Dept. of Food Hygiene,
Animal Health Research Institute, Alex. Lab., Egypt.

OCCURRENCE OF SOME MYCOTOXINS RESIDUES IN MEAT AND MEAT PRODUCTS WITH STUDYING THE EFFECT OF DIFFERENT FOOD PROCESSING METHODS ON MYCOTOXIN STABILITY IN MEAT SAMPLES

(With 5 Tables)

By

HANAA H.A. EL-MOSSALAMI

(Received at 10/2/2010)

تواجد بعض بقايا السموم الفطرية فى اللحوم ومنتجاتها مع دراسة تأثير معاملات الطهى المختلفة على ثباتها فى عينات اللحوم من عدمه

هناء حسينى أحمد سليم المسلمى

تم تجميع اثنتان وثلاثون عينة من اللحوم الطازجة واللحوم المجمدة واللانشون والبيف برجر (8عينات من كل نوع) تجميعا عشوائيا من أماكن مختلفة بمدينة الإسكندرية فى الفترة من شهر أكتوبر الى ديسمبر 2009 لقياس مدى تواجد بقايا السموم الفطرية اللأفلاتوكسين والزيرالينون باستخدام جهاز الفصل الكروماتوجرافي (HPLC)، حيث أظهرت النتائج أن أعلى كمية من بقايا الأفلاتوكسين وجدت فى عينات اللحوم المجمدة 0.87 ميكروجرام /كيلوجرام بينما أقل كمية وجدت فى عينات اللانشون 0.41 ميكروجرام /كيلوجرام ، بينما كانت أعلى كمية من بقايا الزيرالينون وجدت فى عينات اللانشون 2.96 ميكروجرام /كيلوجرام وأقل كمية وجدت فى عينات البيف برجر 2.15 ميكروجرام /كيلوجرام . بالنسبة لدراسة تأثير المعاملات الغذائية المختلفة للحوم علي ثبات السم الفطري الزيرالينون فقد تم حقن 15 ميكروجرام من الزيرالينون في قطعة من اللحم وزنها 200 جرام قبل التجميد عند -20°م ثم استخلاص وقياس كمية الزيرالينون بجهاز الفصل الكروماتوجرافي بعد يوم ثم بعد 30.21.14.7.4 وأخيراً 60 يوما علي التوالي، وأيضا اختبار تأثير المعالجة الحرارية علي تركيز الزيرالينون بطهي اللحوم وغليها عند درجة 100°م لمدة 45 دقيقة، وكذلك تأثير الشواء علي اللهب المباشر لمدة 11دقيقة0 وقد وجد أن كمية السموم المستخلصة تقل بدرجة محدودة جداً مما يعنى أن الزيرالينون مركب ثابت لا يتأثر بالمعاملات الحرارية المختلفة للحوم0 وقد تمت مناقشة الأهمية الصحية للسموم الفطرية وتأثيرها علي الصحة العامة وكذلك الاقتراحات التي يجب وضعها في الاعتبار لتحسين جودة اللحوم ومنتجاتها0

SUMMARY

Thirty-two samples of fresh meat, frozen meat, luncheon as well as beef burger; eight samples of each were randomly collected from different localities of Alexandria city. Collected samples were subjected to detect type of aflatoxin B1 and zearalenone residues were mycotoxins examination. Aflatoxin B1 and zearalenone residues were detected by High Performance Liquid Chromatography (HPLC) with the highest detector average value µg/kg for aflatoxin B1 was 0.87 (frozen meat) and the lowest was 0.41 (luncheon), the respective values for zearalenone were 2.96 (luncheon) and 2.15 (beef burger) µg/kg. In studying the effect of different food processing on stability of zearalenone in meat sample (a piece of fresh beef meat about 200 gm), the amount of zearalenone added to meat sample was 15µg prior freezing at -20°C and extracted, examined by HPLC after one day, 4,7,14,21,30 days and after two months for presence of mycotoxin zearalenone, which added to meat. In order to test the effect of heat treatment on the concentration of the zearalenone in meat were subjected to boiling (at 100°C for 45 minutes) and roasting (on direct flame for 11 minutes). Our results showed limited reduction in the percentage of zearalenone toxin, which is a stable compound and not affected by different food processing methods. The public health significance of the mycotoxins as well as the suggested measures for improving the quality of produced products has been discussed.

Key words: *Meat, meat products, mycotoxins, aflatoxin, zearalenone.*

INTRODUCTION

In recent years a world wide major research efforts aimed at the identification of the toxins produced by moulds growing on foods and feeds, the quantification of these toxins and an evaluation of their biological effects on humans and animals (Rywotycki, 2003; Jean-Denis and Philippe, 2009). Mycotoxins constitute a large number of naturally occurring fungal metabolites with very toxic effect on human and animals. They consist of groups of aflatoxins, trichothecenes, ochratoxins, vomitoxins, citrinin, patulin, penicillic acid and zearalenone (Bullerman, 2004; Toscani *et al.*, 2007; Amézqueta *et al.*, 2009).

