncheon and beef burger). Samples were randomly collected from different markets with divergent localities (Giza and Cairogovernorates) to screen their AFs and OTA contamination.The mycotoxin concentration was assayed using competitive direct enzyme- linked immunosorbent assay (CD-ELISA). According to the location, the survey demonstrated that, the examined samples collected from Giza governorate showed higher aflatoxin residues percentage (83.2%) than in Cairo governorate (77.6%). On the contrary, the incidence of ochratoxin A, was higher in Cairo (84%) than in Giza governorate (77.6%).

INTRODUCTION

Meat is considered a part of balanced diet, contributing with valuable nutrients that are beneficial to one's health. Meat and meat products contain important levels of protein, vitamins, minerals, and micronutrients, which are essentials for the growth and development of humans. Meat is composed of water, amino acids, fats, fatty acids, and small quantities of carbohydrates **Taise *et al*., (2016)**. In addition, meat contains many inorganic compounds that comprise approximately 1% of its total composition **Zenebon *et al*., (2008).** Raw materials, as well as final meat products are exposed to a high risk of microbial contamination at the time of their production, processing, storage and distribution **Laciakova *et al*., (2004).** Mycotoxins are toxic secondary metabolites produced by some species of mould genera which invade

foodstuffs and may grow on foods during storage under favorable conditions of temperature and humidity **Elkak *et al*., (2012).** Nowadays the main mycotoxins of interest are AFs and OTA, as they are mycotoxins of major significance and hence there has been significant research on broad range of analytical and detection techniques that could be useful and practical **Nicholas *et al*., (2009)**. AFs are one of mycotoxins group produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus* molds. They are of greatest concern as they are highly toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. They have been implicated as causative agents in human hepatic and extra hepatic carcinogenesis **INSPQ, (2002).** OTA is mainly produced by *Aspergillus ochraceus* and *Penicillium verucosum*. OTA is mutagenic, teratogenic and nephrotoxic and have been involved in etiology of Balkan Endemic Nephropathy **Vrabcheva *et al*., (2000).** So, it seems necessary to monitor the presence of mycotoxins in food to exclude a frequent exposure of consumers **Shabana *et al*., (2008).** Several methods can be used for AFs detection, as thin layer chromatography, high performance liquid chromatography, gas chromatography, capillary electrophoresis and Enzyme Linked Immuno-Sorbent Assays (ELISA). The lattery one is a simple, specific, sensitive, low-cost and rapid screening method **Turner *et al*., (2009).** Thus, the main objective of this work was to determine AFs and OTA levels in some meat products (minced meat, basterma, kofta, luncheon, and beef burger).

MATERIAL AND METHODS

Sampling:

A total of 250 processed meat samples (50 each of minced meat, basterma, and kofta, luncheon, and beef burger) were collected during the period from June up to December, 2014 from different shops and supermarkets in different localities in Giza and Cairo Governorate. Samples were transferred immediately into cooling ice box to the laboratory. Samples were identified and preserved in sterile polyethylene bags in the refrigerator.

Preparation of Samples:

The samples were stored in a cool place and protected against light. Samples were chilled or kept frozen at -20°C. In case of frozen samples, examination was occurred after samples thawing.

ELISA test procedure:

A-AFs determination:

The quantitative analysis of AFs in examined samples was performed by competitive direct enzyme- linked immunosorbent assay (CD-ELISA) method **Thirumala-Devi *et al*., (2000)**. The method is based on the accurate monitoring of mycotoxins and is suitable for screening large number of samples.The veratox test kits **(RIDASCREEN® AFs, Art No.: R4701-R-Biopharm AG, D-Darmstadt, Germany)** were used.

B-OTA determination:

The quantitative analysis of OTA in examined samples was performed by competitive direct enzyme- linked immunosorbent assay (CD-ELISA) method according to **Bayder *et al.*, (2007)**. The veratox test kits (RIDASCREEN® OTA, Art. No.:R1311-R-Biopharm AG, D - Darmstadt, Germany) were used.

