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Ochratoxin in some Poultry Meat Products

F.S.Hassanin[1], T.Z.Abd- Allah[2], M.El-Shater[3]
[1]Food Control Dept., Faculty of Veterinary Medicine, Benha Univ., Benha, Egypt
[2]General Organization of Veterinary Services, Egypt
[3]Food Hygiene Dept., Animal Health Research Institute, Dokki, Giza, Egypt
E-Mail: faten.mohamed@fvtm.bu.edu.eg

Abstract

One hundred and seventy five of chicken wings, chicken burger, chicken nuggets, chicken pannet, chicken frankfurter, chicken luncheon and chicken thigh (25 of each) were collected from Cairo ,Giza supermarkets for detection of ochratoxin A (OTA) in some poultry meat products. The mean values of OTA detected in the examined samples were 0.29 ± 0.09, 1.63 ± 0.27, 1.04 ± 0.16, 0.43 ± 0.18 , 0.82 ± 0.19 , 0.98 ± 0.25 and 0.68 ± 0.25 ppb for chicken wings, chicken burger, chicken nuggets, chicken pannet, chicken frankfurter, chicken luncheon and chicken thigh, respectively. The results declared that OTA detected in all studied samples of chicken products were within permissible levels, Further studies needed for follow up and monitoring toxicological health hazards accumulation of ochratoxin A in human kidney from other food constituents consumption in Egypt must be followed.

Keywords: Ochratoxin A, Poultry meat products.

1. Introduction

Modern industrial poultry production is the fastest and the most efficient way to obtain high quality products for human consumption of chicken. Poultry meat is known as a highly valuable food due to its biological and nutritional value primarily for the high protein content with a satisfactory amino acid composition and low energy value[4].

Ochratoxin A is a secondary toxic metabolite produced mainly by some species of Aspergillus and Penicillium. These species can grow in different climates. Aspergilli are found in tropical regions, whereas Penicillia are common in temperate regions and can grow when the temperature is as low as 5°C[20] . Once OTA has been formed, it survives most food processing stages such as cooking, fermenting and roasting [10].

Furthermore, processing e.g. heating , ripening and storage do not effect on OTA levels in meat products [12].

The presence of OTA in chicken samples was determined by many authors as [4] in Serbia who recorded that out of 90 liver, kidney and gizzard samples originating from chicken farms located in the different agricultural areas of Serbia, OTA was reported in 23 (38.33%), 17 (28.3%) and 16 (26.6%) samples, respectively, with levels ranging from 0.14 to 3.9 ng/g in liver, 0.1 to 7.02 ng/g in kidneys and 0.25 to 9.94 ng/g in gizzard . the chicken tissue samples must not contain more than (10 ng/g) as recommended by the European Commission. In this concern, [14] reported ochratoxin residues in chicken meat at level of 0.12 ppb in Czech Republic. In Egypt, [13]reported that incidence of ochratoxin in chicken burger and luncheon was 70% and 80%, respectively. The ochratoxin concentration was 0.277 and 0.243 ppb in chicken burger and luncheon, respectively. Therefore, this work was carried out to provide preliminary evaluation of the incidence of OTA in some chicken products chicken wings, chicken burger, chicken nuggets, chicken pannet, chicken frankfurter, chicken luncheon and chicken thigh) by Tandem Mass Spectrometry (LC-MS/MS).

2. Materials and methods

2.1 Samples

Accurately, 25 samples each of chicken wings, chicken burger, chicken nuggets, chicken pannet, chicken frankfurter, chicken luncheon and chicken thigh were randomly collected from Giza and Cairo markets. The samples were stored in at 4°C, until further analysis

2.2 Chemicals

2.2.1 Standard

The standard of ochratoxin A (OTA) used in the present study was purchased from Sigma Aldrich, Steinheim, Germany, while the immunoaffinity columns (IAC) (Ochra Test) from VICAM, Watertown, MA, USA.

2.2.2 Solvents

The HPLC grade solvents acetone, acetonitrile and methanol were purchased from Merck, Darmstadt, Germany.

Deionized water was used and all other chemicals and reagents used were at least of analytical grade

2.3 Apparatus and equipment's

LC MS MS 4000 QTRAP (Applied BioScince): Advanced Linear Ion Trap liquid chromatography technology was used for screening and quantitative analysis of Ochratoxin A. Analyst software was used for sample data analysis. Liquid nitrogen and ultra-high purity (99%) argon gas used in the LC–MS/MS interface

were supplied by TIG (Bangplee, Samutplakarn, Thailand).

