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RESEARCH ARTICLE

Detection of Aflatoxins and Ochratoxin A Residues in Meat Products with Amelioration by Probiotics

Mohamed Karmi
Department of Food Hygiene, Faculty of Veterinary Medicine, Aswan University,
81528 Aswan, Egypt
Corresponding author: Mohamed Karmi, email: karmy99@ yahoo.com

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Abstract

A total of 125 samples of basterma, burger, luncheon, minced meat and kofta (25 / each) were collected from meat markets in Aswan to study the presence of aflatoxins and ochratoxin A using competitive direct enzyme linked immunosorbent assay (CD-ELISA) technique. Moreover, the role of probiotics in residues' detoxification was carried out. The detection rates of aflatoxins versus ochratoxin A residues in the examined basterma, luncheon and minced meat samples were (80% Vs 92%), (92% Vs 80%) and (76% Vs 72%) respectively. The detection rate for both toxins was the same in burger (96%) and kofta (88%) samples. It was found that *Lactobacillus acidophilus* could reduce the aflatoxins and ochratoxin A in experimentally spiked burger to 0.12 µg/Kg (97.2%), 0.57 µg/Kg (61%), respectively, while *Saccharomyces serevisae* could reduce the aflatoxins in spiked burger to 0.17 µg/Kg (96%) and ochratoxin A to 0.43 µg/Kg (71.1%). It could be concluded that meat products represent a potential source of aflatoxins and ochratoxin A for consumers and probiotics significantly decrease the aflatoxins and ochratoxin A in meat products. Monitoring of meat products for mycotoxins and use of preventive compounds should be practiced.

Keywords: Aflatoxins, Ochratoxin A, Meat products, Detoxification, Probiotics.

Introduction

Mould and fungi are widely contaminating food sources as a result of bad hygiene and improper handling of food. Their toxigenic strains, such as *Aspergillus flavus, Aspergillus parasiticus* and *Penicillium* spp., produce highly toxic secondary metabolites known as mycotoxins. These mycotoxins constitute direct potential hazards to human and animal health even with low levels and cause severe economic losses [1]. About 300 mycotoxins are formed by 200 toxic species of fungi, 20 of them occur in meat and meat products. They induce various risks (immunosuppression, hormonal disturbances, carcinogenicity, teratogenicity and mutagenicity) to the consumers. . Liberation of mycotoxins in grains or on meat surface are influenced by several conditions such as species of fungi, species of plant, method of manufacture, duration of storage, atmospheric temperatures, relative humidity and other environmental conditions. Mycotoxins may show antagonistic or synergistic effects in human and animals [2]. Mycotoxins in meat and meat products may occur when animal feeds on contaminated feed or due to other food additives and spices [3]. The four major aflatoxins are called B1, B2, G1 and G2, based on fluorescence (blue or green) and chromatographic analysis. B1 is the most common one and has potent carcinogenic effect [4]. The occurrence of aflatoxins in processed meat was related to the addition of spices to fresh meat [5]. Aflatoxins are linked with carcinogenicity in humans and animals [6]. The adverse effect of aflatoxins on consumers depends on the level and time of exposure, age, gender, health state, strength of immunity, diet and environmental factors [7]. Ochratoxin A is a potent toxin, causes

***Corresponding author e-mail: (karmy99@ yahoo.com), Department of Food Hygiene, Faculty of Veterinary Medicine, Aswan University,**

nephrotoxicity and hepatotoxicity in human and animals [8]. Moreover, it has immunosuppressive, teratogenic and carcinogenic effects where it disturbs cellular physiology in multiple pathways [6]. Lower pH, higher salt content, higher relative humidity, lower temperatures, prolonged storage and ripening times (13-18 months) may help in growth of toxigenic fungi on meat surface [9]. Techniques used in ripening and drying of meat faultily encourage fungal growth although some of them are essential in the flavor of the products [10]. Toxigenic fungi can occur due to feeding animals with contaminated rations or contamination during manufacturing and storage of meat [11]. In Egypt, the most frequent and most common fungal species in meat are Aspergillus and Penicillium [12]. *Aspergillus flavus* represented 10% of all fungal isolates in meat samples in Assiut, Egypt [13]. Meat products get contaminated by the mycotoxins either directly through addition of contaminated spices or indirectly through food animals fed grains and feeding stuffs contaminated by mycotoxins, and subsequently transferred to the consumers (carry-over effects) [14]. Mycotoxins resist autolysis and enzymatic breakdown in the gastrointestinal tract (GIT) of animals, consequently, reside in the meat [15]. The majority of mycotoxins are heat tolerant and therefore decomposition during cooking or processing does not occur [16]. Probiotic such as lactic acid bacteria (LAB) was used to degrade mycotoxins and decrease their bioavailability, beside its beneficial health effects [17]. Perfect study of the weak points in the food chain which can allow the entrance of mycotoxins and toxic fungi should be sought to reduce their contamination [18]. The fungal growth in meat can be prevented by the use of chemical preservatives or by adjusting relative humidity, perfect packaging, or by use of antimicrobial agents, gamma radiation or ozone [19-20]. Monitoring of mycotoxins in meat products and in animal feeds is essential requirement to protect humans and animals from the hazardous products [21]. This study was conducted to determine five mycotoxins including aflatoxins (B1, B2, G1 and G2) and ochratoxin A in some meat products and to trial the use of probiotics such as lactic acid bacteria to eliminate the mycotoxins from meat products.

