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Effect of Ozonated Water on Ochratoxin A levels in Locally Broiler Meat in Baghdad Province

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The main objectives of the present research were to determination of Ochratoxin A in locally broiler meat sold in markets of Baghdad province by ELISA, effect of ozonated water treatment (0.5ppm/15 min.) on the level of Ochratoxin A in positive samples and finally determination of Ochratoxin A after ozonated water treatment. A total of 50 samples were collected randomly from various markets located in different locations of Baghdad province from each sector Al-Kirkh and Al-Rusafa during November 2017 to March 2018. All meat samples were positive for Ochratoxin A and the result showed that there were a significant differences (P d" 0.05) in the residual levels (ppm) of Ochratoxin A between Al-Kirkh and Al-Rusafa before and after ozonated water treatment. The highest Mean ± SE values were recorded in Al-Rusafa (0.648 ± 0.0020), followed by Al-Kirkh (0.636 ± 0.0025) before ozonated water treatment, while the highest Mean ± SE values after ozonated water treatment were recorded in Al-Rusafa (0.346 ± 0.0025), followed by Al-Kirkh (0.332 ± 0.0049). The lowest Mean ± SE values of Ochratoxin A before ozonated water treatment were recorded in Al-Rusafa (0.502 ± 0.0040), followed by Al-Kirkh (0.504 ± 0.0058), while the lowest Mean ± SE values of Ochratoxin A after ozonated water treatment were recorded in Al-Rusafa, followed by Al-Kirkh also at (0.264 ± 0.0040), and (0.266 ± 0.0025) respectively. This research indicated that the poultry meat treated with ozonated water has the advantages methods that it did not affect the colour and texture characteristics of the meat, can be used to eliminate or reduce Ochratoxin A residues at the same time, and can be used in any slaughter house without the need to modify the design of the buildings.

Keyword: Ochratoxin A, Broiler meat, Ozonated water,Mycotoxin.

Mycotoxins are a natural food and feed contaminants, mainly produced by moulds by genera *Aspergillus*, *Penicillum* and *Fusarium* spp. The number of mycotoxins known to exert toxic effects on human and animal health is constantly increasing as well as the legislative provisions taken to control their presence in food and feed. Extensively considered mycotoxins are Aflatoxins (AFs), Ochratoxin A (OTA), Fusarium toxins and Patulin[1].Mycotoxins can enter the food supply in several ways, but these can be grouped into two general routes of contamination, direct or indirect contamination. Direct contamination occurs as a result of mold growth in the food material itself[2]. Almost all foods are susceptible to mould growth during some stage of production, processing, storage or transport, mould growth on foods that are to be consumed directly can result in direct exposure to mycotoxin[3]. Ochratoxin are mycotoxins produced by several species of *Aspergillus* and *Penicillium* spp. Among different types of Ochratoxin, Ochratoxin A is most important, the other being the methyl and ethyl ester of OTA, which is also known as Ochratoxin C and Ochratoxin B [4]. Several lines of evidence suggest that OTA may be carcinogenic to humans.

First, OTA causes a nephropathy aûecting animal that closely resembles a fatal kidney disease in humans, Balkan endemic nephropathy (BEN). Second, OTA is immunotoxic, genotoxic, and carcinogenic in many species. For example, dietary feeding of OTA induces renal adenoma and carcinoma in male mice and rats[5].

Ozone, or triatomic oxygen (O_3), is a powerful disinfectant and an oxidising agent, since 1997, it has been considered as a GRAS (generally recognized as safe) substance and used in a number of applications in the food industry for the destruction or detoxification of chemicals or microorganisms[6]. Ozone can be used as a therapeutic agent and is the most powerful oxidant which can act 3000 times faster than chlorine for inactivating virus, bacteria, fungi and protozoa in drinking water[7].

MATERIAL AND METHODS

Collection of samples

A total of 50 meat samples; comprising locally broiler (30-45 days) were collected randomly from various markets located in different locations of Baghdad province during November 2017 to March 2018, fifty samples (10 samples for each month) 5 samples from each sector Al-Kirkh and Al- Rusafa.All of collected meat samples were locally slaughtered and transported by plastic bags in ice container and were stored at 4°C until analyze by ELIZA.

Determination of OTA using ELISA

The ELISA immunoassay was carried out using OTA ELISA kit provided by MyBioSource, USA.

