EFFECT OF OZONE FUMIGATION IN AFLATOXINS CONTENT OF STORED WHEAT GRAINS

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ABSTRACT

One of the most important factors determined wheat grain quality is aflatoxins contamination during the storage period. Thiess the purpose of this study was to evaluate the effect of ozone gas exposure on the aflatoxins reduction of wheat grains during storage. So, one experiment was conducted with four wheat varieties Sids 12, Misr 1, Sakha 93 as certified local varieties from Agricultural Research Center and Russian imported wheat and two exposure doses (o and 400mg)/ h of ozone gas by using ozone generator model XT-800, Ozone gas purifier generator with three subsamples from each.

The obtained data showed that aflatoxins B1, B2, G1, and G2 were detected in untreated wheat grain samples with the ratio of 17, 2, 76 and 5%, respectively. While, the ratio were 14, 2, 79 and 5% in the ozonated treatment by 400 mg/ h. AFG1was the main aflatoxin (76–79%) from the total aflatoxins content, meanwhile, other AFs gave the lowest values (21-24). Also, total AFs was increased after 90 days compared with 30 days from the storage. After 30 days from storage, Russian variety had the lowest AFs content and on the contrary, the variety of Misr 1 gave the lowest quantity of AFs after 90 days from storage. In general, the aflatoxin reduction were decreased by increasing the stored period.

Keywords: *Aflatoxins; ozone and wheat grains.*

INTRODUCTION

Wheat (T*riticum a estivum*) is one of most important agricultural crops in Egypt. Egyptian wheat production reaches about 8.8 million tons and the total consumption about 18.6 million tons, so about 9.8 million tons were imported in 2012 (Economic Affairs Sector, 2012).

On the other hand, the use of ozone in agricultural sector is more recent. Santos *et al.* (2007) reported that the ozone gas (O_3) can be used as a new alternative modified atmosphere to control and to eliminate pests in grains. However, the efficacy of aqueous ozone in killing food borne pathogenic bacteria (Restaino *et al.*, 1995; Zhao and Cranston, 1995) has been demonstrated. Meier (2011) reported that ozonation treatment has significantly decreased the biological toxicity of certain mycotoxins in contaminated feeds and food grains ensuring safety throughout the food supply chain. Five ppm ozone inhibited surface growth, sporulation, and mycotoxin production by cultures of *Aspergillus flavus* Link: Fr, (Mason *et al.*, 1997). Meanwhile, the growth of *Aspergillus flavaus* was completely inhibited and consequently the total aflatoxin content was decreased when wheat grains was directly treated with ozone gas for 5 and 6 hours, (Abd El Fadel *et al.*, 2011).

So, the aim of this experiment was to study the effect of ozone gas exposure on infected wheat grains with *Aspergillus flavus* on aflatoxin content in the storage period during 90 days.

MATERIAL AND METHODS

One laboratory experiment was performed during 2012 in order to determine the effect of ozone gas on aflatoxin reduction of wheat grains during the storage period. This experiment included eight treatments representing the combinations of two ozone gas exposures (untreated and 400 mg/h) for one hour and four infected wheat grains varieties by *Aspergillus flavus* with three subsamples from each. It was carried in the Regional Center for Food and Feed (RCFF), Agricultural Research Center (ARC), Giza, Egypt.

-Wheat grain samples (*Triticum aestivum*) were obtained as follows as Sakha 93, Sids12, and Misr1 as local variety, Field Crops Research Institute (FCRI) and Russian variety (mixed varieties imported from Russian).

-The ozone gas was produced from air using ozone generator model XT- 800, Ozone gas purilifier generator.

-Toxyigenic strain of *Aspergillus flavus* (NRRL 3518) was the producer of aflatoxin from Regional Center for Food and Feed (RCFF), (ARC), Egypt, according to AACC (2002).

Inoculation of spore suspension and production of aflatoxin in wheat grains:

Sterile wheat samples grains with moisture content 14 % were artificially infected with spores count of *Aspergillus flavus* strain (NRRL 3518) which used in a test for the ability of producing aflatoxin in wheat grains. Number of spores/ml was counted in the collected spore suspension using a Spencer haemocytometer to about 106spores/ml. Spore suspension was inoculated to test grains and give a final density of approximately 3000:3500 spore/g of wheat grains as described by Eisa *et al.* (1996).

Determination of t otal aflatoxin and standard aflatoxin:

Standard aflatoxin were obtained from Sigma Chemical Co.,USA. Diluting solution, Methanol, Trifluoroacetic acid, Lactic acid, Sodium chloride, Boric acid, Potassium Iodide, Sodium thiosulphate and Chloromphnicl were purchased from Sigma Chemical Co., USA. Solid AFB1, AFB2, AFG1 and AFG2 (Sigma, St. Louis, Mo.) were analysis by high performance liquid chromatography (HPLC) in RCFF, (AACC, 2000).