Both intrinsic and extrinsic factors influence fungal growth and mycotoxin production on a given substrate. The intrinsic factors include water activity, PH and redox potential whereas extrinsic factors which influence mycotoxin production are relative humidity, temperature and

availability of oxygen (FAO, 1977; Croubels *et al.*, 2004; Evans *et al.*, 2007).

Mycotoxins are extremely harmful, sometimes lethal to animals and human beings. The toxic potential of several toxin-producing moulds or a limited number of mycotoxins may occurred due to contamination of human food supply whether by direct consumption of these agents or via domestic animals used for meat production (Richard, 2007; Sherif *et al.,* 2009). .

Mycotoxins play an important role in food borne diseases of human and animals.Their toxic effects depend on their composition, they can be hepatotoxic, nephrotoxic, neurotoxic, hemorrhagic, dermatoxic, genotoxic, teratogenic, carcinogenic or have hormonal effects (Bullerman, 2004)

Mycotoxins may also influence meat production and seem to be responsible for alteration of carcass composition and quality. They leave residues in edible tissues (López-Diaz *et al.,* 2001; Petzinger and Weidenbach, 2002). These residues had been detected within the animals exposed to mycotoxins: Aflatoxin B1 and its metabolites, for ochratoxin A and zearalenone. The amount of detectable residues depends on the initial feed contamination, duration of toxin exposure and persistence of the toxin within the animal (Rundberget and Wilkins, 2002; Garcia *et al.,* 2009).

Aflatoxicosis is food poisoning that results from ingestion of aflatoxins in contaminated food (Ali *et al.,* 2005). The aflatoxins are group of structurally related toxic compounds produced by certain strains of the fungi. Under favorable conditions of temperature and humidity, these fungi grow on certain foods, resulting in the production of aflatoxins. The species were the predominant aflatoxin-producing moulds isolated from processed meat products including luncheon. These toxins are usually found together in various proportions. However, aflatoxin B1 is usually predominant and is the most toxic (Aziz and Youssef, 1991; FDA, 1992).

Zearalenone (ZEN) is a potant toxic agent produced by several *Fusarium* species and found to be a common and wide spread contaminant in cereal grains and animal feed stuffs. Various health problems associated with this mycotoxin have been well documented in domestic animals including decreased feed intake, infertility, vaginal prolapse and enlargement of the uterus and mammary glands (Chu, 2003; Välimaa *et al.,* 2010). Furthermore, ZEN has been reported to be potentially carcinogenic. Consequantly, there is increasing concern about

the potential health hazard of ZEN to humans not only from direct exposure to contaminated cereal products, but also through consumption of meat derived from farm animals exposed to ZEN contaminated feeds (Sherif *et al.*, 2009). The main aim of this study survey on determination of mycotoxins (aflatoxin and zearalenone) residues in meat and meat products and effect of some food processing methods on the stability of mycoyoxin zearalenone in frsh meat.

MATERIALS and METHODS

Collection of samples: 32 samples of fresh meat, frozen meat, luncheon as well as beef burger; eight samples of each were randomly collected from different localities of Alexandria city (from October to December 2009).

Reagents:

- Zearalenone (Sigma chemical Co. St. Louis, Mo. USA).
- Aflatoxin B1, B2, G1, G2 (Sigma chemical Co. St. Louis, Mo. USA).
- Solvents–methanol (Burdick & Jackson Laboratories, Inc., Muskeyon, MI 49442).
- Water-glass-distilled, deionized
- Mobile phase: water – acetonitrile (55% - 45%)
- Chloroform-Aldrich Chemical Co., Inc.
- Delivery System: Tolune–Ethylacetate–Formic acid (60%-30%-10%).

Instrument: Beckman, high performance liquid chromatography delivery system (HPLC) was used in the High Institute of Public Health, Alexandria University.

1- Determination of aflatoxin and zearalenone residues in meat and meat products:

A- Extraction of aflatoxin and zearalenone from meat samples according to Roybul *et al.* (1988); Turner *et al.* (2009): The muscular tissues were thoroughly minced to obtain homogenous mixture, then 10 gm sample was weighted and added to 30 ml dist. water. The mixture was homogenate and 1 ml of homogenate was extracted by the following method: 3 ml of isopropand methylene chloride solution (1:9) was added to each test tube contained 1 ml homogenate. Samples were then put in shaker for about 15 min. Solvent layers were separated by centrifugation for 10 min at 2200 r.p.m. The upper organic layer was transferred to clean test tubes and the upper organic phase is saved. The remaining aqueous layer was extracted 2 more times by addition of 3 ml of isopropanol methylene chlorid and shaking centrifugation each time. After separation of the organic layer, the tubes were evaporated to

dryness by the aid of current of air and water bath at 40°C and the final residues was stored at -20°C until analysis.

B- Derivatives formation according to Ahmed (2004): The dansylated derivatives were formed by adding saturated sodium bicarbonate solution (0.5 ml) to the residue (dry film), stoppered and carefully mixed using vortex mixture.1ml of dansyle chloride reagent solution was added using a vortex mixture. After standing for more than 10 hours, the dansyle derivatives were extracted by adding 15 ml HPLC grade water and extracting the mixture with diethyl ether (3x5ml). The combined ether extracts were then evaporated to dryness.