Evaluation of AFs and OTA

The absorbance values obtained for the standards and the samples were divided by the absorbance value of the first standard (zero standards) and multiplied by 100 (percentage maximum absorbance). Therefore, the zero standards are thus made equal to 100 %, and the absorbance values are quoted in percentages. The concentrations of AFs and OTA levels in the tested samples were estimated from standard curve relation optical density versus AFs and OTA standards concentration Fig. (1, 2).

Statistical Analysis:

Data were analyzed and results were reported as mean ± SE. The calibration curve and trend line equation prepared using available software, Percentage, minimum, maximum and mean ± Se were carried out **Zar J.H., (1984).**

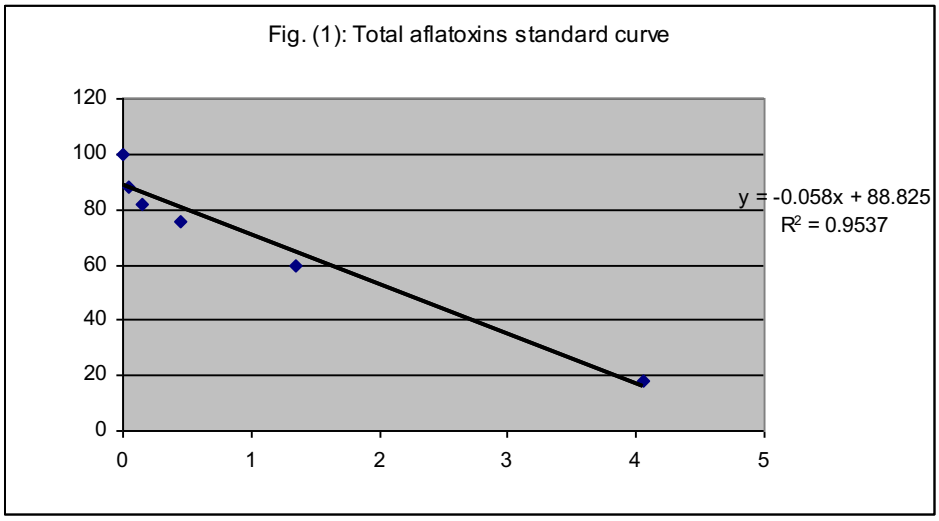

Fig. (1): Calibration curve of AFs standards.

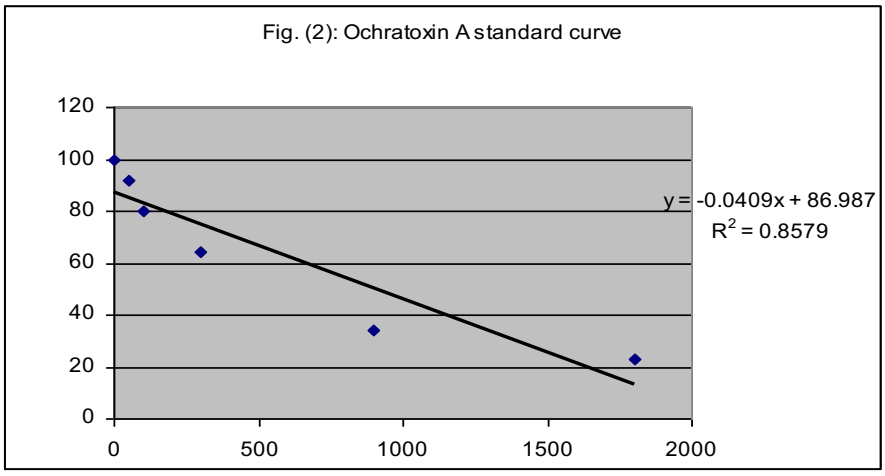

Fig. (2): Calibration curve of OTA standards.

RESULTS

Table (1): Incidence of Total Aflatoxin residues in examined meat product samples. (n=25).

Meat product samples	Giza	Cairo
	Positive samples (%)	Positive samples (%)
Minced meat	19 (76)	16 (64)
Basterma	19 (76)	19 (76)
Kofta	21 (84)	17 (68)
Luncheon	22 (88)	22 (88)
Beef burger	23 (92)	23 (92)
Total	104 (83.2)	97 (77.6)

Table (2): Statistical analytical results of total Aflatoxins residues (ppb) in the examined meat product samples.

