Can ozone fumigation effectively reduce aflatoxin B_1 and other mycotoxins contamination on stored grain?

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Abstract

Mycotoxins are secondary metabolites produced by filamentous fungi that have deleterious effects on human and animal consumers. Cereals are probably the most important source of intake of mycotoxins, because they are susceptible to fungal attack either in the field or during storage and consequently contaminated by mycotoxins under adverse conditions favouring fungal invasion and growth. As a powerful oxidant, ozone has been successfully used for controlling stored-product pests, fungi, and sometime degrading mycotoxins in solution or in vitro (present as pure standards). Despite the above fact, information on the use of gaseous ozone as a mycotoxin-degradation agent for cereal grain preservation is still very limited. Also, it is not clear whether ozone fumigation can effectively degrade mycotoxins on stored grains. In the paper, we put forward wet method of ozone fumigation to degrade mycotoxin contamination on stored grains, and compared the degradation efficacy of three ozone gas treatments-dry method, wet method and aqueous method on aflatoxin B_1 and other mycotoxins in grains. Our results showed that the wet method of ozone fumigation could better degrade mycotoxin-spiked grain sample than the dry method, and aqueous method also had a good mycotoxin-degradation efficacy. To our knowledge, it is the first time to specially report the wet method of ozone fumigation to degrade mycotoxins on stored grains.

Keywords: Ozone gas, Mycotoxin degradation, Stored grain, Wet method

1. Introduction

Ozone, or triatomic oxygen (O_3), is a powerful disinfectant and oxidising agent (McKenzie et al., 1997). 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 destruction or detoxification of chemicals or micro organisms (Silva et al., 2001; Raila et al., 2006; Inan et al., 2007). Ozone gas also could effectively degrade mycotoxins in solution or in vitro (present as pure standards), such as aflatoxins, cyclopiazonic acid (CPA), fumonisin B_1, ochratoxin A (OTA), patulin, secalonic acid D (SAD) and zearalenone (ZEN) (McKenzie et al., 1997), and degradation products are generally harmless. For example, ozone would react across the 8, 9 double bond of the furan ring of aflatoxin through electrophilic attack, causing the formation of primary ozonides followed by rearrangement into monozonide derivatives such as aldehydes, ketones and organic acids (Proctor et al., 2004).

But information on the use of gaseous ozone as a mycotoxin-degradation agent for cereal grain preservation is still very limited. Application of ozone gas in stored grains to decompose the mycotoxins has been reported. Traditionally ozone gas generated by an ozone generator is directly into the spiked or contaminated grains, and then the mycotoxin content is analyzed for determining the treatment efficacy. But in practice, it was found that its efficacy of mycotoxin degradation for mycotoxin-spiked grain sample was not always satisfactory using this traditional method, and sometimes the higher doses of ozone and longer treatment duration were needed to achieve a good result (Luo jianwei et al., 2003; Proctor et al., 2004). For instance, 10 to 12 % (by weight) of ozone gas was used in the study on efficacy of ozonation to degrade aflatoxins in maize reported by Prudente and King (2002).

Our previous work showed that aflatoxin B_1, OTA, ZEN and DON in 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 by us) also has the high ability of degrading the mycotoxins. Therefore, in this paper, we firstly divided the ozone fumigation into a dry method and a wet method, and compared the two methods of the efficacy of degrading AFB_1 in cereal grain substrates. To our knowledge, it was the first to specially report the wet method of ozone fumigation to degrade mycotoxins on stored grains. In addition, for better understanding the ability of different ozone application ways, we also investigated the efficacy of aqueous ozone solution on degrading mycotoxins in grains.

2. Materials and methods

2.1. Grains and sample preparation

Wheat, maize and paddy rice samples were collected from Chinese state grain storage bin. The moisture level in the samples was determined in triplicate by Chinese National Standard Method (GB/T) No. 5497-85 Inspection of grain and oilseeds-Methods for determination of moisture content. All samples were cleaned to remove impurities and stored at room temperature before use.

