Mould And Ochratoxin A In Soybean And Luncheon

Alaa M. Morshdy, Mohamed A. Hussein, kamal El-Desoky and El-Sayed S. El-Sebaey
Faculty of Vet. Medicine, Zagazig University

ABSTRACT

Sixty samples of soybean protein, poultry luncheon and beef luncheon (20 of each) were examined for determination of total mould count and ochratoxin residues. The mean mould counts of were $2.6 \times 10^3 \pm 7.2 \times 10^2$, $1.6 \times 10^2 \pm 0.46 \times 10^2$ and $1.7 \times 10^2 \pm 0.54 \times 10^2$/gm of the examined sample, respectively. The mean of ochratoxin residues in examined samples were 4.23 ± 0.81, 3.06 ± 0.68 and 3.77 ± 0.35 ppb. *As. niger, As. flavus, As. fumigatus, As. terrus, As. ochraechous, penicillium, alternaria, cladosporium, mucor, absidia* were the predominant isolates with varying percentages.

INTRODUCTION

The spores of moulds are always present in the environment and they enable the moulds to survive even in extreme condition. Therefore, it is practically impossible to eliminate them from food. In general the conditions during producing of meat products (ambient temperature, relative humidity, air circulation) are suitable for the development of mould. Consumption of food contaminated with moulds and their toxic metabolites results in development of food-borne mycotoxicosis. The spores of moulds are ubiquitously spread in the environment and can be detected everywhere. *(1).*

Soybean was introduced as a food source in the eastern half of north China in the 11th century B.C. *(2).* Soybean considered as an important animal protein substitute (as filler) so it used in wide scales in production of meat products. Luncheon is a product resulting from mixing of minced meat with filling materials which are added (salt - carbohydrates - dried milk - soyflour - spices), where the end product obtained after cooking give the physical characteristics of luncheon.

Mould toxicity has attracted attention, especially in the fields of agriculture and food industry. Microscopic filamentous fungi often contaminate vegetable and animal products, becoming a source of diseases in man and slaughter animals *(3).* Ochratoxin is one of the most abundant food-contaminating mycotoxins in the world. Human exposure occurs mainly through consumption of improperly stored food products, particularly contaminated grain.

Ochratoxin A (OTA), produced by *Aspergillus ochraceus* and *Penicillium verrucosum*, these fungi are ubiquitous and the potential for contamination of foodstuffs and animal feed is widespread.

Ochratoxin A, the major compound, has been found in more than 10 countries in Europe and the USA. Ochratoxin formation by Aspergillus species appears to be limited to conditions of high humidity and temperature, whereas at least some Penicillium species may produce ochratoxin at temperatures as low as 5° C. OTA has been implicated in a human disease of kidney referred to as Balkan endemic nephropathy, characterized by tubular interstitial nephritis and associated with high incidence of kidney, pelvis, ureter and urinary bladder tumors in some Eastern European countries *(4).*

Recently, the European Food Safety Authority (EFSA) has proposed a new safety value of 120 ng OTA/kg body weight as a tolerable Weekly intake, which corresponds to a TDI of 17.1 ng/kg body weight *(5).* In North African countries the most suspected foods susceptible to be contaminated by OTA are domestic and imported cereals such as wheat and sorghum, olives, poultry products, and spices *(6).* Published data suggested the

evidence association of elevated exposure to OTA with cases of human nephropathies in Tunisia and Egypt *(7,8).* In Morocco, about 2 million of people suffer from chronic diseases of kidney including chronic renal insufficiency and chronic interstitial nephropathy especially in youngs from both sexes. However the etiology of the diseases is not well established. A preliminary survey reported that the Moroccan population could be exposed to OTA *(9).*

MATERIALS AND METHODS

A total of 60 samples of Soybean protein, poultry luncheon and beef luncheon (20 of each) were collected from different markets from Sharkia Governorate. The samples were taken aseptically in sterile polyethylene bags and transferred to centeral laboratory; Faculty of Veterinary Medicine, Zagazig University, and then the following investigation were carried out:

1. Estimation of total mould count in soybean & luncheon *(10).*

2. Mould identification

The colonies of the moulds grown on malt agar were inoculated onto three different agars: czapeck Dox agar, malt agar and malt agar containing 6% Nacl. The moulds were identified based on morphology and growth characteristics according to *(11-15).*

3. Quantitative detection of Ochratoxin residue in examined samples *(16).*

1 50 gm of the samples mixed with 5gm NaCl and 80 ml methanol + 20 ml distilled water.

