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Use of ozone to control fungal pollution in wheat grains

Abd El Fadel, M.G.* ; Seham S. Gad* ; A. A. El-Shibiny* ; M.F. Emara and T.T. El-Sisy****

*** Faculty of Environmental Agric. Sci. (Arish) , Suez Canal University.**

**** Regional Center for Food and Feed, Agriculture Research Center.**

ABSTRACT

The effect of ozone gas on mold and aflatoxin production in wheat grains imported from Argentine, Germany, Ukrainian, Australia and U.S.A and Egyptian wheat grains (Gamaza 7) were investigated. The treatment of wheat grains directly with ozone gas for 5 and 6 hours, the growth of *Aspergillus flavaus* was completely inhibited and consequently the total aflatoxin content was decreased. Cylinders of milling were found to be sources of fungal species pollution, total mould count and Aflatoxin production. The results suggest the use of ozone gas treatment for preservation of wheat grains during storage.

Keywords : Aspergillus, Aflatoxins, ozone gas, storage, wheat.

INTRODUCTION

Unavoidable, natural contaminants in foods may have either chemical or biological origin. Mycotoxins, secondary metabolites of fungi are biological contaminants. Despite efforts to control fungal contamination, toxigenic fungi are ubiquitous in nature and occur regularly in worldwide food supplies due to mold infestation of susceptible agricultural products, such as grains, cereal, nuts, and fruits. Thousands of mycotoxins exist, but only a few represent significant food safety challenges. The natural fungal flora associated with foods is dominated by three genera *Aspergillus*, *Fusarium*, and *Penicillium* may include commensals as well as pathogens. The chemical structures of mycotoxins produced by these fungi are very diverse, as are the characteristics of the mycotoxicoses they can cause ICMSF, (1996).

Aflatoxins may contaminate many crops including corn, peanuts , cottonseed, Brazil nuts, pistachios, spices, copra (dried coconut), and figs with widespread contamination in hot and humid regions of the world. Human aflatoxicoses continue to be an occasional, serious problem. For example, a severe outbreak was reported in Kenya in 2002 CDC, (2004). Half of the maize food samples tested in districts associated with this outbreak had Aflatoxins B_1 levels >20 ppb. This outbreak had at least a 39% incidence of death (317 cases with 125 deaths) resulting from acute hepatotoxicity. (Gong *et al. ,* 2002).

The first line of defense against the introduction of aflatoxins is at the farm level and starts with implementation of good agricultural practices (GAP) to prevent infection. Preventive strategies should be implemented from pre- through postharvest. Preharvest strategies include maintenance of proper planting/growing condition, antifungal chemical treatments and adequate insect and weed prevention. Postharvest measures include use of drying as dictated by moisture content of the harvested grain, appropriate storage conditions, and use of transport vehicles that are dry and free of visible fungal

growth CAC, (2003) and (Qian *et al.,* 2002). While implementation of these precautions go a long way toward reducing aflatoxin contamination of foods, they alone do not solve the problem and should be an integral part of an integrated HACCP-based management system (Lopez-Garcia *et al.,*1999)

Wheat grains are often harvested at a moisture content which can allow the growth of molds and mycotoxin production. If the grains dried to safe moisture content (14-16%), the fungal growth is delayed or inhibited and the toxin production will be inhibited too. Therefore temperature and humidity during wheat storage must be controlled during storage to maintain the grain healthy and prevent food poisoning. Therefore ,different strategies have been developed to prevent fungal growth on food ,one of which is supplementation with ozone gas (Watson and Golding ,1998). Thus, the objective of the present work was to investigate the effectiveness of ozone as preservatives to control the fungal pollution in the imported wheat grains and to determine the effective dose which can be used as fungicide for wheat grains during storage.

MATERIALS AND METHODS

Materials:

Five imported wheat grains *(Triticum aestivum*) different cultivars imported from Argentine, Germany, Ukrainian, Australia and U.S.A were obtained from five locations (Alexandria, Domiata, El-Suwas, El-Sokhna and Cairo) governorate and Egyptian wheat grains (Gemmiza 7) were obtained from El-Gharbia governorate. They were taken from six different Companies since 2009.