Prior to ozonation experiments, grain samples were artificially contaminated with aflatoxin B_1 at a level of 25 $\mu g.kg^{-1}$ with wheat, 50 $\mu g.kg^{-1}$ with paddy rice and 100 $\mu g.kg^{-1}$ with maize according to 5 folds of Chinese hygienic MRLs in grains prescribed in hygienic standard for grains (GB 2715-2005). All spiked grain samples were thoroughly mixed and stored overnight before ozonation. Unspiked grain samples served as negative controls while spiked but untreated samples served as positive controls. To ensure complete recovery of aflatoxin, each sample was spiked with aflatoxins in the same container in which ozonation was carried out. After ozonation, each grain batch was homogenized entirely in 8:2 methanol / water immediately and then ground in a blend jar (Laboratory blender, 8010S, Waring Commercial®, USA) at high speed to ensure complete extraction of residual aflatoxins. This approach was used to reduce unsystematic variations typically associated with sampling of naturally contaminated grain samples and to provide a direct measure of treatment method efficiency (Proctor et al., 2004).

2.2. Ozonation experiments

2.2.1. Ozone gas generator

Ozone gas was generated from laboratory corona discharge ozone generator (Tonglin Corporation, P.R. CHINA) using pure oxygen. The pure oxygen was used because it could produce higher doses of ozone gas than air, and air could produce unfriendly substances such as NOx compounds during corona discharge process (Franco et al., 2008). In this experiment, ozone gas concentrations produced were determined by I_2 method (The industrial standard of the people's republic of China: CJ/T 3028.2-94 for the measure of ozone concentration, output, and specific energy consumption for ozone generator). At selected conditions of 30% generator power and 0.4 $L.min^{-1}$ of oxygen gas, the ozone gas concentrations produced were about 4.8 $mg.L^{-1}$.

2.2.2. Dry method

In the dry method, ozone gas was put directly into the glass reactor. Fifty grams of whole grain samples were placed in the ozone reactor, the check valve of the oxygen cylinder was opened and the flow-rate of oxygen gas was adjusted. Oxygen entered at the bottom of ozone generator and the ozone-rich stream was fed out from the top to the ozone reactor. Grains were exposed to ozone gas which concentrations were 4.8 $mg.L^{-1}$ and exposure time was set 12 h at room temperature. After these treatments, grain samples were extracted as described above.

2.2.3. Aqueous method

In the aqueous method, the reactor was added water, and grain samples were soaked in water, and ozone-rich steam was directly entered into the water where the reactions occurred between aqueous ozone and mycotoxin-spiked grains. Other setups were the same to the dry method. After these treatments, grain samples were air-dried and extracted as described above.

2.2.4. Wet method

In wet method, there was one container full of water between the ozone generator and ozone reactor, and ozone-rich steam produced was firstly put into water and then fed to the ozone reactor where water-vaporized ozone gas reacted with mycotoxin-spiked grain samples. After these treatments, grain samples were extracted as described above.

2.2.5. Aflatoxin B_1 and other mycotoxins including DON, OTA and ZON analysis

After ozone treatment, the entire grain samples of fifty grams were used for aflatoxin B_1 analysis. Each batch of treatment sample was ground using a blend jar (Waring 8010S) and extracted with 200 mL methanol – water (MeOH-H_2O) (8+2) solution containing 5 g NaCl. The combined mixture was blended for 3 min at high speed, and an aliquot of each suspension was then passed through a Whatman No. 4 paper in a porcelain filter under vacuum, collecting in a measuring cylinder. And the sequent analytical procedures were performed according to AOAC official method 2005.08. The determination of other mycotoxins- OTA, DON and ZEN in grains was also performed using HPLC methods with immunoaffinity column clear up. The analysis procedures of OTA, DON and ZEN were respectively performed according to GB/T No. 23502-2009, GB/T No. 23503-2009, and GB/T No. 23504-2009.