RESULTS AND DISCUSSION

According to this study, the aflatoxins B1, B2, G1, and G2 were detected in untreated wheat grain samples with the ratio of 17, 2, 76 and 5%, respectively. While, the ratio were 14, 2, 79 and 5% in the ozonated treatment by 400 mg/ h for above mentioned AFs, respectively (Fig. 1). The main aflatoxin appeared was G1, AFG1 recorded the highest ratio with values ranging from 76 – 79% for the total aflatoxins. Meanwhile, all other AFB1, AFB2 and AFG2 recorded the values varied from 21- 24% for the total aflatoxins and the lowest was B2 (2%).

Results in Table (1), indicated a low percentage in aflatoxines content FB2, AFG1 and AFG2 in wheat grain samples. Data also showed that the ozonation treatment (400 mg/ h) has only significant effect on the content of AFG1 and AFG2 after 30 days from storage. But after 90 days from storage, the use of ozone gas by the dose of 400 mg/ h significant effect on the content of AFB2 and AFG1. Similar results were obtained by El-Desouky *et al.* (2012) who recorded that the amount of AFB1 was decreased with over a longer storage period. Meanwhile, ozone gas is known to possess sporicidal activity (Khadre, *et al.*, 2001) and thereby at higher concentrations could be used to reduce the initial load of contaminants on product (*i.e.* treatment of raw materials) and to disinfect storage and processing areas (Najafi *et al.*, 2009 and Korzun and Sauer, 2008).

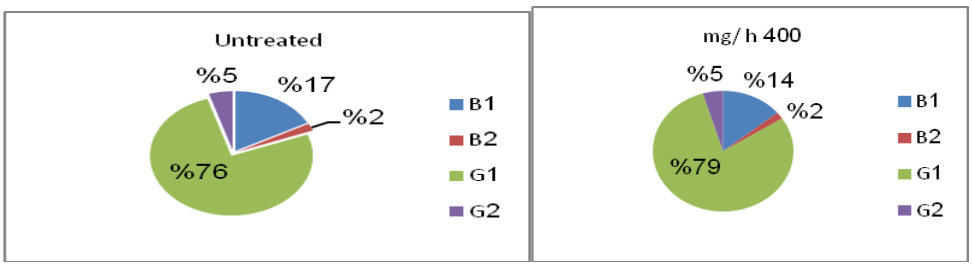


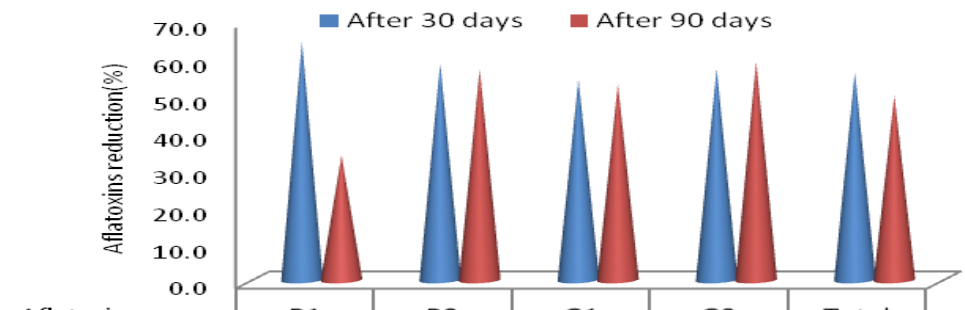
Fig. (1): The percentage of wheat grain aflatoxins content.

With respect to the varieties, Misr 1gave the lowest values (17.2, 3.6, 102.6, 8.3 and 131.7 ppb) for AFs (B1, B2, G1, G2 and total), respectively after 30 days from storage. While, the variety of Russian recorded the lowest AFs content after 90 days by the average of 20.3, 2.0, 119.7, 9.6 and 151.6 for AFB1, AFB2, AFG1, AFG2 and total AFs, respectively. Although the aflatoxin reduction were decreased by increasing the stored period, Fig. (2). The period of storage resulted in aflatoxin reduction from 56 to 49.6% and the reduction of AFB1 in the storage was reported at variable ranges from 64.1 to 33.6, depending on stored period. Graham (1997) reported that ozone gas has advantages over traditional fumigants: Ozone decomposes rapidly (half-life 20–50 min) to molecular oxygen; it leaves no residue; it can be generated on site and it requires no storage and subsequent disposal of chemical containers.

Thus, fumigation with ozone gas can be a good method for achieving sanitation and decreasing initial microbial load in food storage facilities and aid in curbing spoilage on a long term. However, it is clear from this work that ozone gas exposure time higher than 5 min would be required to achieve complete spore kills. These results were in agreement with other studies (Zorlugenc *et al.*, 2008; Antony and Singleton, 2009; Gabler *et al.*, 2010, and Alencar *et al.*, 2012).