Aspergillus flavus NRRL (3518) was obtained from the Agricultural Research Service Culture Collection (Peroia, Illinois, USA). Natural Center for Agricultural Utilization Research (NCAUR) .

Media and Reagents :The following solutions and media were used for mold enumeration and identification :Peptone water, Rose Bengal chloramphenical agar (Biolife, Italy).

Aflatoxins: (B_1, B_2, G_1 and G_2) were obtained from Sigma chemical company (St. Louis, MO USA). Precoated TLC plates (0.2mm thick, 20x20 cm) coated with Silica gel/60, were obtained from Merck (Darmstadt, Germany).

Preservatives: Ozone equipment (FM-300 Mini Ozone Generator) according to WOUDC, (1998). Ozone gas (Concentration 200 mg per hour on ambient air), Plastic bottle (Anti chemical reaction resistance) Size: 5.3 liter

Methods:

Sampling and grain quality testing were carried out according to USDA, (1995 A). Measurement of the temperature, moisture, air conduction and humidity in silos under investigation were carried out according to USDA, (1999 B). Estimation of infested wheat was investigated according to USDA, (2004 D).

Experiment:

Studying the effect of Ozone treatment for different periods on the fungal count and mycotoxins content of wheat before and after storage was carried out as following: using ozone equipment (FM-300 Mini Ozone Generator) Five kilo of wheat sample under study were divided into 5 subsamples and treated as follows:

1. One Kg subsample stored as negative control sample containing normal flora,
2. One Kg subsample artificially inoculated with 10^5 cfu of toxigenic *Aspergillus flavus* NRRL (3518) and stored as positive control sample.
3. One Kg subsample artificially inoculated with 10^5 cfu/100g toxigenic *Aspergillus flavus* and stored for 21 day after which mycotoxin content was estimated.
4. One Kg subsample was artificially inoculated with 10^5 cfu/100g toxigenic *Aspergillus flavus.*
5. One Kg subsample was left to contain normal flora.

Analytical methods: Total mold count & Fungal identification were carried out using Rose Bengal chloramphenical agar and incubated for 5-7 days at 25 ^{o}C. Fungal identification was performed for isolated fungi in Food Safety Lab, Regional Center for Food & Feed, Agriculture Research Center and identified according to (Samson *et al.* 1995)

Estimation of Aflatoxins content was determined using the method of A.O.A.C. (1990 and 1995).

The effect of Ozone treatment for different periods on the fungal count and mycotoxins content of wheat before and after storage: Ozone treatment was applied to samples 3, 4 and 5 using ozone equipment (FM-300 Mini Ozone Generator) which concentration 200 mg per hour on ambient air according to WOUDC, (1998). During which subsamples were withdrawn each 1 hr to estimate the fungal growth as affected by this treatment. After treatment, all samples (1-5) were stored for 3 months through which subsamples were withdrawn each weekly to estimate the total fungal count. The mycotoxin content of the ozone gas treated samples was estimated just after treatment and at the end of every month of storage.

RESULTS AND DISCUSSION

Ozone is capable to be absorbed at wavelengths (ultraviolet radiation) of biologically damaging ultraviolet light. This radiation has been linked to health and environmental concerns. Most of this ozone (90 percent) is found in the stratosphere, the layer of theatmosphe re lying between the altitudes of 10 and 50 kilometers (Desvignes *et al.* 2008) who reported the using of the Oxygreen process which based on wheat grain treatment by ozone (produced in situ), in a closed sequential batch reactor. The Oxygreen process offers a close, homogeneous, and controlled contact between the gas and the grain. It is proposed for use for wheat grain decontamination (insects, fungi, bacteria, mycotoxins, pesticides)**.** Table (1) show thus five mixed samples of the six different wheat grains, so all controls and all treated samples were under detection limit (0.5ppb) for aflatoxins samples in the beginning of the storage period at 25˚C. moisture content of the samples

were modified 18% and stored, also mold count were 6.0 and 4.3 log cfu/g for (control of natural flora sample) and (control infected sample) respectively which infected with 10^5 cfu/g *Aspergillus flavus* NRRL 3518 tixogenic while (infected sample treated) and (natural flora sample treaded) were 4.4 and 6.5 log cfu/g before ozone gas treatment. (Cesare *et al.;* 2009) agreed with us who reported that aflatoxin contamination in corn was reduced by field application of ozone treatment. (Clurkin *et al*. ; 2009) found that ozone-treatment can significantly reduce the level of viable microorganisms on the surface of corn kernels.