2.3. Quality checking

Some physical and biochemical characteristics including water content, color, fatty acid value and germination before and after dry and wet ozonation were examined according to the corresponding national standard methods. Among these, water content of wheat, paddy rice and maize was determined by Chinese National Standard Method (GB/T) reference No. 5497-85. Color and odour were checked using GB/T No. 5492-85. Fatty acidity of paddy rice was determined using GB/T No. 15684—1995. Germination test was determined using GB/T No. 5520－85.

2.4. Statistical analysis

All experiments were replicated three times. The results were evaluated using a Statistical computer program. Differences were considered significant at $P< 0.05$.

3. Results

3.1. Degradation efficacy of ozonation by three ways of ozone treatments

The degradation results of three treatment methods for paddy rice were shown in Fig. 1. Aflatoxin B_1 in paddy rice could be degraded by any method. But wet method gave the highest degradation efficacy, and at 12 h exposure time, the aflatoxin B1 content was reduced 94.4%, the residual content was 2.8 ppb, which was lower than maximum trace tolerable limits (MTLs) of aflatoxin B_1 in paddy rice, which is 10 ppb. The second was the aqueous method and its degradation ratio was 87.4%, and the dry method gave least degradation efficacy, its degradation ratio was 70.8% in the experiment. The degradation results of three treatment methods for maize are shown in Fig. 2. As shown above that the dry method only reduced aflatoxin B_1 52.4%, and aqueous method could reduce it 78.1%, and the wet method could reduce 85.0%. After wet method treatment, the residual content was 15 ppb with 12 h exposure time, and met the grain hygiene standard for maize which is 20 ppb. The degradation results of three treatment methods for wheat are shown in Fig. 3. In this experiment, the aflatoxin B_1 could be reduced more using the aqueous method which degradation level was 92.2%, and the second was the wet method, which degradation level was 85.5%. The dry method only reduced 56.8% with the same treatment conditions. After treatment using wet method and aqueous method, and the residual aflatoxin B_1 was 3.6 ppb and 1.95 ppb respectively and was also below the national grain hygiene standard which is 5 ppb.

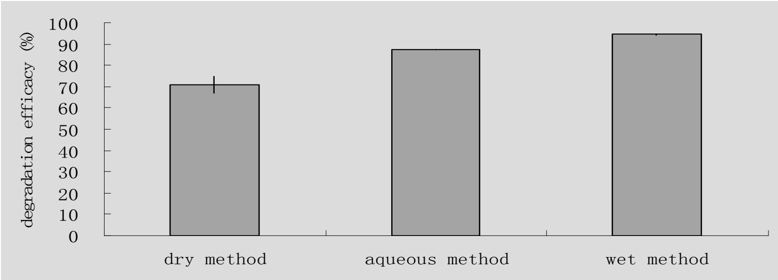

Figure 1 Degradation effect of aflatoxin B_1 in paddy rice using different ozonation methods.

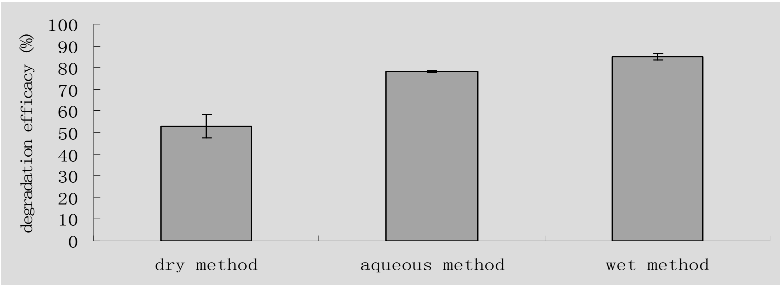

Figure 2 Degradation effect of aflatoxin B_1 in maize using different ozonation methods.