Meat products samples	Giza			Cairo		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
Minced meat	1.998	2.81	2.547±0.0735	0.28	2.43	1.419±0.161
Basterma	2.173	2.85	2.597±0.051	1.223	3.74	2.190±0.174
Kofta	2.43	2.992	2.659±0.041	0.479	2.134	1.306±0.123
Luncheon	1.981	2.75	2.543±0.032	0.452	2.49	1.284±0.133
Beef burger	2.124	2.872	2.579±0.043	0.65	2.83	1.634±0.130

Table (3): Incidence of Ochratoxin A, residues in the examined meat product samples.

Meat product samples	Giza	Cairo
	Positive samples (%)	Positive samples (%)
Minced meat	16 (64)	17(68)
Basterma	21(84)	22(88)
Kofta	21(84)	22(88)
Luncheon	19(76)	20(80)
Beef Burger	20(80)	24(96)
Total	97(77.6)	105(84)

Table (4): Statistical analytical results of Ochratoxin A (ppb) in the examined meat product samples.

Meat product samples	Giza			Cairo		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
Minced meat	0.989	1.981	1.586±0.073	0.078	1.9	0.957±0.151
Basterma	1.62	2.65	1.893±0.051	1.41	3.42	2.306±0.129
Kofta	1.35	1.946	1.668±0.041	0.079	1.888	1.161±0.121
Luncheon	1.45	1.92	1.764±0.032	0.05	2.61	1.463±0.182
Beef Burger	1.188	1.95	1.639±0.043	0.056	1.887	1.064±0.141

DISCUSSION

Mycotoxins, particularly AFs and OTA pose a significant threat to human health. AFs are potent carcinogens and, in association with hepatitis B virus, are responsible for many thousands of human deaths mostly in non-industrialized tropical countries **Shephard, (2006).** OTA is a probable human carcinogen and it was reported to cause urinary tract cancer and kidney damage. As shown in (Table 1). The results were found to be higher than those reported by **Howayda., (2008)** who recorded 20% aflatoxin contamination in minced meat, 26.7% in luncheon , 33.3% in basterma and 40% in burger samples, but extremely higher than those reported by **Ismail and Zaky., (1999)** who investigated 14% aflatoxin residues in

luncheon samples; **Shaltout and Salem., (2000)** found AFs in 20% of minced meat and 13% in kofta sample while **Seham *et al.*, (2013)** stated that AFs contamination was 16% in basterma and 20% in luncheon samples. The results indicated that the highest percentage was at samples of Giza governorate as the contamination with mycotoxins probably originated from other additives than animal tissues or during processing, transport and or storage **Ismail and Zaky,** (**1999**). Moulds could contaminate meat during slaughtering, dressing and handling in slaughter houses where the environmental conditions as air, walls, floors, equipment's and workers hands as well as intestinal contents play an important role in contamination of meat with fungi **El-Shafei, (2004).** The higher incidence of AFs in burger was attributed to the use of different additives especially contaminated spices which may be the main source of AFs **Wafia and Hassan,** (**2000).** Several authors reported that, the main ingredients of meat products are different types of flour (wheat, corn, rice) or dry bread crumbs, egg, some dairy products (milk, butter, yoghurt), vegetable oil and spices **Family time.,** (**2006)** may considered sources of AFs. **Seenapa and Kempton, (1980)** detected AFB1 in 14.4% of black and red pepper, ganger and turmeric in a range of 10-120 ppb. **Fazekas *et al.,* (2005**) reported that 45.45% and 31.82% of ground red pepper contain FB1 (0.14 - 15.7 ppb) and AFB2 (0.22-1.25 ppb). **Baydar *et al.,* (2005)** reported that, the examined soybean samples were contaminated with 0.94 AFB1, 0.18 AFB2 and 0.03 AFG1 ppb respectively. The higher incidence of AFs in luncheon was attributed to the use of different untreated food additives and spices. The frequent contamination of spices and additives used in such meat processing may also represent a source of mycotoxin. Moreover, it has been demonstrated that, the use of spices contaminated with toxigenic mould strains as ingredient in meat products processing may lead to a secondary contamination of the final product with aflatoxins **FAO,** (**2004) and Francis and Warwick, (2009).** It was evident that, the high incidence of AFs residues determined in basterma samples could be attributed to the use of garlic which stimulating AFs production **Bullerman *et al.*, (1969)**, beside the high sodium chloride content (about 8%), storage at room temperature and usage of high proportion of spices that stimulate AFs production **Shaltout, 1996.** While, the lower incidence of AFs in minced meat may be attributed to that it does not contain any spices and food additives.The presence of AFs residues in meat due to ingestion of low levels of AFs over extended periods constitutes a public health hazard; especially AFs have been shown to be carcinogenic **Hassan *et al*., (2004).** Comparing the results of the present study in (Table 2)

