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Ozone as a Solution for Eliminating the Risk of Aflatoxins Detected in Some Meat Products.

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Abstract

The current study was designed to assess the efficiency of ozone (O_3) as a green technology in the detoxification of aflatoxins (AFs) in luncheon and kofta. Both products can deliver AFs to consumers where all samples revealed more than one of the major AF B1, B2, G1, and G2. Kofta contained a higher level (15.2 ppb) of total AFs compared to luncheon (4.8 ppb). By exposing samples to O_3, the degree of detoxification was proportional to O_3 concentration. At 20 ppm O_3, the most detoxified AFs were AFB2 (67.1%) and AFG1 (68.3%) while the reduction in other AFs ranged from 11.6 – 55.2% and 44.7 – 61.4% for luncheon and kofta, respectively. By 40 ppm O_3, the most detoxified AFs were AFG1 (100%) and AFB2 (91.7%) while the reduction in other AFs ranged from 54.6 – 85.7 % and 61.4 – 78.4%, respectively. By the two applied concentrations, O_3 none significantly lowered the pH values of samples. Lipid stability was inversely proportional with O_3 concentration as fat was more stable at 20 compared to 40 ppm O_3, where kofta appeared higher stability compared to luncheon. Ozone appeared able to eliminate or significantly reduce AFs in raw and ready-to-eat meat products with negligible changes in physicochemical properties.

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Fat Stability; Kofta; Luncheon; Meat Products; pH.

Introduction

The safety of meat products is possibly threatened by the presence of chemical pollutants, such as mycotoxins, drugs, and pesticide residues. Mycotoxins are natural contaminants formed by definite species of fungi and their presence in food increases attention in terms of public health and food safety. The more common and the most harmful types of my cotoxins are aflatoxins (AFs), which are the most poisonous produced from some *Aspergillus* species (*A. flavus, A. parasiticus* and *A. nomius*) as secondary metabolites.[1,2] The four major AFs are called AFB1, B2, G1 and G2, based on fluorescence (blue or green) and chromatographic analysis.[3]

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Besides acute toxicity, AFs are linked with cancer in both humans and animals.[4] The harmful effect of AFs on consumers depends on the concentration and exposure time, age, gender, health condition, state of immunity, diet and environmental factors.[5]

Luncheon and kofta (the Egyptian name of meat balls) are popular widely consumed traditional Egyptian meat products. These products are formulated from meat and nonmeat additives.[6] Research works have demonstrated a high incidence of AFs in both products[7,8,9] sometimes with levels passing the regulated limits. AFs are tolerant to traditional industrial processes applied to raw materials;[10,11] so, if raw constituents are polluted, these AFs will occur in processed meals. Besides, the majority of AFs are heat tolerant and therefore decomposition during cooking or processing does not occur.[12]

To protect consumers from harmful effects of AFs, researches have tried some approaches to minimize the hazard. Although many these approaches not only detoxify AFs but also control the growth of microorganisms in food, in the food industry, safety is still the most important goal. Irradiation could decrease the concentration of AFs in food[13,14] but generally, such treatment is not a recommended strategy for AFs detoxification in food industry due to possible molecular reactions.[15,16] So research works are directed towards the green methods of AFs degradations. These studies revealed that ozone (O^3) in one of them can do.[17,18]

Food and Drug Administration of the U.S. and the World Health Organization (WHO) has considered using O_3 technology in food to be safe and effective.[19,20] Consequently, O_3 treatment is regarded as one of many established processes that contribute to the improvement of food product safety and quality. This study was planned to evaluate the effectiveness of O_3 in reducing or eliminating AFs in the traditional meat products luncheon and kofta and its effects (if any) on their, physical properties and lipid stability.

Material and Methods

Collection of Samples

Fifty random samples (100gm for each) of locally produced meat products represented by ready to eat beef luncheon and frozen kofta (25 each) were collected through October 2020 from Assiut city markets, Egypt. The samples transported cooled in their packages to the laboratory and stored frozen until analyses were conducted as soon as possible.