C- Examination of the extract of aflatoxin: The dried samples under test were dissolved in one ml chloroform and 10 µl of each sample were injected in HPLC apparatus, the conditions were used as follows:

- **Flow rate:** 1 ml /minutes.
- **Column:** Reversed-phase Lichrosorp R, C18 steel column (4µm silica).
- **Mobile phase:** consisted of water - acetonitrile (55: 45, V/V).
- **Program:** The mobile phase solutions were sonicated in ultrasonic bath (Heat System Co., Farmingdale, N.Y.) for 15 minutes and filtered through 0.45µm filters from Millipore division (Water-Millipore, Milford, MA).
- **Detector:** The compounds eluted were detected by a 1050 series programmable variable multi-wavelength detector at 200 nm
- **Instrument model:** the output signal generated by the spectrophotometric detector was processed by Integrator Model 427

The concentration of aflatoxin B or G (a percent of recovery) was determined by HPLC using aflatoxin standard curve by comparing the peak areas of each sample extract to the peak areas of the standard solutions and the percent of recovery was calculated.

D- Examination of the extract of zearalenone according to Krska *et al.* (2007): The dried samples under test were dissolved in one ml methanol and 10 µl of each sample were injected in HPLC apparatus. The concentration of zearalenone was determined by HPLC using zearalenone standard curve.

F- Standard solution preparation according to Van Emond (1989): Accurately 10.0 mg of each reference standard were weighted into separate 100 ml volumetric flasks and diluted to volume with methanol and mixed with working solutions 1.0 µg/ ml. The dansyle derivatives were prepared as described above the residue was dissolved in 5ml methanol / chloroform.

2- Effect of some food processing methods on the stability of zearalenone in meat: The effect of some food processing methods on the stability of zearalenone in meat samples, test samples (a piece of fresh beef meat about 200gm), test material (zearalenone, Sigma Chemical Co. St. Lowis, Mo, USA) was studied. Experimental design: First of all the meat samples were tested for the presence of zearalenone residue according to Frisvad and Thane (1993). The NHEL (No Hormonal Effect Level) is 0.05 mg/kg. .

Sample preparation according to Roybul *et al.* (1988); Wilkes and Sutherland, (1998): Beef muscles were shopped into cubes and ground in warring blender. 200 gm ground muscle were weighted into whirl-pack bag then sealed and the tissue was kept until analysis.

Control sample: Sample of fresh beef muscle previously prepared was spiked with zearalenone standard and extracted then examined with HPLC without any food processing methods.

Preparation of the toxin according to Sørensen *et al.* (2008): Dry zearalenone was weighted,the concentration was calculated to give 15 µg/gm. Corn oil was added to the container of dry zearalenone. The mixture was agitated for1 minute and stored at 0°C.

Effect of freezing: Samples of previously prepared fresh beef muscles each 200 gm weight were spiked with zearalenone and put at -20 °C and extracted, examined by HPLC after one day, 4, 7,14, 21,30 days and then after two months for the presence of zearalenone. The final results were recorded and compared with the result of the control samples.

Effect of boiling (Skog *et al.*, 2000): To study the effect of boiling, samples of previously prepared fresh beef muscles each 200 gm weight were spiked with zearalenone and boiled at 100°C for 45 minutes, then extracted and examined by HPLC for the presence of zearalenone. The final results were recorded and compared with the result of the control sample.

Effect of roasting (Rywotycki, 2007): To study the effect of roasting on stability of zearalenone, samples of previously prepared fresh beef muscles each 200 gm weight were spiked with zearalenone and subjected to roasting on direct flame for 11 minutes, then extracted and examined with HPLC. The final results were compared with the results of the control samples.

Statistical analysis: The obtained data was analyzed statistically according to Perrie and Watson (1999)

RESULTS

Table 1: Determination of aflatoxin B1 in meat extracts.

Type of Samples	No. of samples	Aflatoxin positive samples		Amount of AFB1 µg / Kg		
		No .	%	Min.	Max.	Average
Fresh meat	8	ND	ND	ND	ND	ND
Frozen meat	8	2	25	0.72	1.02	0.87
Luncheon	8	1	12.5	0.41	0.41	0.41
Beef burger	8	1	12.5	0.59	0.59	0.59

ND = not detected

Table 2: Determination of zearalenone in meat extracts.

Type of samples	No. of samples	Zearalenone positive samples		Amount of ZEN µg / Kg		
		No.	%	Min.	Max.	Average
Fresh meat	8	1	12.5	3.25	3.25	3.25
Frozen meat	8	2	25	2.76	3.04	2.90
Luncheon	8	2	25	2.77	3.16	2.96
Beef burger	8	1	12.5	2.15	2.15	2.15

Table 3: Effect of freezing on stability of zearalenone in meat samples.