to those of previous scholars work, the levels of AFs in beef burger samples were higher than those reported by **Roushdy *et al.*, (1996)** who detected mean conc. of 0.59 ppb and agree for certain extent with those reported by **Taghreed, (1997) and Howayda, (2008)** who recorded 3.60 ± 4.48 and 3.6±_1.33 ppb respectively but lower than those reported by **Shabana *et al.*, (2008)** who recorded 11.76 ppb. Concerning previous researchers work, the results of luncheon were lower than those detected by **Hassan *et al.*, (1997); Ismail and Zaky., (1999)** who found aflatoxin levels ranged between 0.5-11.1 ppb; **Howayda., (2008)** recorded 3.76±0.87, **Dalia., (2012) and Seham *et al.*, (2013)** recorded 10.4± 5.1 ppb but higher than those detected by **Taghreed., (1997)** recorded 0.063±0.28ppb, **Roushdy *et al.*, (1996)** who recorded 0.41 ppb and **Ali *et al.*, (2005)** who recorded levels ranged between 0.0098-0.0153 ppb) The findings of kofta are consistent with those reported by **Shaltout and Salem, (2000)** who recorded 3 ppb and **Alaa-eldin *et al*., (2015)** who recorded 2.43± 0.95, **Shabana *et al.*, (2008)** who recorded 6.70±0.89 ppb **.** As well as basterma samples results were nearly agreed with the previous work of **Seham *et al.*, (2013)** who recorded 2.3 ± 0.4 ppb and **Alaa-Elden *et al*, (2015)** who recorded 1.33± 0.33 while high incidence was established by **Refai *et al*., (2003)** who recorded 4.53± 0.61; **Howayda., (2008)** recorded 15.85ppb**.** In accord with these facts our results showed lower incidence in minced meat samples than those reported by **Howayda., (2008)** who detected levels ranged between 2.97-8.52ppb, **Shaltout and Salem., (2000)** who recorded 4.1 ppb but higher than data obtained by **Roushdy *et al., (*1996)** who recorded 0.88 ppb. The detected levels of AFs residues in the present samples are compared with the international permissible limits of WHO, FAO and FDA. The results revealed that, the mean of presented samples not exceed the permissible limits (20 ppb) and consequently they are fit for human consumption. From the present data, it could be concluded that, the presence of AFs residues in food of animal origin as meat products may be a result of spices and additives which are common substrates for *A. flavus*, while AFs production in these commodities is almost always a result of poor drying, improper handling, or storage in retail shops and become of public health hazard. Persistence of OTA in the food chain exposure to the compound is a potential human health hazard given that OTA has been experimentally shown to be teratogenic, a potent renal carcinogen, immunosuppressive, an enzyme inhibitor and has been implicated in Balkan endemic nephropathy, a disease characterized by progressive renal fibrosis in humans; it also has been associated with increased incidence of tumors of the upper urinary tract **Vrabcheva *et al*., (2000).**