Table (1): Effect of ozone exposure gas flow rate mg/h and wheat varieties on aflatoxins content of wheat grains.

Exposure rate flow mg/h	Aflatoxins content ppb ± SD After 30 days from treatment				
	B1	B2	G1	G2	Total
Untreated	41.8 ± 16.1	4.8 ± 0.7	184.9 ± 40.8	11.6 ± 2.3	243.0 ± 56.2
400.0	15.0 ± 22.5	2.0 ± 2.1	85.0 ± 58.1	5.0 ± 2.2	107.1 ± 84.1
	Wheat varieties				
Sids 12	60.3 ± 6.3	5.6 ± 0.2	210.1 ± 29.3	11.1 ± 3.0	287.1 ± 38.9
Misr 1	17.2 ± 13.2	3.6 ± 1.4	102.6 ± 22.9	8.3 ± 2.1	131.7 ± 39.6
Sakha 93	23.1 ± 22.2	2.4 ±2.1	112.2 ± 82.7	5.8 ± 2.7	144.1 ± 109.6
Russian	13.2 ± 11.8	2.0 ± 2.0	114.2 ± 64.8	7.9 ± 5.3	137.3 ± 83.9
	After 90 days from treatments				
Untreated	60.8 ± 27.6	4.4 ± 0.7	273.8 ± 62.2	11.2 ± 3.4	350.2 ± 84.5
400.0	40.4 ± 35.1	1.9 ± 1.2	129.6 ± 74.6	4.6 ± 3.4	176.4 ± 108.7
	Wheat varieties				
Sids 12	100.6 ± 3.3	4.4 ± o.6	295.5 ± 38.6	4.6 ± 1.8	405.1 ± 44.3
Misr 1	29.3 ± 18.2	3.1± 0.9	186.0 ± 97.8	12.1 ± 2.0	230.4 ± 118.9
Sakha 93	52.1 ± 10.8	3.2± 2.0	205.6 ± 101.1	5.2 ± 4.4	266.1 ± 118.3
Russian	20.3 ± 8.3	2.0 ± 1.5	119.7 ± 51.0	9.6 ± 4.9	151.6 ± 66.0



Aflatoxins	B1	B2	G1	G2	Total
After 30 days	64.1	58.3	54	56.9	56
After 90 days	33.6	56.8	52.7	58.9	49.6

Fig. (2): Effect of ozone exposure gas flow rate (400 mg/h) on aflatoxins content reduction of stored wheat grains

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تأثير التبخير بغاز الأوزون في محتوى الأفلاتوكسين بحبوب القمح المخزنة.

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يعتبر التلوث بالأفلاتوكسينات واحد من أهم العوامل المحددة لجودة حبوب القمح خلال فترة التخزين. الغرض من هذه الدراسة تقييم تأثير تعرض حبوب القمح لغاز الأوزون للحد من الأفلاتوكسينات أثناء التخزين. لذلك ، تم إجراء تجربة واحدة تشتمل على أربعة أصناف من القمح وهى سدس 12، مصر 1 ، سخا 93 من الأصناف المحلية المعتمدة من مركز البحوث الزراعية وصنف قمح روسي مستورد مع معاملتى التعرض لغاز الأوزون (صفر, 400 مجم/ ساعة) باستخدام جهاز اطلاق غاز الأوزون موديل 800 - XT، بواقع ثلاثة لعينات لكل معاملة .

أظهرت البيانات التي تم الحصول عليها الكشف عن تواجد الأفلاتوكسينات ب1، ب2، ج 1، ج2 في عينات حبوب القمح غير المعالجة بنسبة 17 ، 2، 76 و 5 ٪ على التوالي. في حين ، كانت النسبة 14 ، 2، 79 ، و 5٪ في المعامل بالأوزون بمعدل 400 مجم / ساعة. يعتبر أفلاتوكسين ج2 هو الأفلاتوكسين الرئيسي بنسبة (76-79 ٪) من إجمالي محتوى الأفلاتوكسين ، وفي الوقت نفسه ، أعطت باقى الأفلاتوكسينات الأخرى أقل القيم (21-24) . أيضا ، تم الكشف عن زيادة محتوى الأفلاتوكسينات بعد 90 يوما من التخزين عنه بعد 30 يوما من التخزين سجل الصنف الروسى أدنى قيمة و على العكس من ذلك سجل الصنف مصر 1 أقل قيمة من محتوى الأفلاتوكسينات بعد 90 يوما من التخزين. بشكل عام ، انخفضت نسبة الحد من الأفلاتوكسينات بزيادة فترة التخزين .