Quantitative Measurement of Aflatoxin Residues (AFB1, B2, G1, and G2)

The analysis was carried at the Central Accredited Lab. for Forensics Studies, Faculty of Vet. Med. Assiut University, Egypt following procedures of Cui *et al.*[21]

Aflatoxins Working Standards

Aflatoxins standards AFB1, B2, G1, and G2 (purity ≥ 99.0%) were obtained from Sigma-Aldrich (St. Louis, USA). A vial of AFs mixed standard powder containing 5 mg (2 mg AFB1, 2 mg AFG1, 0.5 mg AFB2, and 0.5 mg AFG2) was used to make the stock standard solution, which was made by dissolving the powder in 10 ml of acetonitrile (99.9% of HPLC grade, Fisher Company USA) to make a stock solution of 500 ppm concentration. Twenty µl of AFs mixture (AFB1, B2, G1, and G2) was dissolved in 10 ml of methanol (99.9% of HPLC grade, Fisher Company, USA) to get AFs (AFB1, B2, G1, and G2) stock solution.[22]

Aflatoxins Extraction

According to the guidelines extractions of AFB1, B2, G1, and G2 from samples have proceeded.[23] A 2 g sample was minced with a tissue grinder, and 20 milliliters of dichloromethane was added to the mixture, which was ultrasonicated for 10 minutes and shaken continuously for 1 hour. Anhydrous sodium sulfate (2 g) was added, and the mixture was centrifuged for 5 minutes at 10,000 rpm. Finally, 10 mL of supernatant was collected and dried in a water bath at 50°C using a Termovap apparatus (American Organomation Associates).

Clean-Up

The cleanup procedures were carried out in accordance with R-Rhône Biopharm's[24] requirements. In a summary, 2 mL methanol and 13 mL phosphate buffer saline were added to the residue and thoroughly mixed together. After that, 1 mL methanol was used to elute the sample. The solution across through the immunoaffinity column at a rate of 1–2 drops per second. The column was rinsed with 10 mL deionized water, and the entire eluate was dried in a water bath at

50°C in a Termovap apparatus. The residue was placed in a water bath at 40°C for 15 minutes, then 100 µl trifluoroacetic acid and 200 µl hexane was added, and it was dried again at 50°C with a gentle stream of nitrogen. Finally, UPLC-FLD was used to detect the level of AFs (B1, B2, G1, and G2) residues.

UPLC-FLD Analysis

Aflatoxins (AFB1, B2, G1, G2) were determined using Acquity (Waters, USA) Ultra-High-Performance Liquid Chromatography (UPLC) with a Fluorescence Detector (FLD, Waters, USA). The evaluation used a chromatographic column (Waters Acquity UPLC BEH C18) with a size of (1.7 m 2.1 mm 100 mm) for analyte separation, a flow rate of 0.4 ml/min, and 10µl injection volume. Methanol, acetonitrile, and water made up the mobile phase (18:18:64). AFG2, AFG1 had excitation and emission wavelengths of 365 and 456 nm, respectively, whereas AFB2 and AFB1 had excitation and emission wavelengths of 365 and 429 nm, respectively. The system was computer-controlled, and the data was analyzed using EMPOWER3 software.

Treatment with Ozone

Generation of Ozone

Ozone gas was generated from a Cold Plasma Ozone Generator (Longetviy, Canada), using oxygen at a flow rate of 0.25 L/minute, with a working voltage of 220 volts, at ambient temperature. The ozone generator was controlled to generate O_3 at a required concentration (20 or 40 ppm). The concentrations of O_3 were calibrated by iodometric titration method where generated O_3 was injected to KI solution for 5 minutes and titrated against sodium thiosulphate. The concentration of O_3 was calculated from the equation recommended by Chasanah *et al.*[25]

$$C_{ozone} = R \times V_t \times N_t / V_{gas}$$

Where

C_{ozone} is the concentration of O3 (g / L).

R is the ratio of the analytical mol and the reactant of a balanced chemical equation, V_t is the volume of titrant (L). N_t is the normality of sodium thiosulfate (mol / L), and V_{gas} is the volume of air.

Treatment with Ozone

Samples were prepared to simulate retail conditions, where 5 mm thick luncheon slices (10) / 5 meatballs (kofta) weighing (20 g each) were placed in a perforated foam plate separately and warped with a plastic net. Each type of sample was divided into two subgroups. Each subgroup was submitted to a separate dose of 20 or 40 ppm. Meatballs/ luncheon slices (4 foam plates) were placed into vacuum package bags separately and either sealed immediately (control) or treated with O_3. Before passing O_3, the bag was air evacuated using a suction plumb then connected to the current of O_3 for 5 minutes. While samples were exposed to O_3, the bag was agitated to allow all surfaces to be adequately exposed to the O_3 gas. Ozone was left to react for 20 minutes then the bags were evacuated via a tube into a 2% KI solution to prevent passing excess O_3 to the environment.[26]

Effect of Ozone on Aflatoxins and Physico -chemical Attributes

Treated and control samples were submitted to AFs, pH, and thiobarbituric acid (TBA) analysis.