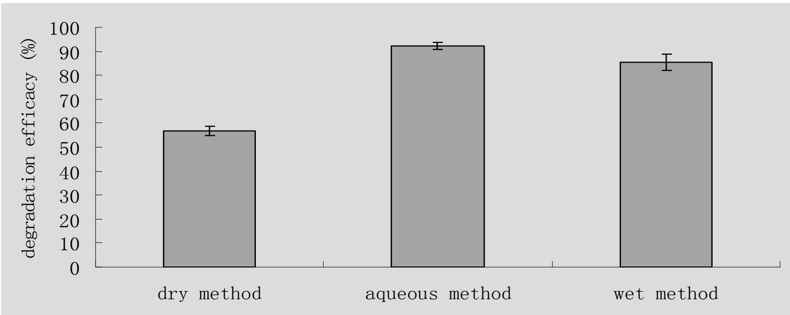

Figure 3 Degradation effect of aflatoxin B_1 in wheat using different ozonation methods.

The efficacy of different ozone treatment methods on DON, OTA and ZEN in grains was also investigated (data not shown). Similar to the results obtained from AFB_1, the wet method or aqueous method degraded mycotoxins in grains more than dry method.

3.2. Effect of dry and wet methods of ozone gas on the characteristics of cereal grains.

Table 1 showed the changes of some physical and biochemical characteristics of wheat, paddy rice and maize after dry and wet ozone treatment, and no ozone treatment grains were served as control samples. Different treatment methods had distinct effects on grain water content. Compared with control samples, water content of wheat, paddy rice and maize was reduced 1.78-2.7% after dry ozone method, and increased 0.44-1.62% after the wet method. Germination tests showed that grain germination was reduced after ozonation whatever wet or dry method. Compared with the dry method, the wet method seems to decrease more the germination capability. Few effects were observed for grain odour after wet and dry treatment method. However, the color appeared somewhat white after wet method compared with dry method and no obvious color changes were observed between grain treated by dry method and control sample.

Table 1 Some physical and biochemical characteristics of grains after dry and wet ozone treatment methods.

Treatment method	Grain type	Water content %	Germination rate %	Fatty acidity (mg KOH/100g)	color	odour
No ozone treatment	wheat	11.80	97	/	normal	normal
	Paddy rice	10.76	97	7.10	normal	normal
	corn	15.56	99	/	normal	normal
Dry ozone treatment	wheat	9.90	30	/	normal	normal
	Paddy rice	8.98	86	7.20	normal	normal
	corn	12.86	60	/	normal	normal
wet ozone treatment	wheat	12.24	34	/	somewhat white	normal
	Paddy rice	12.38	79	7.38	somewhat white	normal
	corn	16.08	38	/	somewhat white	normal

4. Discussion

As a promising treatment method, 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 (Raila et al., 2006). As we have showed that aflatoxin B_1 in stored grain also could be directly degraded by ozone gas. This result was in agreement with other studies. Traditional application of ozone gas, called dry method by us, was that directly feeding it into stored grain after ozone production without any pretreatment. In this experiment, aflatoxin B_1 was not effectively degraded within limited ozone concentrations and exposure time using dry method. The initial level of approximately 5 fold of aflatoxin B_1 MTLs in Chinese national grain hygiene standard in the ozone treated contaminated sample was only further reduced 50-70% after 12 h exposure, and the residual concentrations were still higher than Chinese national grain hygiene standard. This may indicated that dry method has low ability of degrading AFB_1 in stored grain with the relative low ozone concentrations and limited exposure time.