Amount of zearalenone added	Amount recovered after freezing							Control sample (sample + toxin without freezing)
	After 1 day	4	7	14	21	30	60	
15 µg	11.6	11.5	11.1	11	10.1	9.8	8.51	13.3
% of recovery	77.3	76.6	74	73.3	67.3	65.3	56.7	88.7
of reduction%	22.7	23.4	26	26.7	32.7	34.7	43.3	11.3

Table 4: Effect of boiling on zearalenone stability in fresh meat samples

Amount of zearalenone added	Amount recovered after boiling				Control sample (sample + toxin Without boiling)
	Sample 1	Sample 2	Sample 3	Sample 4	
15 µg	13.18	13.30	13.17	13.21	13.3
% of recovery	87.9	88.7	87.8	88.1	88.7
of reduction%	12.1	11.3	12.2	11.9	11.3

Table 5: Effect of roasting on zearalenone stability in fresh meat samples.

Amount of zearalenone added	Amount recovered after roasting		Control sample (sample + toxin without roasting)
	Sample 1	Sample 2	
15 µg	10.78	10.75	13.3
% of recovery	71.9	71.7	88.7
of reduction%	28.1	28.3	11.3

DISCUSSION

Aflatoxin extracted from meat samples:

Aflatoxins are considered as potent carcinogens and known to cause death in cattle. In addition, they may be involved in some human disease conditions (Atlas-Ronald, 1995). More specifically, AFB1, is one of the most potant aflatoxins. They are responsible for liver cancer in laboratory animals and even human-beings. They have been linked to a wide variety of human health problems. FDA (1992) established maximum allowable levels of total aflatoxins in food commodities at 20 ppb (Bahgat, 1999).

Our results revealed that aflatoxins failed to be detected in any of the examined fresh meat samples. In frozen meat samples 2(25%) out of 8 tested samples were positive and the amounts of aflatoxin B1residues were 0.72 and 1.02 µg/kg (Table 1). Nearly similar results were obtained by Moustafa (1994) who recorded 1.07 µg/kg. Our results are lower than those of Fargaly (1998) and Altalhi and Albashan (2004) who recorded 2.63 and 3.6 µg/kg, respectively.

As regarded to luncheon out of 8 samples tested 1(12.5%) was positive and the amount of aflatoxin extracted from the positive luncheon sample was 0.41 µg/kg. Our results are lower than those of Aziz and Youssef (1991), Ismail and Zaky (1999) and Ali *et al.* (2005) who recorded 4, 2.23 and 1.53 µg/kg, respectively. Nearly similar results were obtained by Moustafa (1994) and Zaky *et al.* (1995) who recorded 0.46 and 0.35 µg/kg.

Concerning beef burger, only one sample (12.5%) was positive and the amount of aflatoxin was 0.59 µg/kg. Nearly similar result was obtained by Moustafa (1994) who recorded 0.68 µg/kg. On the other hand, Aziz and Youssef (1991) recorded higher results 8 µg/kg.

In looking to (Table 1) we find that the amount of aflatoxin extracted from fresh meat, frozen meat, luncheon and beef burger samples were small as compared with the limit permissible 0.02 mg/kg (Bahgat, 1999) but in spite of these results it is still very dangerous, the presence of aflatoxin residue in meat although over toxicosis resulting from ingestion of residue contaminated food products is a relatively uncommon occurrence, the long term health effect from repeated exposure to low concentrations of certain chemical are of much greater concern (Stephen and Charles, 1985; Atlas-Ronald, 1995; Herzallah, 2009).

The mode of action of aflatoxin B1 and the activities of its biologically active intermediates by binding of compounds to protein, DNA and RNA can interfere with normal cellular functions, resulting in initiation of carcinogenesis or necrosis if the interference is sever enough. Most reports support the feed for activation of aflatoxin B1 by microsomal enzymes for binding of AFB1 to macromolecules, as well as for carcinogenesis or mutagenesis (Kolb, 1984; Hsieh and Atkinson, 1991; Farhadian *et al.*, 2010).

From the public health point of view, the presence of natural toxicants, such as mycotoxins, in animal food products is greatly affect human health. At high levels in feeds, these mycotoxins may cause loss or illness of farm animals, through development of animal toxicosis such as aflatoxicosis. At lower levels in feed, these mycotoxins may have no apparent effect on live stock production, but their residues and related substances from the consumption of animal food products may pass a health to humans. These health hazards as compared with the possible health hazards from the direct intake by humans of cereal and other food crops that contain mycotoxins (Kuiper-Goodman, 1991; Deneo-Pellegrini *et al.*, 1996; Dalié *et al.*, 2010). In addition, aflatoxins have

been incriminated mainly on circumstantial evidence, hepatocellular carcinoma, acute hepatic failure and other possible effects of continuous or intermittent dietary exposure to aflatoxins. Such exposure has serious implications for immune and hepatic functions and is detrimental to growth and development (Pestka and Bondy, 1990; Hendrickoe, 1991; Bahgat, 1999; Bouhet and Oswald, 2005).