Effect of Ozone on Aflatoxins

After estimation of AFs levels, AFs degradation percentage (AFsD %) was calculated from the formula

AFs D %= (Control level – Treatment level) x100/ Control level

Measurement of pH

The pH values were estimated according to Elbazidy *et al.*[27] where a 5g sample was homogenized for 10-15 seconds in 20 ml distilled water, and the pH of the slurry was measured with a calibrated pH (by buffers of pH 7.0 and 4.0) digital pH meter (Gallenhamp No.101284) at ambient temperature.

Determination of Thiobarbituric Acid (TBA)

5g sample was homogenized with 15 ml of deionized distilled water using a stomacher for 10 seconds at the highest speed, as described by DU and Ahn.[28] One ml from the homogenate was mixed with 50 µl butylated hydroxyl anisole (7.2%) and 1 ml each of 15mM 2-thiobarbituric acid and 15% trichloroacetic acid. The mixture was vortexed, then incubated for 15 minutes in a boiling water bath to produce color.

It was then cooled for 10 minutes under running water, vortexed again, then centrifuged for 15 minutes at 2500 g. At 531 nm, the absorbance of the resultant supernatant solution was measured against a blank containing 1 mL deionized water and 2 mL TBA–TCA solution. The reading was multiplied by 7.8 to obtain the value of TBA (mg of malonaldehyde (MDA) /kg of the sample).

Statistical Analysis

In each test, the mean and standard deviation values were determined for each group. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to look for normality in the data, which revealed a non-parametric (not normal) distribution. To compare two groups in unrelated samples, the Mann-Whitney method was employed. $P \leq 0.05$was used as the significant threshold. IBM® SPSS® Statistics Version 26 for Windows was used to conduct the statistical analysis.

Results

The distribution of AFs in fifty random samples of two locally produced meat products (beef luncheon and frozen kofta) shows that AFs nearly recorded by parallel levels for both products. AFB1 was recorded in 96% and 88% of kofta and luncheon, respectively. Furthermore, 88% of kofta and 92% of luncheon revealed AFB2 while AFG2 was detectable in 84 and 88%, respectively. On the contrary, AFG1 was predominated in kofta samples (64%) compared to 12% in luncheon (Table 1).

Table 1: Distribution of aflatoxins in meat products

Products	Aflatoxins									
	AFB1		AFB2		AFG1		AFG2		Total	
	No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%
Luncheon	22	88	23	92	3	12	22	88	25	100
Kofta	24	96	22	88	16	64	21	84	25	100

No. +ve Number of positive

Levels of AFs in meat products illustrated in Table 2, the mean of AFs was 4.8 and 15.2 ppb, for luncheon and kofta, respectively.

The samples and their response to ozonation were listed in tables 3 and 4. For ready-to-eat luncheon, treatment with 20ppm O_3, AFB2 resulted in a reduction of 67.1% followed by AFB1 and AFG2 where reductions were 55.2 and 34.3%, respectively. At 40 ppm, AFG1 appeared the highest detoxification degree where none of the exposed samples revealed the detectable level of the toxin (100% reduction). Also, exposure to O_3 resulting a reduction of 85.7, 83.8, and 54.6 for AFB2, B1, and G2, respectively (Table 3 and Figure 1).

Table 2: Levels of aflatoxins (ppb) in meat products

	Luncheon					Kofta				
	Aflatoxin (ppb)					Aflatoxin (ppb)				
	B1	B2	G1	G2	Total	B1	B2	G1	G2	Total
Min.	0.03	0.047	1.93	0.591	1.032	0.686	0.098	1.534	0.071	2.651
Max.	3.7	6.788	3.555	8.738	15.284	4.115	4.06	52.938	9.457	53.674
Mean	1.136	1.105	2.478	2.781	4.761	2.085	0.81	15.894	2.709	15.162