Table 1: Effect of ozone gas treatment of six different wheat grains on mold growth by treating 8 hours of ozone at the beginning of storage at 25°C for 90 days.

Storage period (days)	Wheat samples	Mold count (log cfu/g) after ozone treatment									Aflatoxin (ppb)				
		0 hr.	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	7 hr.	8 hr.	B1	B2	G1	G2	Total
0	Control of natural flora sample	6.0	#	#	#	#	#	#	#	#	*	*	*	*	*
	Control infected sample	4.3	#	#	#	#	#	#	#	#	*	*	*	*	*
	Sample Counted Aflatoxin	#	#	#	#	#	#	#	#	#	*	*	*	*	*
	Infected sample treated	4.4	3.8	3.1	2.3	1.0	<1	<1	<1	<1	*	*	*	*	*
	natural flora sample treated	6.5	4.0	2.4	1.3	<1	<1	<1	<1	<1	*	*	*	*	*

*** = Under detection limit (0.5ppb)**
 # = Not determined

The data presented in Table (2) show the effect of ozone gas used as preservative for two mixed samples of the six different wheat grains on mould growth after storage at 25˚C for 7 days. It could be noticed that the increase in ozone gas treatment time reduced the mould count to less than 1.0 log cfu/g. The results could show that ozone gas retreatment of the samples every month to increased effect on mould count.

Table 2: Effect of ozone gas used as preservative for six different wheat grains on mold growth after 7 to 90 days of storage at 25°C.

Wheat samples		Storage period (days)											
		7	14	21	30	36	42	49	60	66	72	84	90
Control of natural flora sample	Mold count log cfu/g	6.5	7.2	6.1	6.0	6.0	5.5	4.8	4.8	5.2	5.3	5.8	6.5
Control infected sample		4.9	5.3	4.5	4.1	4.0	4.0	3.5	3.5	4.0	4.1	4.5	5.0
Infected sample treated		<1	<1	<1	<1	<1	<1	1.5	1.6	1.9	1.8	2.0	2.0
natural flora sample treated		<1	<1	<1	<1	<1	<1	<1	1.0	1.4	1.5	1.6	1.5

 # = Not determined

On the other hand the aflatoxin content of the five mixed samples of the six different wheat grains were under detection limit (0.5ppb) at the beginning of the storage period at 25˚C. Table (3) shows that storage at 25˚C for 21 days, total aflatoxin concentration ranged from 300ppb to 0.0 ppb. After 90 days all samples were under detection limit (0.5ppb) for aflatoxin after ozone gas treatment. (Raila *et al.* 2006) reported that drying grains by active

ventilation with an ozone air mixture, at O (3) concentration of 700 ppb the drying period was reduced by about 20 %, and contamination was reduced by up to 2.2 times. (Gaou *et al.* 2005) agree with this result too.

Table 3: Effect of ozone gas treatment of six different wheat grains on aflatoxins content during storage at 25°C.

Storage period (days)	Wheat samples	ozone treatment	Aflatoxin (ppb)				
			B1	B2	G1	G2	Total
0	Control of natural flora sample	#	*	*	*	*	*
	Control infected sample	#	*	*	*	*	*
	Sample Counted Aflatoxin	#	*	*	*	*	*
	Infected sample treated	#	*	*	*	*	*
	natural flora sample treated	#	*	*	*	*	*
21	Control of natural flora sample	#	51.3	6.9	17.4	14.4	90
	Control infected sample	#	126.5	34	35.5	24	230
	Sample Counted Aflatoxin	0 hr.	160	30	85	25	300
		1 hr.	86.4	38.6	26	29	180
		2 hr.	42	20	19	19	100
		3 hr.	42	9.6	14	4.4	70
		4 hr	9.6	5.1	2.5	2.8	20
		5 hr.	1.75	1.5	1	1	5
		6 hr.	*	*	*	*	*
		7 hr.	*	*	*	*	*
		8 hr.	*	*	*	*	*
	Infected sample treated	#	*	*	*	*	*
	natural flora sample treated	#	*	*	*	*	*
60	Control of natural flora sample	#	61.6	28	23	27	140
	Control infected sample	#	150	50	25	25	250
	Sample Counted Aflatoxin	#	*	*	*	*	*
	Infected sample treated	#	*	*	*	*	*
	natural flora sample treated	#	*	*	*	*	*
90	Control of natural flora sample	#	45	19	16	20	100
	Control infected sample	#	152.5	48	25	20.5	246
	Sample Counted Aflatoxin	#	*	*	*	*	*
	Infected sample treated	#	*	*	*	*	*
	natural flora sample treated	#	*	*	*	*	*