Table (3) revealed the incidence of OTA in the examined meat products. It was obvious that the highest incidence of OTA was at beef burger which is higher than recorded by **Dalia, (2012)** who recorded higher incidence in basterma. The results of kofta and minced meat were extremely higher than those reported by **Shaltout and Salem, (2000)** (6.66%) while **Ali *et al*., (2005)** recorded (10%) in luncheon samples but **Shabana *et al.,* (2008)** not detected OTA in all tested samples. These different incidences of ochratoxin may be related to the amount of additives used in the processing, level of additives contamination with ochratoxin and the amount of ochratoxin residues which may be present in animal muscles. The present results in (Table4) were nearly similar to those reported by **Hassan *et al*., (1997) and Shaltout and Salem, (2000)** whose results were 1.5 ppb in minced meat and 6.8 ppb in kofta samples. In contrast to **Shabana *et al*., (2008)** who do not detect OTA residues in all tested samples. While, **Dalia, (2012)** recorded a higher result in Basterma and luncheon and 45% of sausage samples were positive to OTA with lowest value (3 ppb). **Lacumin *et al.,* (2009)** recorded higher levels (18 ppb). Complete elimination of mycotoxins from food commodities seems an impossible goal, it is important to ensure that levels should not threaten human health **Reddy *et al*., (2008).** Regarding to OTA, it is highly stable to heat treatment and not destroyed even at 200 C. In addition, OTA was classified as a possible human carcinogen (Group 2B) by International Agency for Research on cancer "IARC" **(IARC, 1993).** Occurrence of OTA in meat and meat products can be ascribed to an indirect transmission via the ingestion of OTA contaminated feed or to direct contamination due to mould growth in the outer layers of meat products. **Krogh., (1987)** reported that 36% and 19% of soybean and soya flour contained OTA in level of 500 ppt. in another hand, **Langseth., (1999)** reported that 15 out of 108 wheat samples contained OTA residues**. Rao., (2000)** said that 1 of 10 wheat samples and 3 from 28 mixed cereal samples were contaminated with OTA in Dubai. The frequent contamination of spices and additives used in such meat processing may also represent a source of mycotoxin **Refai *et al*., (2003)**. The obtained aforementioned data showed that all tested samples had OTA level lower than the permissible limit (5 ppb) **FAO.,(1995, 1997) ;WHO.,(2002)** and consequently they are fit for human consumption.

CONCLUSION

In conclusion, this study warrants the global public health hazards of consumption of contaminated meat products with mycotoxins. This requires a strict control against possible meat products contaminations with mycotoxins. It is also extremely important to maintain a safety low mode level of aflatoxin B_1 in the feeds of meat producing and dairy animals. The internationally-compatible continuous training and surveillance programs uprising among correspondent personnel are at most important to cope against possible contamination. More importantly, storage conditions of feeds must be widely available and be subjected to regular and serious investigation. It is necessary, however, to apply an ideal recommended limit of possible AFs and OTA contaminations in meat products. Application of Good Agricultural Practices and Good Veterinary Practices by agriculture and also the Hazard Analysis and Critical Control Points (HACCP) system as a draft code of practice for pre-harvest and postharvest control of cow's feed and in spices and meat products processing are effective.

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الكشف عن بقايا الافلاتوكسينات الكلية والاوكراتوكسين أ في.
بعض منتجات اللحوم

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الملخص العربى

. في هذه الدراسة تم فحص ٢٥٠ عينه من منتجات اللحوم المختلفة وهى لحوم مفرومه، لحوم البسطرمة , كفته , اللانشون و البيف برجر عشوائيا من اسواق عديده ممثله لمناطق مختلفة من محافظات (الجيزة والقاهرة) لاختبارها لمعرفه مدى وجود سموم الافلاتوكسينات والاوكراتوكسين أ وتحديد مستويات بقاياها باستخدام جهاز الأليزا التنافسي المباشر حسب تعليمات الشركة المجهزة لمواد الاختبار. أوضحت النتائج ان مدى تواجد سموم الافلاتوكسين كا 104 (83.2%) و105(84%) للاوكراتوكسين أ في محافظه الجيزة والقاهرة على التوالي. كما أظهرت نتائج فحص عينات منتجات اللحوم أن أعلى تركيز لبقايا الافلاتوكسينات كانت (0.041± 2.659) في عينات كفته الجيزة وأعلى تركيز للاوكراتوكسين أ (0.129 ±2.306) في عينات البسطرمة التي تم تجمعيها من القاهرة. وقد تم مناقشه اهميه تواجد السموم الفطرية الافلاتوكسينات والاوكراتوكسين أ من الناحية الصحية للمستهلك وكذلك التوجيه باتخاذ الممارسات الصحية السليمة للحفاظ على صحه المستهلك بتقديم منتجات لحوم مأمونه خاليه من مسببات الامراض.