2.4 Extraction procedure for ochratoxin A

The samples were extracted according to the method as described by [6] with few modifications. The sample 15 g was blended (15 min) in 50 ml of acetonitrile - water (45:05, v/v), using high speed blending and then the extract was filtered through filter paper. About 5 ml of the filtrate was mixed with 50 ml of phosphate buffer saline (PBS) and filtered through a glass microfiber. Then 10 ml of the filtrate was passed through immunoaffinity columns. OTA was eluted from the column by passing 1.5 ml of methanol (HPLC grade) and collected in a vial. The eluate was evaporated until dryness at 40°C and residues were dissolved in 1 ml of mobile phase i.e. acetonitrile : water : acetic acid (47/51/2, v/v/v) for HPLC analysis

2.5 Standard solution preparation

Stock standard solutions of each analyte were prepared at concentrations of approximately 200 mg/L in MeCN or MeOH, depending on solubility, and stored in amber-glass vials at −10 °C. A working standard mixture of all analytes (Mix-1) was prepared at 5 mg/L in MeCN, except for a mixture of NIV and DON (Mix-2), which was separately prepared at 50 mg/L in MeOH. These working standard solutions were appropriately diluted to provide 11-point calibration standards in mobile phase A–B (1:1, v/v). For the recovery and precision experiments, Mix-1 and Mix-2 served as high spiking solution. Middle and low spiking solutions were prepared by dilution of the high spiking solution in MeCN.

2.6 Sample preparation

a. 10 ± 0.05 g milled sample was added in 50 mL Teflon centrifuge tube, with water which used for the reagent blank. For a spiked sample, the required volume of spiking standard solution was added .

b. 10 ml water was added to 10% formic acid in MeCN (10 mL), then vortex the tube for 30 s.

c. The tube was shaked with an automatic horizontal shaker at maximum speed for 1 h to fully disperse the sample. then anhydrous $MgSO_4$ (4 g) + NaCl (1 g) + tri-Na (1 g) + di-Na (0.5 g) was Added . Then, shake the tube immediately and vigorously by hand for 1 min; and centrifuge the tube at 3400 rpm (approximately 2171 rcf) for 5 min.

d. For d-SPE clean-up, transfer 8 mL of the MeCN extract (upper layer) into a 15 mL centrifuge tube containing anhydrous $MgSO_4$ (1.2 g) + C18 (0.25 g) + Al-N (0.25g) + PSA (0.4 g).

e. the tube was shaked vigorously by hand for 1 min. then, the tube put in the centrifuge at 3400 rpm for 5 min.

f. 5 mL of the extract was transferred to a glass tube. Then, evaporate to dryness at 40°C under a stream of N_2.

g. 1 mL of mobile phase A–B (1:1, v/v) was added to reconstitute the extract, and then vortex for 1 min. Filter the extract using 0.2 µm nylon syringe filter (Chrom Tech Inc., Apple Valley, MN, USA) into an auto sampler vial.

2.7 Method validation

It was performed according to the European Commission [5] were used as for guidelines and criteria to assess the method validation. Selectivity was determined from Retention time, ion ratios, and identification-points (IP) for each analyte.

Matrix effect of each analyte was estimated by calculating the difference of the linear best-fit slope, obtained from the matrix-matched calibration curve and solvent-based standards calibration curve, divided by the slope of the solvent-based standards calibration curve.

Recovery and precision studies were conducted by spiking chicken muscle samples with 10 replicates at each spiking level: 10 (low), 50 (middle), and 100 µg/kg (high), on three separate days. For NIV and DON, which generally showed low precursor ion signal intensities in ESI+, we used spiking levels of 100 (low), 500 (middle), and 1000 µg/kg (high) throughout the quantitative validation. To check for possible interferences and carry-over, the first and the last injections were reagent blanks. Recoveries were calculated from matrix-matched standard calibrations.

2.8 Instrument and condition

Applied Biosystems 4000 Q TRAP® LC/MS/MS System triple quadrupole/ linear ion trap mass spectrometer system.

2.9 Software

Analyst 1.5.1 Portion Copyright ® 2005 Versant Corporation.

2.10 Column

S/N : ODS-H-OL3-36550 B /N : 008-4424-BO
Desc Synergic 4 µ Fusion – RB – 80 A
Size 50 x 2 mm 4 micron

Total injection Time: 20 minutes. Flow rate: 0.5 ml/min. Column temperature: 40 °C. Injection volume: 5 µ. LOD (Level of detection): From 0.01 to 0.03 ppb. LOQ (Level of quantitation**):** From 0.05 to 0.06 ppb

2.11 Statistical analysis

Data obtained were statistically analyzed for descriptive statistics, Q squares and ANOVA test at significant level of $P < 0.05$ using [17]

3. Results

3.1 Quality control parameters

The LC MSMS performance parameters like linearity, Limit of detections (LOD), Limit of quantifications (LOQ), repeatability and reproducibility of OTA are presented in Table (1).