Extraction of OTA in poultry meat

According to[8], Chickens were sacrificed by cervical dislocation, meat were collected, weighed and frozen for subsequent OTA analysis. One gram of raw materials, homogenized with 0.5 of 1 M H_3PO_4 and 3ml of ethyl acetate, the samples were gently mixed and centrifugation 1 mint. 2000rpm at room temperature.Transferred supernatant ethyl acetate and supplemented with additional 3ml ethyl acetate.After mixing and centrifugation, ethyl acetate layers were combined and supplemented with 3ml of 0.65M $NAHCO_3$. Vortexed and continued to be mixed for another 15 mint. and centrifugation 5 mint. 2000rpm, 1 ml of lower aqueous phase transferred and heated in a water bath at 100 R"C for 3 mint., shaken and cooled. After cooling 4ml of distill water was added. An aliquot was then diluted with 0.13M. $NAHCO_3$.

Calculation of ozone concentration output (ppm/in water) of the ozone generator

The ozone concentration (ppm) in water generated by the ozone generator was carried out by using CHE-Mets-Kit (USA) according to the procedure by[9]. In this experiment a small plastic jar was used. The plastic cover had one ozone gas inlet port to inject the ozone gas into the water using aeration stone (Diffuser), and distributed it evenly throughout the water (micro-bubbling). The ozone generator was fed with 1 litter per minute (600mg/h.) of compressed air as a feed gas.The meat samples were wrapped in gauze then submerged into the ozonated water.The ozone water was diffused within the meat samples for 15 mint.

Statistical Analysis

The data were analyzed using one way ANOVA. Differences were considered significant at (P d" 0.05). SPSS (version 22) was used for statistical assessments.

RESULTS

The ELISA analyses revealed that all 50 meat samples were positive for Ochratoxin A (Table 1). The highest range values (ppm) of Ochratoxin A were recorded in Al-Rusafa (0.64 - 0.65), followed by Al-Kirkh (0.63 - 0.64) at March/ 2018, while the lowest range values (ppm) of Ochratoxin A were recorded in Al-Rusafa (0.49 - 0.51), followed by Al-Kirkh (0.49 - 0.52) at November/2017.

Out of the 50 positive samples were determine the effect of ozonated water (0.5ppm/15 min.) on Ochratoxin A residues (ppm) in meat samples (Table 2). The highest range values (ppm) of Ochratoxin A were recorded in Al-Rusafa (0.34 - 0.35), followed by Al-Kirkh (0.32 - 0.35) at March/2018, while the lowest range values (ppm) of Ochratoxin A were recorded in Al-Rusafa (0.25 - 0.27), followed by Al-Kirkh (0.26 - 0.27) at November/2017.

When comparing the mean levels of Ochratoxin A in meat samples between the study months (November 2017 to March 2018), the

results revealed that there were a significant differences (P d" 0.05) between all study months (Table 3). The highest levels were recorded in March, followed by February, January, December, and November in Al-Rusafa sector before ozonated water treatment (0.648 ± 0.0020), (0.596 ± 0.0025), (0.540 ± 0.0032), (0.518 ± 0.0020), and (0.502 ± 0.0040) respectively, while the highest mean levels of Ochratoxin A after ozonated water treatment were recorded in March, followed by February, January, December and November also (0.346 ± 0.0025), (0.314 ± 0.0025), (0.274 ± 0.0051), (0.266 ± 0.0025), and (0.264 ± 0.0040) respectively.

Moreover, the results showed that, the highest mean levels of Ochratoxin A Al-Kirkh sector were recorded in March, followed by February, January, December, and November also before ozonated water treatment (0.636 ± 0.0025), (0.588 ± 0.0037), (0.536 ± 0.0025), (0.516 ± 0.0025), and (0.504 ± 0.0058) respectively, while the highest mean levels of Ochratoxin A after ozonated water treatment were recorded in March, followed by February, January, December and November (0.332 ± 0.0049), (0.308 ± 0.0037), (0.276 ± 0.0040), (0.266 ± 0.0040), and (0.266 ± 0.0025) respectively.

DISCUSSION

The present study showed that all meat samples were positive for OTA when analyses by the ELISA. The highest levels of OTA in meat were recorded atAl-Rusafa, followed by Al-Kirkh.The differences between Baghdad sector could be attributed to several reasons such as difference in age of animals, the site that animals come from, source of water, type of rearing, feed component, farmers having low formal education and differences in withdrawal times.