Zearalenone extracted from meat samples:

The recorded data in Table 2 showed that only one sample (12.5%) was positive in a concentrated of 3.23 µg/kg for zearalenone in fresh meat.These results are lower than those reported by Abdel Hamid (1990) and Moustafa (1994) who recorded 4.11and 3.09 µg/kg. In frozen meat samples 2 (25%) out of 8 tested samples in a concentrated of 3.04 and 2.76 µg/kg were positive. Nearly similar results were obtained by Ramadan (1990) and Moustafa (1994) who recorded 3.11 and 2.65 µg/kg. As for luncheon samples 2 (25%) were positive for zearalenone the levels in a concentrated of 2.96 µg/kg. Nearly similar results were obtained by Abdel Hamid (1990) and Moustafa (1994) who recorded 2.86 and 3.01 µg/kg. In beef burger samples for zearalenone could be detected in 1(12.5%) was positive where its amount in a concentrated of 2.15 µg/kg. These results are higher than those reported by Moustafa (1994) who recorded 1.87 µg/kg. .

Our results revealed that the amount of zearalenone detected were less than the permissible limit which was 0.05 mg/kg (Bahgat, 1999). But as a zearalenone is a mycotoxin found in grain products and it is anabolic veterinary drug used commercially in cattle and sheep for increasing the rate of weight gain and improving feed efficiency, this involves possible health risks if harmful residues remain in the meat and meat products intended for human consumption (Anon, 1986; Cavret and Lecoeur, 2006). Many countries prohibit or restrict the use of any hormone active substance for growth promotion and fattening in food producing animals (Jose *et al.,* 1987; Krska *et al.,* 2007).

Zearalenone and some of its derivatives have been shown to competitively bind to estrogen receptors in a number of in vitro system. Binding to specific receptors has been demonstrated in rat, mouse and bovine uterus, in rat mammary gland, in human breast cancer cells, in rat mammary tumor and in rat hypothalamus. Binding also occurs to specific receptors in rat liver (Powell-Jones *et al.,* 1981). Synthesis of uterine estrogen-induced protein (IP), is one of the earliest events following estrogen binding (Reiss and Kaje, 1981). An increase in the uterine RNA synthesis as well as an increase in the RNA polymerase

activity was observed following the interperitoneal administration of zearalenone to rats (Tashiro *et al.*, 1980; Kolb, 1984; Ting Zhou *et al.*, 2010).

The effect of zearalenone in most animals is indistinguishable from the effects of large doses of estrogen. The resulting syndrome is called hyperestrogenism and clinical signs appear as abnormal reproductive activity, including prolonged estrus, anestrous, infertility, increase udder or mammary development, abnormal lactation. Abortion, mastitis, vulvovaginitis, hemorrhage and rectal or vaginal prolapses are thought to be secondary complications (Stoloff *et al.*, 1981). If it is ingested in sufficient quantity, it can severely damage the entire digestive tract and cause rapid death due to internal hemorrhage. zearalenone has been implicated in human diseases alimentary toxic aleukia and pulmonary hemosiderosis (Haschek *et al.*, 2002; Massart *et al.*, 2008).

Effect of different food processing methods on Zearalenone stability in meat samples:

Effect of freezing: Table 3 shows when15 µg of zearalenone were added to 200 gm meat sample and exposed sample to freezing for one day11.6 µg were recovered and the percent of recovery was 77.3 and that of reduction was 22.7. After 4 days the amount recovered was 11.5 µg percent of recovery 76.6, percent of reduction was 23.4. After 7days the amount recovered was 11.1 µg with a percent of recovery 74, a reduction percent of 26. After 14 days the amount recovered was 11 µg percent of recovery was 73.3, percent of reduction was 26.7. After 21 days the amount recovered was 10.1 percent of recovery was 67.3, percent of reduction was 32.7. After 30 days the amount recovered was 9.8 µg, percent of recovery was 65.3, percent of reduction was 34.7. After 60 days the amount recovered was 8.51, percent of recovery was 56.1, percent of reduction was 43.3. our results reveal that very little decrease in the amount of zearalenone spiked in meat samples after almost 60 days study, this means that zearalenone is a stable compound and this agree with what was reported by Mastura *et al.* (1981); Skog *et al.* (2000); Rywotycki (2007).

Effect of boiling: The amount of zearalenone revealed in meat samples subjected to boiling for 45 minutes was 13.18 µg with a percent of recovery 87.9 and the percent of reduction 12.1. For sample 2, the amount of zearalenone found was 13.30 with a percent of recovery 88.7 and a reduction percent 11.3. For sample 3, the amount of zearalenone found was 13.7 with a recovery percent 87.8 and reduction percent 12.2,

for sample 4 the amount of zearalenone found was 13.21 with a recovery percent 88.1 and reduction percent was 11.9. From the above mentioned results we found that the reduction percent was very limited and most of toxins were heat stable. This means that heating at 100°C for 45 minutes does not affect the zearalenone compound. These results agree with that reported by Bata and Orsi (1981); Skog *et al.* (2000); Zinedine and Mañes (2009).

Effect of roasting: The results were, for sample one, the amount of zearalenone found was 10.78 with a recovery percent 71.9 and a reduction percent 28.1. For sample two, the amount of zearalenone found was 10.75 with a recovery percent 71.7 and reduction percent 28.3. These results agree with that reported by Rywotycki (2007) and Faradian *et al.* (2010).