Table (1) The LC MSMS parameters for the analysis of ochratoxin A in chicken products :

Ochratoxin A Linearity (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	Precision (%RSD) Repeatability
Chicken wings	0.01	0.05	9
Chicken burger	0.02	0.05	8
Chicken nuggets	0.02	0.05	9
Chicken pannet	0.03	0.06	9
Chicken frankfurter	0.01	0.06	8
Chicken luncheon	0.02	0.05	8
Chicken thigh	0.01	0.05	8

LOD : Level of detection LOQ : Level of quantitation

Table (2) Fortified levels of ochratoxin A in chicken samples :

Sample	Fortified level (mg/kg)	Recovery %	RSD %
Chicken wings	0.2	86	7
Chicken burger	0.2	83	8
Chicken nuggets	0.2	88	10
Chicken pannet	0.2	85	9
Chicken frankfurter	0.2	96	8
Chicken luncheon	0.2	87	7
Chicken thigh	0.2	92	10

RSD: relative standard deviation SD: standard deviation

Method precision was determined in terms of repeatability and reproducibility at two different concentrations on the same day with three-replicated analysis of spiked samples. The results have shown good linear response with coefficient of determination for all the analyzed mycotoxins. The recoveries were as curtained by spiking 2mg/kg of OTA in non-contaminated chicken samples as shown in Table (2). The method has shown good recoveries and all the spiked samples have recoveries in the range of 83 – 96% with RSD varies from 7to 10% Table (2).

3.2 Calibration curve

Calibration curve was performed in the following 5 points 0.5, 1, 5, 50 and 100 µg/ml (ppb). The results for the first daughter (Analyte: OchraA1 (404.334/239.200 Da)) was in the following table (3) . The obtained Regression Equation: was $y = 219 x + -26.9$ ($r = 0.9999$)
Fig (1) showed typical LC MSMS Diagram of ochratoxin in Chicken Burger sample.

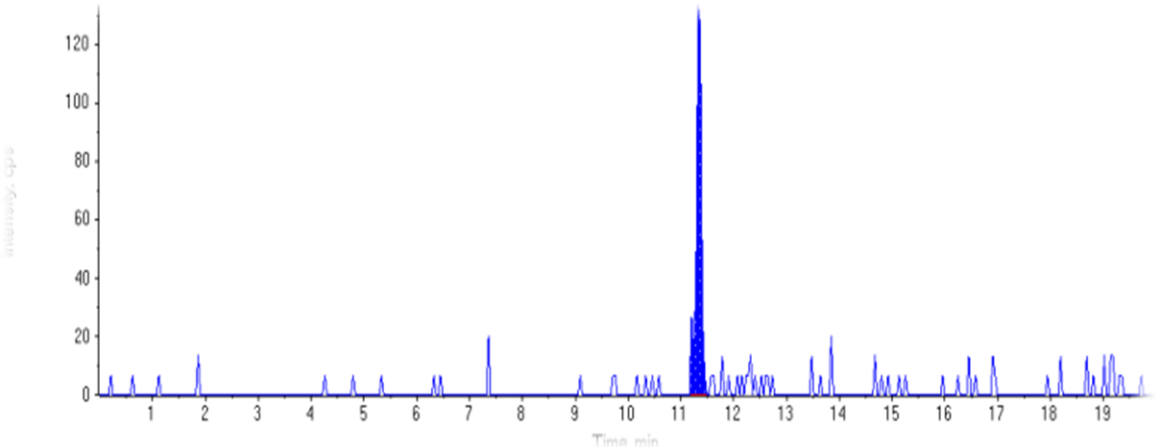

Fig (1) Typical LC MSMS Diagram of ochratoxin in Chicken Burger sample.

Table (3) Calibration curve parameter and result of Ochratoxin in LC MSMS system (Analyte: OchraA1 (404.334/239.200 Da))

Expected Concentration	Number of Values	Mean Calculated Concentration	% Accuracy
0.5	1	0.42	84.8
1	1	1.63	162.9
5	1	4.34	86.8
50	1	50.15	100.3
100	1	99.95	99.9

3.3 Incidence and residues of ochratoxin

Incidence of ochratoxinin chicken products samples showed that the level of OTA was ranged from 12 to 28 %. Statistically, there is no significant alteration between studied products Table (4).

Chicken Burger was contained the higher content of OTA residues 1.63 ppb, followed by Chicken Nuggets, Luncheon and Frankfurter (1.04, 0.98 and 0.82 ppb, respectively. Then, Chicken Thigh and Pannet (0.68 and 0.43ppb, respectively). Lastly Chicken Wings, OTA residues was 0.29 ppb.