2 filteration

3 10 ml of the filtrate + 40 ml distilled water followed by another filteration using microfibre filters.

4 10 ml of the filtrate pass over the affinity column (ochratest TM) produced by VICAM (313pleasant street, Watertown, MA 02472 USA. www. Vicam.com).

5 The column washed with 10 ml (8ml wash buffer+ 2 ml water).

6 Elution of Ochratoxin from the column was carried out using 1.5 ml eluting solution and collect in a cuvette.

7 The cuvette was calibrated in flurometer and results are calculated in ppb.

RESULT AND DISCUSSION

A- Mould in examined samples

The results achieved in Table 1. revealed tht sixty samples of soybean, poultry luncheon and beef luncheon, 20 of each, used for determination the average number of filamentous fungi. The contamination varied between the examined samples. The mean $\pm$ standard error of examined Soybean sample was $2.6x10^3 \pm 7.2 x10^2$, while that of poultry luncheon were $1.6x10^2 \pm 0.46 x10^2$. On the other hand mold counts was $1.7x10^2 \pm 0.54 x 10^2$ in examined meat luncheon. These counts were nearly similar to that obtained was recorded by several studies *(17,18)* on contrary higher results were recorded *(19).* The contamination load related to the hygienic level of the supermarket where slicing happening and no significant different between poultry and beef luncheon but examination of soybean revealed a higher counts .This may be attributed to the heat treatment of luncheon during processing which destructed the filamentous fungi in addition to the chemical added during processing of luncheon.

The results given in Tables 1,2 showed that the diversity of isolated fungi was relatively high, thus probably reflects that fungal conidia are air - borne and are therefore easily spread. Important reservoir can be humans, soil, dust, raw materials, drains equipment surfaces and ventilation duct *(20).*

Aspergillus niger could be isolated from soybean, poultry luncheon and beef luncheon with a percentages of 14 (70%), 9 (45%) and 6 (30%), while the numbers of isolates were 16 (25%), 16 (26.2%) and 14 (37.8%), respectively. *Aspergillus flavus* could be isolated with a percentages of 2 (10%), 3 (15%) and 4 (20%) while the number of isolates were 4 (6.25%), 3 (4.9%) and 4 (10.8%), respectively.

Aspergillus fumigates could be isolated with percentages of 2 (10%), 2 (10%) and 3 (15%) while the number of isolates were 2 (3.12%), 6 (9.8%) and 3 (8.1%), respectively. On the other hand *Aspergillus terrus* isolated with a percentages of 4 (20%) , 2 (10%) and 1 (5%) and the number of isolates were 8 (12.5%), 6 (9.8%) and 2 (5.4%), respectively while *Aspergillus ochraechous* only isolated from soybean with a percentages of 8 (40%) and number of isolates were 12 (18.75%) .

Aspergillus found on various substrates and is common indoor air ***(21)***. The occurrence is typical high in warmer climates where Aspergillus occur on meat products ***(22)***.

Penicillium were isolated frequently with percentages of 10 (50%), 12 (60) and 6 (30%) and number of isolates were 8 (12.5%), 14(18%) and 6 (16.6%), respectively of examined soybean, poultry luncheon and beef luncheon Penicillium species are common on various substrates including processed meat ***(15)***.