Materials and Methods

Samples

A total of 125 meat samples including 25 samples, each of basterma, Burger, luncheon, minced meat and kofta were collected from meat markets in Aswan during 2018. Samples were put in ice box with ice until transfer to the laboratory. Samples were preserved in sterile polyethylene bags in the refrigerator.

Analysis

Competitive direct enzyme linked immunosorbent assay (CD-ELISA) was used for analysis of aflatoxins [22] and ochratoxin A [23]. The veratox test kits, RIDASCREEN®OTA, No. R1311-R-Biopharm AG, Darmstadt, Germany were used. Ten gram of each samples were ground and extracted with 50 ml of 70% methanol and mycotoxins were analyzed according to manufacturer instructions. The absorbance was measured at 650 nm using an ELISA reader.

Manufacture of Burger

Burger was manufactured using raw ingredients previously tested for its freedom from aflatoxins and ochratoxin A residues. Aflatoxin and ochratoxin A standards (100 µl/L) were added to burger ingredients, incubated for 12 hours and different concentrations were determined in zero time [24]. Then treated with different concentrations of *Lactobacillus acidophilus* and *Saccharomyces serevisae* (1% and 3%) for experimental evaluation of the efficacy of probiotic in amelioration of aflaotoxines and ochratoxines in meat products

Probiotics

Inocula of Lactic acid bacteria including *Lactobacillus acidophilus* and *Saccharomyces serevisae* were prepared and added to burger formula in two different concentrations; 1% and 3%. *Lactobacillus acidophilus* was obtained from Chr. Hansen`s Lab, Denmark, while, *Saccharomyces serevisae* was obtained from baker`s shops as baker`s yeast [25].

Results

Data in Table (1) reveals that aflatoxins residues were detected in 20 (80%) basterma, 24 (96%) burger, 23 (92%) luncheon, 19 (76%) minced meat and 22 (88%) kofta samples. Concentration of aflatoxins residues (µg/Kg) in the examined samples was ranged from 2.3 to 2.9 with a mean of 2.6 ± 0.05 in basterma, 2.5 to 2.9 with a mean of 2.7 ± 0.04 in burger, 2 to 2.7 with a mean of 2.3 ± 0.03 in luncheon, 2 to 2.8 with a mean of 2.5 ± 0.07 in minced meat and 2.5 to 3 with a mean of 2.7 ± 0.04 in kofta. Table (2) shows that ochratoxin A residues were detected in 23 (92%) basterma, 24 (96%) burger, 20 (80%) luncheon, 18 (72%) minced meat and 22 (88%) kofta samples. concentrations of ochratoxin A residues (µg/Kg) in the examined samples were ranged from 1.5 to 3.5, 0.07 to 2, 0.05 to 2.7, 0.05 to 2 and 0.08 to 2 in the examined basterma, burger, luncheon, minced meat and kofta samples with means of 2.5 ± 0.15, 1.04 ± 0.14, 1.4 ± 0.16, 1.03 ± 0.14 and 1.04 ± 0.13, respectively. For the experimental trials to evaluate the impact of probiotics in mycotoxine amelioration , results in Table (3) reveals the ability of probiotics to reduce the aflatoxins in spiked burger, *Lactobacillus acidophilus* at a concentration of 1% reduced aflatoxins concentration from 1.20 (71.8%) on the 2nd day to 0.12 (97.2%) on the 6th day post inoculation. However, a concentration of 3% of *Lactobacillus acidophilus* reduced aflatoxins concentration from 1.23 µg/Kg (70.7%) to 0.14 µg/Kg (96.7%) on the 2nd to the 6th day. On the other hand, inoculation of *Saccharomyces serevisiae* reduced the aflatoxins concentration from 2.85 µg/Kg (33%) to 0.17 µg/Kg (96%) and from 1.68 µg/Kg (60%) to 0.17 µg/Kg (96%) on the 2nd to 6th day when used at a concentration of 1% and 3%, respectively. Table (4) shows *Lactobacillus acidophilus* (1%) reduce the ochratoxin A in spiked burger from 1.35 µg/Kg (5.6%) to 0.57 µg/Kg (61%) on the 2nd to 6th day, respectively and by using *Saccharomyces serevisiae* from 1.32 µg/Kg (2.2%) to 0.43 µg/Kg (71.1%) in the 2nd to 6th day, respectively.