Aqueous ozone was another application way of ozonation, which was widely applied in food processing industry and water treatment (Wei Kuangji et al., 2007; Franco, et al., 2008; Suarez et al., 2007). 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 (von Gunten, 2003). It was found that aqueous ozone was very effective in significantly reducing organic pollutants in water or microbial loads on live catfish entering the plant. Mycotoxins in solutions also could be greatly degraded using aqueous ozone (Young et al., 2006), therefore the decontamination efficacy of aqueous ozone on mycotoxin-contaminated grains was expected. Herein we investigated the efficacy of aqueous ozone water on degradation of mycotoxin contaminated grains, and the results showed that aqueous ozone was very effective in significantly reducing mycotoxins in grains. Generally the efficacy of aqueous ozone method was superior to the dry method and the AFB_1 in the three types of grain could all be reduced about 80% or above, especially for wheat. We believed in that aqueous ozone would provide a good solution to processing the contaminated-mycotoxin grains in the grain processing industry.

For storage environment, aqueous ozone was not suitable, but the strong ability of degrading mycotoxins by the aqueous method seemed to indicate that the oxidation ability of ozone gas may be related with water, in other words, the water or water vapour appeared to play an important role in the reaction between mycotoxin-contaminated grains and ozone. Young et al. (2006) also mentioned the role of water in ozone reaction with DON. So the water-vaporized ozone gas was investigated about its ability of degrading mycotoxins in stored grains. The water-vaporized ozone gas was produced by being bubbled through water, and the application method of ozone gas was named the wet method, which was used to differentiate with traditional method-dry method. The results above showed that wet method proved to be an effective method. Generally the efficacy of the wet method was superior to the dry method and close to or better than the aqueous method. The possible mechanism was attributed to the reaction of water with ozone gas. When ozone gas contacted with water, the reactions might occur as below.

$$O_3+H_2O \rightarrow HO_3^{+}+OH- \quad (1)$$
$$HO_3^{+}+OH- \rightarrow 2HO_2 \quad (2)$$
$$O_3+HO_2\cdot \rightarrow \cdot OH+2O_2 \quad (3)$$
$$HO\cdot+HO_2\cdot \rightarrow H_2O+O_2 \quad (4)$$

In these reactions, the free radicals were produced such as OH which had the stronger oxidation ability than ozone gas. So the oxidation ability of ozone gas would be greatly enhanced after water-vaporization.

We also investigated the water content of grain after dry and wet method, and from the above results we could see that the water loss was significant after dry method, and after wet method, the grain water content did not also be elevated significantly as expected. Generally the water loss should be avoided during grain storage because of its direct relation with economy loss, but the higher water content would affect the grain storage safety because this would significantly increase possibility of mould development. Wet method could not lead to the water loss of grains and also did not lead to the significant increases of grain water content. Germination tests showed that ozonation significantly affected the capability of grain germination. Germination ability is very important for grains used as seed, and if a seed grain loses its ability to germinate, it has little value as a seed to be planted. Therefore ozone fumigation could only be used for the preservation of the stored grains, but could not be used as storage of seeds. Ozone fumigation, wet method or dry method, has little effect on grain odour, but has somewhat effect on grain color especially for the wet method. Other biochemical characteristics such as crude protein and RVA profile characteristics were also determined (data not shown), and detrimental effects were not observed. The effects of ozone treatment on physical and biochemical characteristics were also studied by other researchers. In general, there were no detrimental effects on grain characteristics after ozone treatments with certain treatment conditions. For example, the results observed by Mendez et al. (2003) showed that treatment of grains with 50 ppm ozone for 30 d had no detrimental effect on popping volume of popcorn, fatty acid and amino acid composition of soybean, wheat, and maize, milling characteristics of wheat and maize, baking characteristics of wheat, and stickiness of rice. Desvignes et al. (2008) also found that application of ozone treatment (10 g/kg) of common wheat before milling, the required energy at breaking stage whatever the grain hardness was significantly reduced (by 10-20%) and without changes in the flour yield.

In a word, ozone in managing stored grains has potential usefulness, and as a promising method for degradation of mycotoxins used in storage environment of grains, the wet method should be further developed in the future.

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