Alternaria was isolated with incidence 4 (20%), 2 (10%) and 1 (5%), the numbers of isolates were 8 (12.5%), 6 (9.8%) and 2 (5.5%) of Soya bean, poultry luncheon and beef luncheon, respectively. *Alternaria* has been recorded from cold stored and frozen meat ***(23)***. Cladosporium was obtained from Soybean, poultry luncheon and beef luncheon with incidence of 3 (20%), 2 (10%) and 1 (5%), the number of isolates were 4 (6.25%), 12 (19.6%) and 3 (8.3%), respectively. Caldosporium was obtained and less frequent from luncheon meat ***(19)*** and from other meat products such as sausage, beef burger, basterma , minced meat and frozen meat ***(25)***. Mucor isolated only from Soybean sample with incidence of 2 (10%) and number of isolates were 2 (3.12%), while Absidia only detected with incidence of 1 (5%) from meat luncheon.

The unidentified species from soybean samples were 5 (25%) which illustrates the effect of heat treatment that destruct the most species and only a few species contaminate the processed product after processing from the environment.

Ochratoxin residues in examined samples

Ochratoxin could be produced by different moulds as *Aspergillus ochraechous* and *penicillium verrucosum* when they grow on the surface of foods such as sausage, dry ham and other meat products during both ripening and storage ***(25)***.

Ochratoxin residues were detected in all examined samples within varying limits. Table 4 showed that the minimum, maximum and mean $\pm$ SE in examined soybean were 2.8, 6.8 and 4.32 $\pm$ 0.81 ppb, 1, 4.7 and 3.06 $\pm$ 0.68 ppb in examined poultry luncheon, while in meat luncheon were 3.3 , 5.51 and 3.77 $\pm$ 0.35 ppb. Ochratoxin residues were detected on the casing of fermented sausage within 3 to 18 ppb during processing ***(26)***.

The number of samples which exceed the maximum permissible limite in soybean were 2 samples, while only one sample exceed the maximum permissible limite in meat luncheon.

We can concluded that the additive must be of high quality to obtain safe product and must be examined for determination of ochratoxin residues before entering in luncheon processing.

Table 1. Statistical analytical results of total mould count/gm of examined samples (N= 20).

	Soya bean	Poultry luncheon	Beef luncheon
Min.	$2x10^2$	10	3x10
Max.	$6x10^3$	$4x10^2$	$6x10^2$
Mean	$2.6x10^3$ **	$1.6x10^2$ NS	$1.7x10^2$ NS
SE	$7.2x10^2$	$0.46x10^2$	$0.54x10^2$

NS : Non significant correlation. ** : Significant correlation at $p < 0.01$
SE : Standard error. N : Number

Table 2. Incidence of isolated moulds in examined samples (N= 20).

Fungal strain	Soya bean		Poultry luncheon		Beef luncheon	
	No.	%	No.	%	No.	%
As. niger	14	70	9	45	6	30
As. flavus	2	10	3	15	4	20
As. fumigatus	2	10	2	10	3	15
As. terrus	4	20	2	10	1	5
As. ochraechous	8	40	-	-	-	-
Penicillium	10	50	12	60	6	30
Alternaria	4	20	2	10	2	10
Cladosporium	3	15	2	10	1	5
Mucor	2	10	-	-	-	-
Absidia	-	-	-	-	1	5
unidentified spp.	5	25	-	-	1	5

Table 3. Summarized numbers and percentages of identified mould isolates in examined samples.

Fungal strain	Soya bean		Poultry luncheon		Meat luncheon	
	No.	%	No.	%	No.	%
As. niger	16	25	16	26.2	14	38.8
As. flavus	4	6.25	3	4.9	4	11.1
As. fumigatus	2	3.12	6	9.8	3	8.3
As. terrus	8	12.5	6	9.8	2	5.5
As. ochraechous	12	18.75	-	-	-	-
Penicillium	8	12.5	14	18	6	16.6
Alternaria	8	12.5	6	9.8	2	5.5
Cladosporium	4	6.25	12	19.6	3	8.3
Mucor	2	3.12	1	1.6	1	2.77
Absidia	-	-	-	-	1	2.77
Total	64	100	61	100	36	100