Table 1: Aflatoxins residues (µg/Kg) in the examined meat products obtained from Aswan markets using CD-ELISA **during 2018**

Meat product	Number of examined samples	Positive samples	Percentage of positive samples	Minimum level	Maximum level	Mean ± SE*
Basterma	25	20	80	2.3	2.9	2.6 ± 0.05
Burger	25	24	96	2.5	2.9	2.7 ± 0.04
Luncheon	25	23	92	2	2.7	2.3 ± 0.03
Minced meat	25	19	76	2	2.8	2.5 ± 0.07
Kofta	25	22	88	2.5	3	2.7 ± 0.04

*Standard Error, CD-ELISA: competitive direct enzyme linked immunosorbent assay

Table 2: Ochratoxin A residues (µg/Kg) in the examined meat products obtained from Aswan markets during 2018 using CD-ELISA.

Meat product	Number of examined samples	Positive samples	Percentage of positive samples	Minimum level	Maximum level	Mean ± SE*
Basterma	25	23	92	1.5	3.5	2.5 ± 0.15
Burger	25	24	96	0.07	2	1.04 ± 0.14
Luncheon	25	20	80	0.05	2.7	1.4 ± 0.16
Minced meat	25	18	72	0.05	2	1.03 ± 0.14
Kofta	25	22	88	0.08	2	1.04 ± 0.13

*Standard Error
CD-ELISA: competitive direct enzyme linked immunosorbent assay

Table 3: Ability of probiotics to reduce aflatoxins residues in experimentally spiked burger using CD-ELISA.

Probiotic concentration	Reduction quantity (µg/Kg) and percentage							
	0 day	%	2nd day	%	4th day	%	6th day	%
Lactobacillus acidophilus 1%	4.27	0	1.20	71.8	0.50	88.3	0.12	97.2
Lactobacillus acidophilus 3%	4.20	0	1.23	70.7	0.46	89	0.14	96.7
Sacharomyces serevisiae 1%	4.26	0	2.85	33	1.73	59.4	0.87	79.5
Sacharomyces serevisiae 3%	4.25	0	1.68	60	0.66	84.5	0.17	96

CD-ELISA: competitive direct enzyme linked immunosorbent assay

Table 4: Ability of probiotics to reduce ochratoxin A residues in experimentally spiked burger using CD-ELISA..

Probiotic concentration	Reduction quantity (µg/Kg) and percentage							
	0 day	%	2nd day	%	4th day	%	6th day	%
Lactobacillus acidophilus 1%	1.43	0	1.35	5.6	1	3	0.64	55.2
Lactobacillus acidophilus 3%	1.46	0	1.42	2.7	0.8	45.2	0.57	61
Sacharomyces serevisiae 1%	1.35	0	1.32	2.2	1.20	11.1	0.66	51.1
Sacharomyces serevisiae 3%	1.49	0	1.45	2.6	1.39	6.7	0.43	71.1