*** = Under detection limit (0.5ppb)** **# = Not determined**

Figure (1) illustrates the effect of ozone gas treatment for 8 hours on the mould. Total mould count growth was decreased to less than 1.0 log cfu/g. Modeling experiments were performed to evaluate bactericide and fungicide effects of small ozone concentrations (0.3 mg/l of water and 47 mg/m^3 of air)

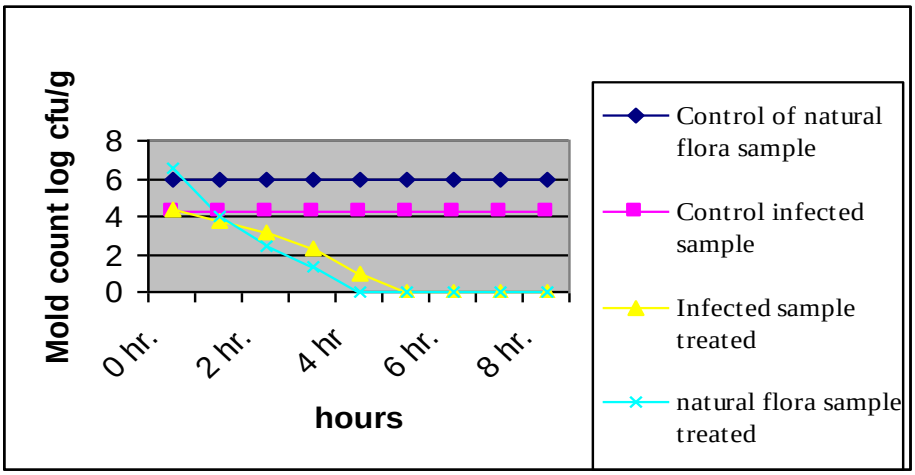

Figure 1: Effect of ozone gas treatment after ionization for 8 hours

The effect of ozone gas treatment on the mould growth during storage for 90 days at 25˚C on (infected sample treated) and (natural flora sample treaded) was decreasing slightly after 42 days of storage period which showed in Figure (2).

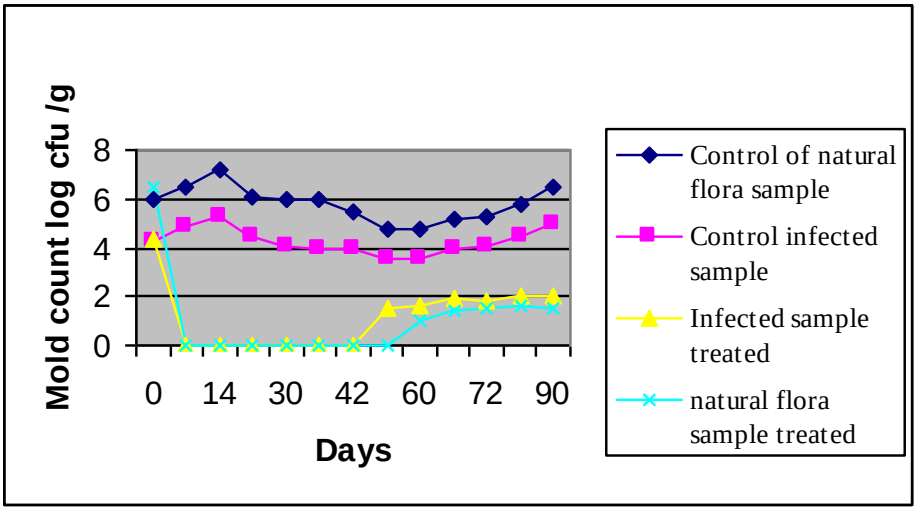

Figure 2: Mold count after ozone treatment of wheat grains during storage at 25ºC for 90 days after ionization