Table 3: Effect of ozone on Aflatoxin levels in luncheon

		Aflatoxin (ppb) / Ozone dose (ppm)													
		AFB1			AFB2			AFG1			AFG2			Total	
luncheon Samples	Cont.	20ppm O_3	40ppm O_3	Cont.	20ppm O_3	40ppm O_3	Cont.	20ppm O_3	40ppm O_3	Cont.	20ppm O_3	40ppm O_3	Cont.	20ppm O_3	40ppm O_3
1	1.84	1.221	0.737	0.107	0.062	UD.	UD.	UD.	UD.	3.238	1.424	1.358	5.185	2.707	2.096
2	1.969	0.491	0.248	0.171	0.123	0.053	UD.	UD.	UD.	1.25	1.199	0.828	3.39	1.814	1.129
3	0.814	0.2955	UD.	UD.	UD.	UD.	UD.	UD.	UD.	2.399	2.35	1.35	3.214	2.645	1.35
4	3.046	0.286	0.277	3.499	0.318	0.29	UD.	UD.	UD.	8.738	4.582	3.059	15.284	5.186	3.626
5	0.533	0.471	0.037	0.076	0.062	0.038	UD.	UD.	UD.	0.614	0.527	0.418	1.223	1.061	0.493
6	0.929	0.758	0.465	6.788	0.372	0.266	UD.	UD.	UD.	4.698	3.492	3.112	12.416	4.622	3.844
7	UD.	UD.	UD.	0.13	0.02	0.017	UD.	UD.	UD.	0.892	0.828	0.653	1.032	0.848	0.671
8	0.076	0.056	UD.	0.246	0.2	0.053	3.555	3.417	UD.	0.591	0.562	0.243	4.468	4.235	0.296
9	0.795	0.078	0.053	0.047	0.02	0.01	UD.	UD.	UD.	0.714	0.608	0.436	1.557	0.707	0.499
10	3.006	1.993	UD.	1.523	1.031	0.048	UD.	UD.	UD.	1.904	1.541	0.123	6.433	4.566	0.17
11	0.732	0.467	0.359	1.092	0.304	0.223	UD.	UD.	UD.	UD.	UD.	UD.	1.824	0.771	0.583
12	0.03	0.014	UD.	0.838	0.669	UD.	1.93	1.43	UD.	1.715	1.496	0.863	4.513	3.609	0.863
13	1.038	0.403	0.081	0.521	0.439	0.328	UD.	UD.	UD.	7.647	5.154	3.835	9.206	5.995	4.245
14	0.073	0.034	UD.	1.346	1.284	0.508	UD.	UD.	UD.	2.881	2.877	2.291	4.301	4.195	2.799
15	3.7	1.763	0.758	0.814	0.764	0.624	UD.	UD.	UD.	6.169	1.939	1.168	10.684	4.466	2.549
Mean ppb	1.327	0.595	0.215	1.229	0.405	0.176	2.743	2.424	UD.	3.104	2.041	1.41	5.649	3.162	1.681
P- value	-	0.060	0.001**	-	0.154	0.007**	-	0.439	0.102	-	0.312	0.060	-	0.120	0.001**
Red.%		55.16	83.8		67.05	85.68		11.63	100		34.25	54.57		44.03	70.24
SD		0.9961	1.44		0.9329	2.422		3.742	1.016		0.9885	15.42		2.003	13.19