By comparing the above mentioned results with the control sample, we found that only small amount of zearalenone toxin was affected by roasting which means that roasting has a processing limited effect on zearalenone stability. By studying the effect of roasting on different food, we found that zearalenone toxin is a stable compound and is not affected by different food processing. This means that once the toxin was found in meat it is very difficult to get rid of it which means that the only way to control this toxin is by primary preventing its existence in meat. As some animals are sensitive to concentrations in the feed as low as 0.01 ppm (Mirocha *et al.,* 1983; Honikel, 2003; Fuchs *et al.*, 2008), exposure of animals to zearalenone should be kept as low as possible, food sources must be controlled and presence of small quantities of zearalenone in food must be restricted. However residues of carcinogenic mycotoxins, such as aflatoxin B1 and zearalenone, when present in animal products pose a threat to human health and their levels should be monitored and controlled (Chu, 1991; Hussein and Brasel, 2001; Acosta *et al.,* 2009). Because mycotoxins are natural contaminants of food and their exposure is more likely to occur in world where poor methods of food handling and storage are common, their formation is unavoidable and methods of controlling are usually preventive (Horchner *et al.,* 2006).

REFERENCES

Abdel Hamid, A.M. (1990): Occurrence of some mycotoxins (Ochratoxin A, Citrinin, Zearalenone and Vomitoxin) in various Egyptian Feeds, Arch-Tierermahr, 40(7): 247-264.

Acosta, R.; Rodriguez-Martin, A.; Martin, A.; Nűñez, F. and Asensio, M. A. (2009): Selection of anti fungat protein-producing molds from dry-cured meat product. International Journal of Food Microbiology, 135(1): 39-46.

Ahmed, I.A. (2004): Mycotoxins / Detection and Analysis by Classical Techniques. Encyclopedia of Food Microbiology, P. 1526-1532.

Ali, H.M. Fatma; Farghaly, M.R. and Hammad, A.M. (2005): Mycological investigations in beef and chicken luncheon. Beni-Suef Vet. Med. J. 15(2): 98-102.

Altalhi, A. and Albashan, M. (2004): Mycological study on fresh and frozen meat in Taif City, Saudia Arabia. Assiut Vet. Med. J. 50(102): 22-32.

Amézqueta, Susana; González-Peñas, Elena; Murillo-Arbizu, Maria and de Cerain, Adela, López (2009): Ochratoxin A decontamination: Areview. Food Control, 20(4): 326-333.

Anon, M.N. (1986): Methods for the Microbiological Examination of Foods. The American Public Health Association, Washington, D.C.

Atlas-Roland, M. (1995): Hand book of Microbiological of Food. 2nd, (ed.) RC Press. Inc. Florida USA.

Aziz, N.H. and Youssef, Y.A. (1991): Occurrence of aflatoxins and aflatoxins-producing moulds in fresh and processed meat in Egypt. Food Addit. and Contam., 8(3): 321-332.

Bahgat, M.F. (1999): Mycotoxins. A potential Universal everlasting threat: "Consumer Perspective" 5th ed. Sci. Cong. Egyp. Soc. Cattle Dis. Assiut, P., 22-35.

Bata, A. and Orsi, F. (1981): Stability of zearalenone with respect to the possibility of detoxication. Acta Aliment, 10: 161-162.

Bouhet, S. and Oswald, I.P. (2005): Contaminants on the intestinal epithelial cell-derived innate immune response.Veterinary Immunology and Immunopathology, 18 108(1-2): 199-209.

Bullerman, L.B. (2004): Mycotoxins: Classification. Encyclopedia of Food Microbiology, P. 1512-1520.

Cavret, S. and Lecoeur, S. (2006): Fusariotoxin transfer in animal. Food and Chemical Toxicology, 44(3): 444-453.

Chu, F.S. (1991): Mycotoxins: Food contamination mechanism, carcinogenic potential and preventive measures. Mutation Research\ Genetic Toxicology, 259(3-4): 291-306.

Chu, F.S. (2003): Mycotoxins / Toxicology. Encyclopedia of Food Science and Nutrition, P. 4096-4108.

Croubeles, S.; Daeseleire, E.; De Baere, S.; De Backer, P. and Courtheyn, D. (2004): Residues in Meat and Meat products Feed and Drug Residues. Encyclopedia of Meat Sciences, P. 1171-1187.

Dalié, D.K.D.; Deschamps, A.M. and Richard-Forget, F. (2010): Lactic acid bacteria-Potential for control of mould growth and mycotoxins: A review. Food Control, 21(4): 370-380.

Deneo-Pellegrini, H.; De Stefani, E.; Roco, A.M. Maria and Carzoglio, J.C. (1996): Meat consumption and risk of lung cancer; A case-control study fromUruguay. Lung Cancer, 14(2-3): 195-205.

Evans, T.J. and Gupta, R.C. (2007): Tremogenic mycotoxins. Veterinary Toxicology, P.1004-1010.

FAO, (1977): Food Agriculture Organization "The occurrence of Aflatoxin and Patulin". Conference on Mycotoxin, 3: 321-325.