Previous studies confirmed the present results by reported that the Ochratoxin are fungal secondary metabolites that contaminate grains,

Table1. Levels of Ochratoxin A residues (ppm) in broiler meat samples

Sector	No. of samples	Months	Range
Al-Kirkh	5	November/2017	0.49 - 0.52
	5	December/2017	0.51 - 0.52
	5	January/2018	0.53 - 0.54
	5	February/2018	0.58 - 0.60
	5	March/2018	0.63 - 0.64
Al- Rusafa	5	November/2017	0.49 - 0.51
	5	December/2017	0.51 - 0.52
	5	January/2018	0.53 - 0.55
	5	February/2018	0.59 - 0.60
	5	March/2018	0.64 - 0.65
Total	50		

Table 2. Effect of ozonated water treatment on Ochratoxin A residues (ppm) in meat samples

Sector	No. of samples	Months	Range
Al-Kirkh	5	November/2017	0.26 - 0.27
	5	December/2017	0.26 - 0.28
	5	January/2018	0.27 - 0.29
	5	February/2018	0.30 - 0.32
	5	March/2018	0.32 - 0.35
Al- Rusafa	5	November/2017	0.25 - 0.27
	5	December/2017	0.26 - 0.27
	5	January/2018	0.26 - 0.29
	5	February/2018	0.31 - 0.32
	5	March/2018	0.34 - 0.35
Total	50		

Table 3. Comparisons of Ochratoxin A residues (ppm) in meat samples between the months of the study period before and after ozonated water treatment

Treatments	Mean ± SE					
	Al-Kirkh sector			Al-Rusafa Sector		
Months	Before	After	Dec. %	Before	After	Dec. %
November	0.504 ± 0.0058 e	0.266 ± 0.0025 c	47.2	0.502 ± 0.0040 e	0.264 ± 0.0040 c	47.4
December	0.516 ± 0.0025d	0.266 ± 0.0040 c	48.4	0.518 ± 0.0020d	0.266 ± 0.0025 c	48.6
January	0.536 ± 0.0025 c	0.276 ± 0.0040 c	48.5	0.540 ± 0.0032 c	0.274 ± 0.0051 c	49.2
February	0.588 ± 0.0037b	0.308 ± 0.0037 b	47.6	0.596 ± 0.0025b	0.314 ± 0.0025 b	47.3
March	0.636 ± 0.0025 a	0.332 ± 0.0049 a	47.7	0.648 ± 0.0020 a	0.346 ± 0.0025 a	46.2

legumes, coffee, dried fruits, beer, wine, and meat[10,11] mentioned that the highest amounts of OTA were found in the blood, while the distribution within the tissues follows the pattern "kidney Ã liver Ã muscle Ã fat.Another study showed that the residues of OTA accumulated in all organs, with high levels in liver and kidneys and low levels in muscle. In particular, in chickens fed 0.5 mg of OTA per animal weekly for four weeks, after the first two weeks of exposure, OTA was found at 0.28 and 0.20 ng/g in breast and thigh muscle, respectively. Then, OTA residue in muscle increased slightly, reaching its maximum value after four weeks, when the concentration of OTA was 0.84 ng/g in muscles[12].

The reason for increased the levels of OTA in poultry meat in March due to in this month rain fell many with poor storage of feed in fields, this result in growth of mould in feed that lead to produce mycotoxin and transfer to poultry tissues. Moulds are present in soil and plant debris, and its spores are spread by wind currents, insects, and rain, they are frequently found in/on foods together with their associated mycotoxins[13], so the environmental variations can affect the concentration of OTA residues.[14] suggested that development of toxicogenic moulds in the animal feed and tissues is favored by factors such as condensation, heating, leakage of rain water, insect infection.

Ozone gas had been used in decontamination of fungi and mycotoxin contaminated stored grain. Generally ozone gas treatment could significantly prevent fungi development and consequently reduce mycotoxin production[15]. Previous work showed that Aflatoxin B1, OTA, ZEN and DON in ozone aqueous solution could be rapidly and completely degraded within a few tens of seconds using ozone gas. It may be implied that water vaporized ozone gas (called wet ozone gas) has the high ability of degrading the mycotoxins (Wang *et al*., 2010) .When ozone gas as a water additive was dissolved in water, the oxidation of organic and inorganic compounds in water during ozonation could occur via ozone or OH radicals or a combination thereof[16] It was found that aqueous ozone was very effective in significantly reducing organic pollutants and microbial organs in water. Mycotoxins in solutions also could be greatly degraded using aqueous ozone[17].

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