UD. Undetectable

SD Standard deviation

Table 4: Effect of ozone on Aflatoxin levels in Kofta

		Aflatoxin (ppb) / Ozone dose (ppm)													
		AFB1			AFB2		AFG1			AFG2			Total		
Kofta Samples	Cont.	20ppm O_3	40ppm O_3	Cont.	20ppm O_3	40ppm O_3	Cont.	20ppm O_3	40ppm O_3	Cont.	20ppm O_3	40ppm O_3	Cont.	20ppm O_3	40ppm O_3
1	1.838	1.115	0.835	0.914	0.318	0.061	19.291	5.216	3.911	0.958	0.04	UD.	23.001	6.69	4.808
2	1.273	1.239	0.93	0.248	0.152	UD.	43.241	8.252	7.74	1.901	0.932	0.444	46.663	10.575	9.115
3	1.474	0.737	UD.	0.38	0.221	0.046	38.399	13.266	4.109	UD.	UD.	UD.	40.254	14.225	4.155
4	0.736	0.703	0.142	UD.	UD.	UD.	52.938	12.565	10.71	UD.	UD.	UD.	53.674	13.269	10.852
5	4.115	1.965	1.534	0.302	0.086	UD.	UD.	UD.	UD.	5.456	2.358	1.961	9.873	4.41	3.496
6	3.914	3.084	2.889	0.098	0.087	0.075	6.527	3.271	2.579	3.182	1.635	1.289	13.72	8.077	6.833
7	4.079	2.774	1.19	3.288	1.439	0.179	UD.	UD.	UD.	9.457	2.716	UD.	16.826	6.93	1.369
8	1.893	0.651	0.222	4.06	0.285	0.177	2.337	0.75	0.524	0.072	0.065	UD.	8.362	1.75	0.924
9	1.143	0.81	0.238	0.153	0.026	0.033	4.409	3.568	1.73502	1.102	0.923	0.641	6.807	5.327	2.646
10	1.967	1.816	1.702	0.112	0.112	UD.	1.534	UD.	UD.	4.153	4.044	3.97	7.765	5.972	5.673
11	0.917	0.585	0.328	0.527	0.513	0.211	14.891	11.227	8.744	0.458	0.162	0.04	16.794	12.487	9.324
12	3.688	0.875	0.412	0.128	0.1	0.058	2.646	UD.	UD.	3.046	2.728	1.447	9.51	3.703	1.917
13	0.686	0.552	0.302	0.116	0.054	0.006	UD.	UD.	UD.	1.849	1.839	1.167	2.651	2.445	1.475
14	2.389	1.419	0.96	0.735	0.218	0.051	10.689	4.782	2.711	3.836	2.263	1.624	17.649	8.682	5.347
15	1.887	1.281	0.681	0.869	0.241	0.091	11.754	3.235	2.411	2.753	1.435	1.148	17.263	6.193	4.333
Mean ppb	2.133	1.307	0.824	0.852	0.275	0.07	17.388	5.511	3.764	2.94	1.626	1.056	19.388	7.382	4.818
P-value	-	0.033*	0.001**	-	0.046*	<0.001**	-	0.083	0.015*	-	0.086	0.020*	-	0.003**	<0.001**
Red. %		38.72	61.37		67.72	91.71		68.31	78.35		44.69	64.08		61.92	75.15

UD. Undetectable

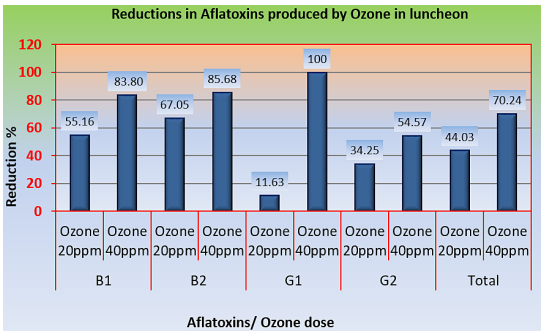

Fig. 1: Reductions in Aflatoxins produced by Ozone in luncheon

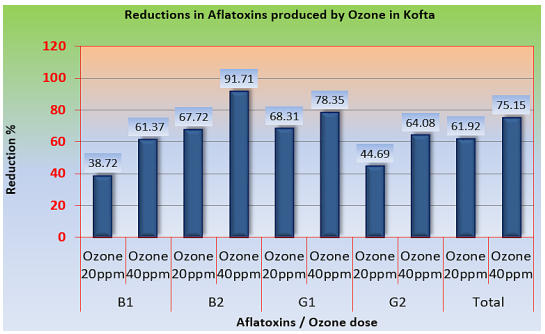

Fig. 2: Reductions in Aflatoxins produced by Ozone in Kotfta

For the raw meat product (kofta), the responses of AFs to O_3 were summarized in table 4 and figure 2. At 20 ppm O_3, the highest reduction was achieved in AFG1 and AFB2 (68.31% and 67.7%) respectively, followed by AFG2 and AFB1 (44.69% and 38.72%) respectively. Also at 40 ppm O_3, the highest reduction was achieved in AFB2 and AFG1 (91.71% and 78.35%) respectively, followed by AFG2 and AFB1 (64.08% and 61.37%) respectively.