CD-ELISA: competitive direct enzyme linked immunosorbent assay

Discussion

Aflatoxins residues were detected in 80%, 96%, 92%, 76% and 88%of basterma, burger, luncheon, minced meat and kofta samples, respectively. These results were higher than those reported by Ebaid [26], whose results were 33.3%, 40%, 33.3% and 26.7% in basterma, burger, luncheon and minced meat, respectively. However, our results were extremely higher than 20% in minced meat and 13% in kofta recorded by Shaltout and Salem [27], 14% in luncheon stated by Ismail and Zaky [12] and 16% and 20% respectively documented in basterma and luncheon by Abdel-Shafi *et al*. [28]. In the current study, the mean concentration of aflatoxins was 2.6 µg/Kg (µg/Kg = ppb) in basterma. This is similar to 2.3 µg/Kg recorded by Abdel-Shafi *et al*. [28], higher (1.33 µg/Kg) than Alaa Eldin *et al*. [29] and lower than 4.5 and 15.8 µg/Kg reported by Refaie *et al*. [30] and Ebaid [26], respectively. Mean concentration aflatoxins was 2.7 µg/Kg in burger in the current study which is higher than (0.59 µg/Kg) that reported by Roushdy *et al*. [31], nearly similar to those recorded by Hegazi *et al*. [32], Hassan [33] and lower than those documented by Ebaid [26] and Aziz and Youssef [5]. In luncheon examined in the current study, the mean aflatoxins' concentration was 2.3µg/Kg. This is similar (3.7 µg/Kg) to Ebaid [26], and higher than 0.063 µg/Kg, 0.41 µg/Kg and 0.153 µg/Kg previously cited by Hassan [33], Roushdy *et al* [31] and Ali *et al*. [34]. While, it was lower than those recorded by Abdel-Shafi *et al*. [28] and Ismail and Zaky [12]. Aflatoxins' concentration in minced meat was 2.5 µg/Kg which is higher (0.88 µg/Kg) than that of Roushdy *et al*. [31] of, and lower (4.1 µg/Kg) than that of Shaltout and Salem [27]. The mean aflatoxins' concentration in Kofta in the current study was 2.7µg/Kg. This is similar to

3µg/Kg and 2.4 µg/Kg, respectively recorded by Shaltout and Salem [27] and Alaa Eldin *et al*. [29] and lower than 6.7 µg/Kg and 13 µg/Kg, respectively stated by Shabana *et al*. [35] and Shaltout *et al*. [1]. Percentage of ochratoxin A residues was 92%, 96%, 80%, 72% and 88% in basterma, burger, luncheon, minced meat and kofta samples, respectively. Ochratoxin A was recorded by; Abd-Elghany and Sallam [21] in 100% of luncheon and burger, and in 67% and 10% of luncheon as recorded by Hort *et al*. [36] and Ali *et al*. [34], respectively. However, Ochratoxin A was detected by Shaltout and Salem [27] in 6.66% of kofta and minced meat and by Iacumin *et al*. [37] in 45% of sausage. Mean concentrations of ochratoxin A were 2.5, 1.04, 1.4, 1.03 and 1.04 µg/Kg in basterma, burger, luncheon, minced meat and kofta samples, respectively. Concentrations of 1.5 µg/Kg, 4.55 µg/Kg, and 7.83 µg/Kg were respectively reported by Shaltout and Salem [27] in minced meat, Gareis *et al*. [38] and Markov *et al*. [39] in sausage.

Abd Elghany and Sallam [21] detected ochratoxin A at concentrations of 7.8 µg/Kg in sausage, 5.23 µg/Kg in luncheon and 4.55 µg/Kg in burger. However, concentrations of 56-158 µg/Kg and 240 µg/Kg were previously documented by Sorensen *et al*. [40] in meat products and Hassan *et al*. [41] in minced meat. The concentration of aflatoxins recorded in the investigated samples in the current study does not exceed the international permissible limits of 20, 15 and 20 µg/Kg respectively listed by FDA [42], WHO [43] and FAO [44]. Moreover, the concentration of ochratoxin A recorded in the screened samples in the current study does not exceed the international permissible limits (5 µg/Kg) previously recorded by FAO [45] and WHO [43].

Inoculation of *Lactobacillus acidophilus* into the experimentally spiked burger with standard aflatoxins caused a significant rate of reduction in detected toxin by the time, ranged from 71.8% on the 2nd day to 88.3% on the 4th day and reached a maximum reduction of 97.2% on the 6th day of cold storage. There is no difference in the effect between 1% and 3% concentration of *Lactobacillus acidophilus*, while the reduction rate in case of *Saccharomyces serevisiae* was ranged from 33% on the 2nd day to 59.4% on the 4th day and reached a maximum reduction of 78.5% on the 6th day. This reduction effect was increased in case of 3% concentration which was 60%, 84.5% and 96% on the 2nd, 4th and 6th, respectively. The higher is the concentration of *Saccharomyces serevisiae* the higher is the reduction effect of the toxin. Inoculation of *Lactobacillus acidophilus* into the experimentally spiked burger with standard ochratoxin A caused a reduction of the toxin by the time, ranged from 5.6% on the 2nd day to 3% on the 4th day and reached a maximum reduction of 55.2% on the 6th day of cold storage with no significant difference in the effect between 1% and 3% concentration of *Lactobacillus acidophilus,* while the reduction rate in case of *Saccharomyces serevisiae* was ranged from 2.2% on the 2nd day to 11.1% on the 4th day and reached a maximum reduction of 51.1% on the 6th day which significantly increased to 71.1% on the 6th day in case of 3% concentration of the *Saccharomyces serevisiae*. Human exposure to mycotoxins occurs frequently due to consumption of mould-contaminated agriculture products or transmission from feed to meat [46]. Mycotoxins transferred to the consumers by ingestion of contaminated food or by inhalation of toxigenic spores or by direct contact. Mycotoxins contaminate cereal grains before harvest or during storage [6]. The formation of mycotoxins by the fungus depends on nutrients, the specific enzymes and environmental conditions. There is association between the growth of fungi and the production of aflatoxins, so that conditions suitable for fungal growth are favorable for toxin production. Moisture content (water activity, w_a) and the temperature of the food are very important factors in fungal growth [47]. The occurrence of aflatoxins in processed meat was related to the addition of spices to fresh meat [5]. The majority of mycotoxins are heat tolerant and therefore not affected by cooking or processing [16]. Moulds contaminate meat during slaughtering, dressing and handling in slaughter houses where the environmental conditions as air, walls, floors, equipments and workers hands as well as intestinal contents play an important role in contamination of meat [48]. The contamination of meat with mycotoxins