Table 4. Statistical analysis results of ochratoxin residues in ppb of examined samples (N.= 5)

	Soya bean	Poultry luncheon	Meat luncheon
Min.	2.8	1	3.3
Max.	6.8	4.7	5.51
Mean	4.23 ^{NS}	3.06 ^{NS}	3.77 ^{NS}
SE	0.81	0.68	0.35
No. of sample exceed permissible limt (5 ppb) acc.to **E.M.R.P. (2007)**	2	-	1

NS: Non significant correlation **E.M.R.P:** European mycotoxin regulation project

REFERENCES

1.Mižáková A, Pipová M and Turek P (2002): The occurrence of moulds in fermented raw meat products. Czech J. Food Sci., **20**: 89–94.

2.Hymowitz T and Kaizuma N (1981): Soya bean protein electrophoresis profiles from 15 Asian countries or regions: hypotheses on paths of dissemination of the Soya bean from China. Economic Botany 35, 10– 23.

3.Laciaková A and Laciak V (1994): Možnosti eliminácie mikroskopických vláknitých húb dezinfekčnými prostriedkami.Vet. Med. (Czech), 39: 723–731.

4.Pfohl-Lesskowicz A, Petkova-Bacharava T, Chernozemsky I N and Castegnaro M., (2002): Balkan endemic nephropathy and associated urinary tract tumors: A review on etiological causes and the potential role of mycotoxins. Food Additives and Contaminants, 19, 282-302.

5.European Food Safety Authority – EFSA. (2006): Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to ochratoxin A in food (Question No. EFSA-Q-2005-154), the EFSA Journal, p. 365.

6.Grosso F, Saïd S, Mabrouk I, Fremy J M, Castegnaro M and Jemmali M(2003): New data on the occurrence of ochratoxin A in human sera from patients affected or not by renal diseases in Tunisia. Food and Chemical Toxicology, 41, 1133–1140.

7.Maaroufi K, Achour A, Hammami M, El May M, Betbeder A M and Ellouz F (1995): Ochratoxin A in human blood in relation to nephropathy in Tunisia.Human & Experimental Toxicology, 14, 609–615.

8.Wafa E. W, Yahya R S, Sobh M A, Eraky I, El-Baz M. and El- Gayar H A M (1989): human ochratoxicosis and nephropathy in Egypt: A preliminary study. Human & Experimental Toxicology, 17, 124-129.

9.Filali A, Betbeder AM, Baudrimont I, Benayada, A, Souleymani R and Creppy EE (2002): Ochratoxin A in human plasma in Morocco: A preliminary survey.Human and Experimental Toxicology, 21, 241–245.

10.APHA (American Public Hehalth Association) (1985): Compendium of methods for the microbiological examination of food, 2nd Ed. American Public Health Aossociation , Washington D.C.

11.Ainsworth GC, Sparrow FK and Sussman AS (1973): A taxonomic review with keys: Ascomycetes and Fungi Imperfecti. In: Fungi, vol. IV. Academic Press, New York.

12.Pitt JI (1987): Penicillium viridicatum, Penicillium verrucosum, and production of ochratoxin A. Appl. Environ. Microbiol. 53 (2), 266–269.

13.Pitt, JI and Hocking AD (1997): Fungi and Food Spoilage. Blackie Academic & Professional, London.

14.Samson, RA, Hoekstra ES, Frisvad JC and Filtenborg O (2002): Introduction To Food- and Airborne Fungi, 6 edn. Utrecht, Centraalbureau voor Schimmelcultures.

15.Samson RA, Hoekstra E S, Frisvad J C and Filtenborg O (2004): Introduction to Food and Airborne Fungi, seventh ed. Centraalbureau Voor Schimmelcultures, Wageningen, The Netherlands.