After treatment with O_3, the physical status of meat products is expressed in pH values as illustrated in table 5. By the two applied concentrations of O_3, the pH values were none significantly ($p > 0.05$) affected. The response of fat to O_3 treatment in raw and ready-to-eat meat products was illustrated in table 6. At 20 ppm O_3 fat was more stable compared to 40 ppm where the raw product (kofta) appeared higher stability compared to ready-to-eat (luncheon). In kofta samples treated with 20 ppm O_3, 83.3% (10/12) of samples agreed with the allowed level of TBA in meat products (not more than 0.9 mg MDA/ Kg) while, at 40 ppm O_3 58.3% (7/12) of treated samples still meeting the allowed limit. In the case of treated luncheon, 69.2% (9/13) and 23.1% (3/13) of treated luncheon met the allowed level of TBA for 20 and 40 ppm O_3, respectively.

Table 5: Effect of Ozone on pH in luncheon & Kofta

.	pH values					
	Luncheon			Kofta		
Samples No	Cont.	20ppp O_3	40pp O_3	Cont.	20pppO_3	40ppO_3
1	5.19	5.19	5.11	5.65	5.2	5.11
2	5.27	4.87	4.71	4.96	4.95	4.89
3	5.37	5.37	5.36	4.88	4.8	4.67
4	4.82	4.81	4.8	4.9	4.89	4.72
5	6.15	6.11	6.11	4.95	4.90	4.89
6	6.1	6.08	6.07	5.14	5	4.99
7	6.28	6.27	6.20	4.54	4.48	4.35
8	6.1	6.06	5.94	5.1	4.95	4.94
9	6.31	6.27	6.26	5.14	5.13	5.01
10	5.98	5.92	5.91	5.03	5.03	5.02
11	6.03	6.02	6.01	5.01	4.99	4.98
12	5.97	5.98	6	5.84	5.78	5.76
13	6.02	5.95	5.91	4.75	4.74	4.63

14	6.24	6.19	6.13	5.1	5.08	5.04
15	5.33	5.32	5	5.53	5.09	5.06
Mean	5.81	5.76	5.7	5.1	5	4.93
P-value	-	0.633	0.372	-	0.350	0.158

Table. 6: Stability of fat in luncheon & Kofta treated with ozone

	TBA values (mg MDA/g)					
	Luncheon			**Kofta**		
Samples No.	**Cont.**	**20ppp O_3**	**40pp O_3**	**Cont.**	**20pppO_3**	**40ppO_3**
1	0.245	0.745	1.073	0.31	0.683	0.982
2	0.497	0.523	0.719	1.522	2.11	2.21
3	0.346	0.618	1.411	0.32	0.391	0.497
4	0.593	0.845	1.25	0.245	0.553	1.1
5	1.183	1.789	1.819	0.795	0.931	1.61
6	0.562	0.853	1.121	0.446	0.547	0.946
7	0.252	0.949	1.232	0.315	0.547	0.668
8	1.181	1.338	1.539	0.416	0.547	0.689
9	0.076	0.278	0.509	0.088	0.179	0.315
10	0.277	1.287	1.691	0.456	0.461	0.598
11	0.772	0.878	1.1	0.527	0.578	0.669
12	0.278	0.606	0.697	0.668	1.305	1.507
13	0.646	1.136	1.317	0.431	0.467	0.901
14	0.535	1.08	1.681	0.911	1.805	1.814
15	0.278	0.348	1.482	0.906	1.279	1.486
Mean	0.515	0.885	1.243	0.557	0.825	1.066
P-value	-	0.007**	<0.001**	-	0.065	0.004**

Discussion

Contamination with AFs represents one of the problems threatening food industries due to their serious harms to human health. Recently, both B- and G-type AFs are categorized as Group 1 mutagens by the International Agency for Research on Cancer (IARC).[29] In the preliminary work of the current study, the AFs load of luncheon and kofta was evaluated. Table1 summarized the distribution of AFs in the two assessed products. With exception of AFG1 which predominated in kofta samples (64%) comparing to 12% in luncheon, other AFs nearly recorded by parallel levels for both products. AFB1 is the highest lethal mycotoxin for humans.[30] It was recorded in 96% and 88% of kofta and luncheon, respectively. Furthermore, 88% of kofta and 92% of luncheon revealed AFB2 while AFG2 was detectable in 84 and 88%, respectively.

At least two of the four major AFB1, B2, G1 and G2 were detected in 100% of luncheon and kofta with a mean of 4.8 and 15.2 ppb, respectively, table 2. The findings of present study coordinate with those of Karmi,[8] Shaltout *et al.* [31] and Soliman *et al.*[32] in exploring the role of luncheon and kofta in delivering AFs to consumers with their findings for total AFs are higher than current study.