Farhadian, A.F. Saneh; Jinap, S.; Abas, F. and Sakar, Z.I. (2010): Determination of polycyclic aromatic hydrocarbons in grilled meat. Food Control, 21(5): 606-610.

Faragaly, R.M. (1998): Some studies on the aflatoxin-producing aspergillii in meat-cold stores. Assuit Vet. Med. J. 38(76): 111-120.

FDA, (1992): Food and Drug Administration "Aflatoxins and Aflatoxicosis". Food borne Pathogenic Microorganisms and Natural Toxins 2nd ed. Book. USA.

Frisvad, J.C. and Thane, U. (1993): Liquid colum chromatography of mycotoxins. Journal of Chromatography Library, (54): 253-372.

Fuchs, S.; Sontag, G.; Stidl, R.; Ehrlich, V.; Kundi, M. and Knasműller, S. (2008): Detoxification of Patulin and Ochratoxin A, Two abundant mycotoxins, by Lactic acid bacteria. Food and Chemical Toxicology 46(4): 1398-1407.

Garcia, Daiana; Ramos, Antonio, J.; Sanchis, Vicente and Marin, Sonia (2009): Predicting mycotoxins in foods: Areview. Food Microbiology, 26(8): 757-769.

Haschek, Wanda, M.; Voss, Kenneth, A. and Beasley, Val R. (2002): Selected Mycotoxins Affecting Animal and Human Health. Handbook of Toxicologic Pathology (2nd Edition), P. 645-699.

Hendrickoe, R.G. (1991): Clinical implications of food contaminated by aflatoxins. Ann. Acad. Med. Singapore, 20(1): 84-90.

Herzallah, Saqer, M. (2009): Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UVdetectors. Food Chemistry, 114(3): 1141-1146.

Honikel, K.O. (2003): Man-made residues and environmental contaminant in Meat. The 2nd Int. Cong. Of Food Hyg. And Human Health. Fac. Vet. Med., Assiut Univ., PP.: 54-63.

Horchner, P.S.M.; Brett, D.; Gormley, B.; Jenson, L. and Pointon, A.M. (2006): HACCP-based approach to the derivation of an on-farm food safety program for the Australlian red meat industry. Food Control, 17(7): 497-510.

Hsieh, D.P. and Atkinson, D.N. (1991): Disfuranoid mycotoxins, their genotoxicity and carcinogenicity. Adv. Exp. Med. Biol., 283: 525-532.

Hussein, H.S. and Brasel, J.M. (2001): Toxicity, metabolism and Impact of mycotoxins on humans and animals. Toxicology, 167(2): 101-134.

Ismail, M.A. and Zaky, Z.M. (1999): Evaluation of the mycological status of Luncheon meat with special reference to aflatoxigenic moulds and aflatoxin residues: Mycopathologia, 146(3): 147-154.

Jean-Denis, B. and Phillippe, G. (2009): Mycotoxins in meat and Processed Meat Products. Safety of Meat and Processed Meat, Food Microbiology and Food Safety, Springer-Verlag New York, P. 83.

Jose, A.D.; Hocking, A.D. and Pitt, J.I. (1987): The mycoflora of some Australian food. Food Technology in Australia, 33(2): 55-60.

Kolb, E. (1984): Recent Knowledge on the mechanism of action and metabolism of mycotoxins. Z. Gesanate. Inn. Med., 39(15): 353-358.

Krska, R.; Welzig, E. and Boudra, H. (2007): Analysis of Fusarium toxins in feed. Animal Feed Science and Technology, 137 (3-4): 241-264.

Kuiper-Goodman, T. (1991): Risk assessment to humans of mycotoxins in animal derived food products. Vet. Hum. Toxicol., P. 325-332.

López-Diaz, Teresa-Maria; Santos, Jesús-Angel; Garcia- López, Maria-Luisa and Otera, Andrés (2001): Surface mycoflora of a Spanish fermented meat sausage and toxigenicity of *Penicillium* isolates. International Journal of Food Microbiology, 68(1-2): 69-74.

Massart, F.; Meucci, V.; Saggese, G. and Solidani, G. (2008): High growth rate of girls with precocious puberty exposed to estrogenic mycotoxins. The Journal of Pediatrics, 152(5): 690-695.

Mastura, Y.; Yoohizama, T. and Merooka, N. (1981): Effect of food additives and heating on the decomposition of Zearalenone in wheat flour. J. Food- Huj. Soc. Japan, 22: 293-298 (in Japanees).

Mirocha, C.J.; Pawlosky, R.A.; Chatterjee, K.; Watson, S. and Hayes, W. (1983): Analysis for *Fusarium* toxins in various samplesimplicated in biological narfare in South East Asia. J. Assoc. Off. Anal. Chem., 66(6): 1285-1299.

Moustafa, R.M.K. (1994): Mycotoxin residues in meat and meat products. Ph.D. Thesis, Department of Food Hygiene, Fac, Vet. Med., Cairo Univ., Egypt.