probably comes from food additives than animal tissues or during processing, transport or storage [49]. The genus Lactobacillus was frequently involved in the antifungal activity of LAB as probiotics [50], which remove the mycotoxins from meat through physical binding of the toxin to the cell wall or cell wall components [51]. The genus Saccharomyces also reduces the mycotoxins from meat through physical adsorption of the toxin on surface of cell wall [52]. The cell wall polysaccharides (Glucan and mannan), proteins and lipids adsorb mycotoxins through hydrogen bonding, ionic or hydrophobic interaction [53]. Strategies should be designed to prevent the entrance of mycotoxins to meat products through reduction of contamination of meat products, additives and spices with fungal spores and through monitoring of all products for the presence of higher levels of mycotoxins higher than permissible limits.

Conclusion

Most samples of meat products were nearly positive for aflatoxins and ochratoxin A residues, so that, increased health hazards to consumers. It is advisable to monitor the meat and its products for the presence of these mycotoxins. The use of probiotics as additive in meat products is very profitable where it is significantly decrease the bioavailability of aflatoxins and ochratoxin A residues levels in meat through physical binding and biological inhibition of the toxins.

Conflict of interest

The author declared that there are no competing interests.

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الملخص العربي

الكشف على متبقيات الأفلاتوكسينات و الأوكراتوكسين (أ) فى منتجات اللحوم مع الغاء سميتها باستخدام البروبيوتك

محمد كرمى

قسم الرقابة الصحية على الأغذية ـ كلية الطب البيطرى- جامعة أسوان- الرمز البريدى: ٨١٥٢٨ أسوان – مصر

تم تجميع عدد ١٢٥ عينة من البسطرمة, البرجر, اللانشون, اللحم المفروم و الكفتة (٢٥ عينة من كل نوع) من محلات اللحوم بأسوان لدراسة مدى تواجد سموم الأفلاتوكسينات الكلية و الأوكراتوكسين أ وجدوى استخدام البروبيوتك لالغاء سميتها. أوضحت النتائج ان معدل تواجد الافلاتوكسين مقابل الاوكراتوكسين (أ) فى البسطرمة, اللانشون و اللحم المفروم كان (٨٠% مقابل ٩٢%), (٩٢% مقابل ٨٠%) و (٧٦% مقابل ٧٢%), على التوالى. و كان معدل تواجد نوعى التوكسين ٩٦% فى البرجر و ٨٨% فى الكفتة. تجريبيا أستطاعت بكتريا اللاكتوباسيلس أسيدوفيلس أن تخفض نسب الأفلاتوكسينات الكلية و الأوكراتوكسين أ فى البرجر التجريبى بنسبة وصلت الى ٩٧.٢% و ٦١% على التوالى بينما أستطاعت بكتريا السكارواميسيس سيرفيسى أن تخفض الأفلاتوكسينات الكلية و الأوكراتوكسين أ فى البرجر التجريبى بنسبة وصلت الى ٩٦% و ٧١.١% على التوالى. تشكل منتجات اللحوم خطورة كبيرة على صحة المستهلكين لكونها مصدرا لسموم الأفلاتوكسينات الكلية و الأوكراتوكسين أ لذلك يجب اختبارها لمدى تواجد متبقيات هذه السموم كما ينصح باستخدام البروبيوتك لالغاء سميتها و خفض نسبتها فى منتجات اللحوم.