Contamination of meat and meat products with AFs occurs through two routes. The first comes from feeding of the animals with contaminated feed which accumulates toxin residues in their tissues. The second way is the contamination that can occur during the processing, preservation and distribution of meat and meat products.[33] The Food and Agriculture Organization (FAO) focus on the hidden route of mycotoxins pollution

to meat products through the contaminated animal feedstuffs. Mycotoxins are estimated to contaminate up to 25% of the world's food crops and an even larger percentage of animal feed stuffs.[34]

The products under investigation are stored meat products where fungi constitute a significant division of their flora. The incidence of the various toxigenic genera in luncheon and kota has been documented by several research works. In addition, *in vitro* studies have proven the mycotoxins producing ability of these genera.[9,32,35,36] The generation of AFs is directly tied to the proliferation of aflatoxigenic fungus.[37] Also reports cleared that AFs are especially problematic in hot (30 to 40°C) dry climates.[5] Such favorable environmental conditions predominate the climate of the year in Assiut Governorate. Meanwhile, luncheon and kofta are formulated from meat and nonmeat additives. Related studies appeared that several AFs co-occur in contaminated commodities of meat cuts,[38] spices[13] and meat additives[39] so the occurrence of AFs in such formulated products was not surprising.

The detected AFs (AFB1,B2,G1 and G2) are characterized by their high temperature of hydrolysis (268°C-269°C) for AFB1, (244°C-246°C) for AFG1, (268°C-289°C) for AFB2 and (240°C-247°C) for AFG2.[40] These temperatures not achieved during processing of luncheon or by any cooking method for kofta. Consequently, the high AFs detoxification resistance to heat treatment necessitates the development of alternative effective methods.

In the experimental part of the present study, O_3 was tried as a green approach to control the hazard of AFs in meat products. The samples and their response to ozonation were listed in tables 3 and 4. As a post-processing step for ready to eat luncheon, the degree of detoxification was proportional to O_3 dose, table 3. At 20ppm O_3, AFB2 was the most liable to treatment with a resulted reduction of 67.1% followed by AFB1 and AFG2 where reductions were 55.2 and 34.3%, respectively. At 40 ppm, AFG1 appeared the highest detoxification degree where none of exposed samples revealed detectable level of the toxin (100% reduction). Also exposure to O_3 was effective in minimizing the hazard of other three AFs with a resulting reduction of 85.7, 83.8 and 54.6 for AFB2, B1 and G2, respectively (figure 1).

For the raw meat product, O_3 was applied as a processing step in raw kofta. The responses of AFs to O_3 were summarized in table 4 and figure 2. As for luncheon, the detoxification ability appeared proportional to O_3 concentration. At 20 ppm O_3 the highest reduction was achieved in AFB2 (67.7%). Also the same toxin was the most reduced (91.7%) by 40 ppm O_3.The present findings shared the observation with Agriopoulou *et al.*[15] that AFG1 appeared to be the most sensitive to O_3 especially at 40 ppm O_3 but not well coordinate with the observation of Proctor *et al.*$_3$ that the rate of degradation was higher for AFB1 and AFG1, compared to AFB2 and AFG2.The variation of response of AFs to O_3 may due to difference in food matrix, concentration of O_3, conditions of applications and whether samples were naturally or artificially contaminated.

Gaseous ozone had the ability to degrade AFs in many commodities and operation conditions. The degradation percentage ranged from 24 to 100% according O_3 dose and kind of food.[40] Also by O_3, the rate of degradation of AFB1 varied from 25%[43] to 96.6%[44] in agriculture products. For AFB2 the degradation rate was 84.5 %[44] while that recorded by Luo *et al.*[45] was 70.9%. Meanwhile, AFG1 could be degraded by O_3 by rate of 70.6%.[45]

Within the analyzed meat products, none of the samples revealed levels passing the permissible limit of total AFs (20ppb) recommended by FAO [46] for human foods. By the respect, it is well established that there is no AFs dose below which tumour development is not possible. To put it another way, only a zero amount of exposure will result in no harm to public health.[47] Besides, AFB1, B2, G1, G2 were detected in the samples characterized by high potency of toxicity, carcinogenicity, and mutagenicity.[2] The hepatocarcinogenic and immunosuppressive properties of AFB1 consider AFB1 is the most dangerous of the group.[48] Therefore the intake should be reduced to as low as reasonably achievable levels. For the same reasons the European Union regulation recommended not more than 2ppb AFB1 and not more than 4 ppb of total AFs in foods for human consumption.[49, 50]

In the current study, by 40 ppm O3 the most harmful AFB1 could be completely detoxified in 35.7% (5/14) of luncheon and 6.7% (1/15) of kofta samples.