16.Pestka J J (1988): Enhanced surveillance of food born mycotoxins by immunochemical assay. J. Assoc. Anal. Chem. 71 : 1075-1081.

17.Ismail, MA and Zaky ZM (1999): Evaluation of the mycological status of luncheon meat with special reference to aflatoxigenic moulds and aflatoxin residues. Mycoparthologia 146: 147-154.

18. Hafez T A (2003): Mold decontamination in Egyptian meat products handled at room temperature. Ph. D. Thesis, Faculty of Vet. Med., Suez Canal Univ., Egypt.

19.Abdel-Rahman HA, Yossef H and Hefnawey Y (1984): Mycological quality of meat products in Egypt. Assiut Vet Med J; 12(24): 153–159.

20.Scholte RPM, Samson RA and Dijksterhuis J (2002): Spoilage fungi in the industrial processing of food. In: Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O. (Eds.), Introduction to Food- and Airborne Fungi, 6 edn. Centaalbureau voor Schimmelcultures, Utrecht, pp. 339–356.

21. Samsom R A, Hoekstra E S, Lund F, Filtenborg O and Frisvad J C (2002): Methods for the dectection isolation and characterisation of food-borne fungi. In: Samson, R. A., Hohmann, S., Frisvad, J. C., Filtenborg, O. (eds.), Introduction to Food- and Airborne Fungi, 6 edn. Centralbureau voor Schimmelcultures, Utrecht, pp. 283-297.

22.Comi G , Orlic S Redzepovic S Vrso R. , Iacumin L (2004): Moulds isolated from istrain dried hema at the pre- ripeniu and repening level. Int. J. Food Microbial. 96 , 29-34.

23.Nassar A and Ismail M A (1994): Psychrotrophic and mesophilic fungi isolated from imported frozen lean meat in Egypt. J. Food Safety; 14: 289-295.

24.Roushdy S, Ibrahim A, Aldanaf N, Hammad H and Moustafa R (1996): Mycotoxin residues in meat and meat products. Vet Med J, Giza; 44(2): 181–187.

25.Cantoni C ; Comi G ; Chiesa , L. Iacumiu, L. (2007): Muffee ocratossiuna anegli insaccati crudi stagianati. Industrie Alimet xlvi (gemaid 1-4).

26.Lacumin L; Chiesa L; Boscolo D; Manzano M; Cantoni C and Orlic S. (2009): moulds and Ochratoxin A on surface of artisanal and industrial dry sausage. J. Food Microbiol. 26: 65-70.

الملخص العربى
الفطريات و الأوكراتوكسين فى فول الصويا واللانشون

علاء المرشدى- كمال الدسوقى- محمد عبد الله حسين- سيد السباعى
كلية الطب البيطرى – جامعة الزقازيق

فى هذا البحث تم فحص عدد ٦٠ عينة (٢٠ من كل من فول الصويا بروتين- لانشون الدجاج- اللانشون البقرى) وذلك لتحديد كم التلوث الفطرى لهذه العينات ووجد أن متوسط عدد الفطريات ٢,٦ × ١٠^٣ + ٧,٢ × ١٠^٢ ، ١,٦ × ١٠^٢ + ٠,٤٦ × ١٠^٢ و ١,٧ × ١٠^٢ + ٠,٥٤ × ١٠^٢ على الترتيب ٠ وكان متوسط بقايا الاوكراتوكسين فى العينات التى تم فحصها ٤,٢٣ + ٠,٨١ ، ٣,٠٦ + ٠,٦٨ و ٣,٧٧ + ٠,٣٥ جزء فى البليون على التوالى وكان من أهم المعزولات أسبرجلس نيجر ، اسبر جلس فلافس ، أسبرجلس نيومجاتس ، أسبرجلس فيربس ، أسبرجلس أوكراشيس ، بنسليم ، النانارياً ، كلادسبورم ، ميوكر ، الابسيديا كما تم مناقشة الأهمية الصحية لتلك المعزولات وكذا الشروط الصحية لإضافات اللحوم ٠