Perrie, A. and Watson, L. (1999): Statistics in Veterinary and Animal's Science 1st Ed. The Bark well Science. Rd, UK.

Pestka, J.J. and Bondy, G.S. (1990): Alteration of immune function following dietary mycotoxin exposure. Can. J. Physiol. Pharmacol., 68(7): 1009-1016.

Petzinger, E. and Weidenbach, A. (2002): Mycotoxins in the food chain: The role of Ochratoxins. Live stock Production Science, 76(3): 245-250.

Powell-Jones, W.; Rae-Fold, S. and Lucier, G.W. (1981): Binding properties of Zearalenone mycotoxin to hepatic estrogen receptors. Mol. Pharmacol., 20: 35-42.

Ramdan, M. (1990): Toxin producing fungi in imported frozen meat. M.V.Sc. Thesis Vet. Med. Cairo, Univ.

Reiss, N. and Kaeje, A.M. (1981): Identification of the major component of the estrogen induced protein of rat uterus. J. Biol. Chem., 265: 5741-5749.

Richard, John, L. (2007): Some major mycotoxins and their mycotoxicoses An over view. International Journal of Food Microbiology, 119(1-2): 3-10.

Roybul, J.E.; Munns, P.K.; Moris, W.J.; Hurlbat, J.A. and Sbimoda, W. (1988): Determination of Zeronol / Zearalenone and their metabolites in edible animal tissue by liquid chromatography. J. Assoc. Off. Anal. Chem., 71(2): 263-277.

Rundberget, Thomas and Wilkins, Alistair, L. (2002): Determination of *Penicillium* mycotoxins in foods and feeds using Liquid Chromatography-mass Spectrometry. Journal of Chromatography A, 964(1-2): 189-197.

Rywotycki, R. (2003): Meat nitrosamine contamination level depending on animal breeding factors. Meat Science, 65(1): 669-676.

Rywotycki, R. (2007): The effect of baking of various kinds of raw meat from different animal species and meat with functional additives on nitrosamine contamination level. Food Chemistry, 101(2): 540-548.

Sherif, S.O.; Salama, E.E. and Abdel-Wahhab, M.A. (2009): Mycotoxins and Child health: The need for health risk assessment. International Journal of Hygiene and Environmental Health, 212(4): 347-368.

Skog, K.; Solyakova, A. and Jâgerstad, M. (2000): Effects of heating conditions and additives on the formation of heterocyclic amines with reference to amino-carbolines in a meat Juice model system. Food Chemistry, 68(3): 299-308.

Sørensen, L.M.; Jacobsen, T.; Nielsen, P.; Frisvad, J.C. and Koch, A.G. (2008): Mycobiota in the processing areas of two different meat products. International Journal of Food Microbiology, 124(4): 58-64.

Stephen, G. and Charles, W.G. (1985): Some moulds associated with meat in cold storage lokess in Iowa. Proc. Acad. Sci., 44: 37-43.

Stoloff, L.; Wood, G. and Corter, L. (1981): Zearalenone, An estrogenic toxin in: Veterinary Pharmacology and Therapeutics. J. Dairy Sci., 64: 2426-2430.

Tashiro, F.; Kawabata, Y.; Noor, M. and Ueno, Y. (1980): Zearalenone estrogen receptor interaction and RNA synthesis in rat uterus. In medical mycology. Supl. 8 pp. 31-320 Fischer. Stuttgart / New York.

Ting Zhou, Jianwei He; Christopher, Young, J. and Peter, M. (2010): Chemical and biological transformations for detoxification of Trichothecene mycotoxins in human and animal Food Science and Technology, 21(2): 67-76.

Toscani, T.; Moserita, A.; Dossena, A.; Dall' Asta, C.; Simoncini, N. and Virgili, R. (2007): Determination of Ochratoxin A in dry-cured meat products by a HPLC-FLD quantitative method. Journal of Chromatography B, 855(2): 242-248.

Turner, N.W.; Subrahmanyam, S. and Piletsky, S.A. (2009): Analytical methods for determination of mycotoxins: A review. Analytica Chimica Acta, 632(2): 168-180.

Välimaa, Anna-Liisa; Kivistä, Anniina, T.; Leskinen, Piia, I. and Karp, Matti, T. (2010): A noval biosensor for the detection of Zearalenone family mycotoxins in milk. Journal of Microbiological Methods, 80(1): 44-48.

Van Emond, H.P. (1989): Current situation on regulations for mycotoxins overviews of tolerance and status of standard methods of sampling and analysis. Food Additives and Contaminants, 6: 139-188.

Wilkes, Jon, G. and Sutherland, John, B. (1998): Sample preparation and high-resolution separation of mycotoxins possessing carboxyl groups. Journal of chromatography B: Biomedical Sciences and Applications, 717(1-2): 135-156.

Zaky, Z.; Ismail, M. and Refaie, R. (1995): *Aspergillus flavus* and aflatoxin residues in Luncheon meat. Assiut Vet. Med. J., 33(66): 114-118.

Zinedine, A. and Mañes, J. (2009): Occurrence and Législation of mycotoxins in food and feed from Moroco. Food Control, 20(4): 334-344.