AFB2 was completely detoxified in 14.3% (2/14) of luncheon and 21.4% (3/14) of kofta samples. AFG1 was completely eliminated in 100% (2/2) of luncheon and 16.6 % (2/12) of kofta. AFG2 was eliminated from 23.1% (3/13) of kfota samples but its residues continued in luncheon (tables 3 and 4). Ozone damages the furan ring's hypertoxic site in AFs.[45] As a result, their molecular structures alter, resulting in products with lower molecular weight, fewer double bonds, and lower toxicity.[51] The mechanism of AFs detoxification by O_3 varies with their structural differences.[52] When O_3 attacks furan ring double bond at C8–C9 of AFB1 and AFG1, results in the creation of primary ozonides. The first reaction of O_3 happens at various locations of AFB2 and AFG2 molecules because of the absence of susceptible double bonds.

Regarding meat the pH is the main technical attributes that drive consumer purchasing decisions. For accepted quality meat products, pH should not exceed 6.0.[53] The physical status of meat products after treatment with O_3 expressed in pH values was illustrated in table 5. By the two applied concentration of O3, the pH values were none significantly ($p > 0.05$) affected. Some of treated samples appeared slight but not significant reduction in pH of compared with control. The same observation was also recorded by Stivarius *et al.*[54] and Lyu *et al.*[51] for vacuum packaged beef and Mercogliano et al [55] for poultry meat treated by O_3. The mechanism that lower pH in some conditions of O_3 treatment has not been clear established but it is reported that by its own O_3 does not change pH.[56]

Ozone is preferred to other detoxification due to its precursors being abundant, can be used in a gaseous or aqueous form, and does not produce residue on the product.[57] Also does not need delivery (generated on-site) and without harmful disposal.[58] The response of fat to O_3 treatment in raw and ready to eat meat products was illustrated in table 6. At 20 ppm O_3 fat was more stable compared to 40 ppm where the raw product (kofta) appeared higher stability compared to ready to eat (luncheon). In kofta samples treated with 20 ppm O_3, TBA values were none significantly ($p>0.05$) affected and 83.3% (10/12) of samples agreed with ES [51] for TBA in meat products (not more than 0.9 mg MDA/Kg). At 40 ppm O_3, the TBA values were significantly ($p<0.05$) raised but 58.3% (7/12) of treated samples still meeting the allowed limit of ES.[51] Comparatively, 69.2% (9/13) and 23.1% (3/13) of treated luncheon met the ES [51] criteria for 20 and 40 ppm O_3, respectively.

Previous studies reported that the pro-oxidant effect of O_3 is possible but it is not selective. In other words, compared to other oxidizing agents, O_3 does not work selectively oxidizing definite enzymatic systems but as a common oxidizing agent.[59] Meanwhile, when in the presence of inorganic and/or organic substances, O_3 reacts quickly to produce a wide range of oxidized molecules, which then vanish in a matter of seconds.[60]

Cardenas *et al.* [61] and Muhlisin *et al.* [62] related the significant rise in TBA values in chicken and duck breast meat, respectively to the long exposure to O_3. On the contrary, Lyu *et al.* [51] revealed lowering values of TBA after the combined pretreatment CO and O_3 vacuum-packaged beef. Also, Pirani[63] revealed that O_3 had no effect on the oxidation of fat in the treated sausage. The differences in effects of O_3 on fat in experimental studies can be attributed to the different methodological approaches,[43] exposure time, temperature and moisture content of food.[64] Furthermore, the critical role played by environmental conditions, such as humidity and temperature on efficacy of O_3 has to be considered as it is known that little variations in these parameters can significantly modify its activity[65]

Conclusion

From achieved data it could be concluded that application of O_3 has given promising results for the serious problem of AFs residue in meat products. Ozone appeared able to eliminate or significantly reduce AFs in raw and ready-to-eat meat products with negligible changes in physicochemical properties (pH). However, much care is required as preserving chemical quality is very dependent on O_3 concentration. Since every O_3 application is unique, in vitro toxicological studies should be conducted to screen the effects of degradation products if any on human before starting large scale applications.

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Conflict of Interest
The authors declare no conflict of interest.

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