The effect of ozone gas treatment on total aflatoxin during ionization for 8 hours on (sample counted aflatoxin) so Figure (3) illustrates the decreasing of total aflatoxin to under detection limit (0.5ppb).

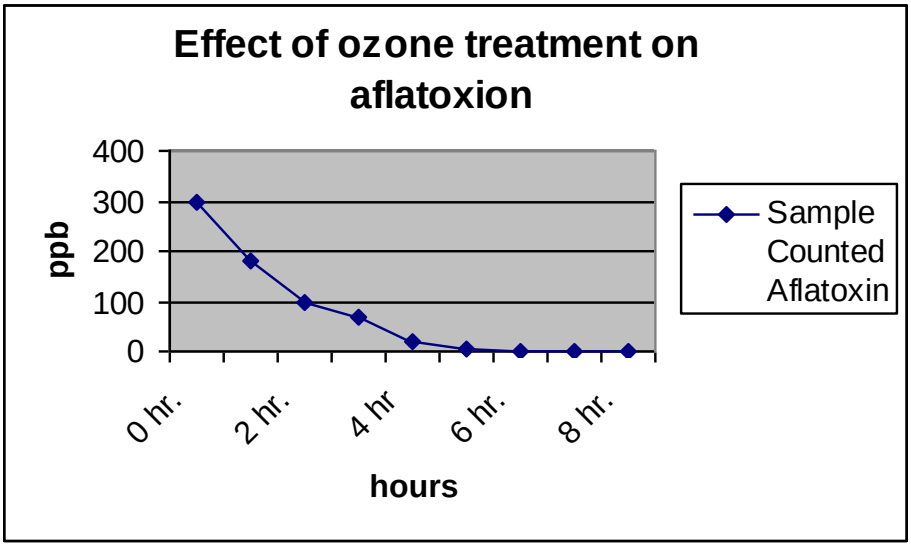

Figure 3: Aflatoxion during ionization for 8 hours

Conclusion

Therefore, it can be recommended the use of ozone to control the fungal pollution in wheat grains during storage under room temperature.

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استخدام غاز الأوزون لمكافحة التلوث الفطري فى حبوب القمح.

مجدي غانم عبد الفضيل، سهام صلاح الدين جاد*، أيمن عبد المجيد الشبيني* ، محمد فهمي زكى عمارة** و تامر توفيق السيسى****

*** كلية العلوم الزراعية البيئية بالعريش- جامعة قناة السويس**

**** المركز الاقليمى للأغذية و الأعلاف- مركز البحوث الزراعية**

تم دراسة تأثير غاز الأوزون على نمو الفطريات وإنتاج السموم الفطرية في عينات حبوب القمح المستوردة من (استراليا ، الأرجنتين ، ألمانيا ، أمريكا ، أوكرانيا) و القمح المصري (جميزة ٧) و قد استخدام غاز الأوزون لمدة (٥) ساعات مباشرة و وجد انه يثبط نمو *Aspergillus flavus* تماما مع عدم إنتاج سموم وأيضا استخدام لمدة (٦) ساعات مباشرة و وجد أنة يثبط إنتاج السموم الفطرية المنتجة في القمح. تعتبر سيليندرات الطحن من أهم مصادر زيادة التلوث الفطري و السموم الفطرية المنتجة. لذلك توصى النتائج باستخدام معاملة غاز الأوزون في حفظ حبوب القمح من نمو الفطريات وإنتاج سمومها .

قام بتحكيم البحث

أ.د / احمد عبد العزيز الرفاعى	**كلية الزراعة – جامعة المنصورة**
أ.د / محمد عبد الرازق النواوى	**كلية الزراعة – جامعة عين شمس**