Table (4) Incidence of ochratoxin in chicken products samples

Type of sample	Samples	Positive samples	Percentage (%)
Chicken wings	25	4	16 a
Chicken burger	25	6	24 a
Chicken nuggets	25	5	20 a
Chicken pannet	25	6	24 a
Chicken frankfurter	25	7	28 a
Chicken luncheon	25	7	28 a
Chicken thigh	25	3	12 a
χ^2			0.958#

a Insignificant difference between similar using Fischer Exact Probability test at $P < 0.05$. # Insignificant difference using chi square probability test at $P < 0.05$. * Significant difference at $P < 0.05$ using ANOVA test.

4. Discussion

Ochratoxin A is the greatest probable mycotoxin involved in the Balkan nephropathy (BEN) endemic and many renal tumours could be involved in the same pathologies in many countries [3]. OTA risks assessment for animal and human health depend on the duration and OTA exposure level. Exposure to OTA differ considerably depending on different factors, among which food-processing systems. These systems are often traditional and typical of the different geographicalareas [19]. Generally, mycotoxins are stable compounds, and particularly, OTA is a moderately heat stable molecule which can survive most food processing operations so, it appears in final and derived products [2]. Table (5) showed that incidence of OTA in studied products was ranged from 12 to 28 %. There is no significant alteration between percent of OTA in studied products. The highest OTA residue was found in chicken burger (1.63 ppb), followed by chicken nuggets (1.04 ppb), luncheon (0.98 ppb) and frankfurter (0.82 ppb), chicken thigh (0.68 ppb) , pannet (0.43ppb) and finally chicken wings (0.29 ppb).

Table (5) Ochratoxin residues in selected positive chicken samples

Type of sample	No. of +ve Samples	OTA mean (µg/kg)	Range (µg/kg)	
			Min	Max
Chicken wings	4	0.29 ± 0.09 d	0.07	0.71
Chicken burger	6	1.63 ± 0.27 a	0.13	2.24
Chicken nuggets	5	1.04 ± 0.16 b	0.32	1.87
Chicken pannet	6	0.43 ± 0.18 c	0.08	0.89
Chicken frankfurter	7	0.82 ± 0.19 b	0.24	1.56
Chicken luncheon	7	0.98 ± 0.25 b	0.41	1.42
Chicken thigh	3	0.68 ± 0.25 c	0.39	1.12
P <		0.015*		

The data confirm some of those of the previous studies obtained by [4] in Serbia who recorded that the incidence of OTA in chicken liver, kidney and gizzard samples from chicken was 38.33%, 28.3% and 26.6%, respectively, with levels ranging from 0.14 to 3.9 ng/g in liver, 0.1 to

7.02 ng/g in kidneys and 0.25 to 9.94 ng/g in gizzard. In Czech Repulic, reported that ochratoxin residues was 0.12 ppb in chicken meat. while the incidence is so lower than that reported in Egypt by [13] who reported that incidence of ochratoxin in chicken burger and luncheon was 70% and 80%, respectively. The residue of ochratoxin was very low in their study, where OTA residues were 0.277 and 0.243 ppb in chicken burger and luncheon, respectively. This could be explained by the fact that in our work we used Tandem Mass Spectrometry (LC-MS/MS) which consider highly sophisticated and accurate technique for determination of OTA residues.

Although, none of the studied chicken products samples contained more than the maximum level of OTA (10 ng/g) recommended by the European Commission [4], but OTA inhibit protein synthesis and lipid peroxidation by oxidative processes[9]. So, it may generate nephrotoxic, neurotoxic and immunotoxic effects. The mechanisms by which OTA is carcinogenic are not entirely elucidated and two hypotheses are still under discussion: .(i) an indirect mechanism which would result in a classification as an epigenetic carcinogen [18] or (ii) due to direct covalent binding of OTA on DNA, suggesting genotoxic mechanisms being involved in the carcinogenicity[11]. Although there is evidence for a time- and dose-dependent induction of DNA lesions in vivo when applying the 32P-postlabeling technique, the chemical identities of adducts and metabolites implicated, need to be elucidated [15] .This could be increased the risk assessment of OTA to human consummation. The limited data concerning monitoring OTA residues in chickens and its products in Egypt make difficult to compare these results with others obtained specially with different analytical methodology.

In conclusion, ochratoxin A analysis in chicken products in this investigation could be regarded to natural occurrences of ochratoxicosis in poultry. The actual concentration of ochratoxin A in chicken products is generally very low. However, the daily intake of OTA through chicken meat depends on (1) the concentration in the food, (2) the amount consumed, (3) the frequency of consumption and (4) the consumers' preferences. To protect human health and reduce economic losses, the control of mycotoxins in food and feed has become a major objective for producers, authorities and researchers work.

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