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Effect of ozone on aflatoxins detoxification and nutritional quality of peanuts



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ABSTRACT

Aflatoxins are a group of secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* with carcinogenicity, teratogenicity, and mutagenicity. Aflatoxins may be found in a wide range of agri-products, especially in grains, oilseeds, corns, and peanuts. In this study, the conditions for detoxifying peanuts by ozonation were optimised. Aflatoxins in peanuts at moisture content of 5% (w/w) were sensitive to ozone and easily degraded when reacted with 6.0 mg/l of ozone for 30 min at room temperature. The detoxification rates of the total aflatoxins and aflatoxin B₁ (AFB₁) were 65.8% and 65.9%, respectively. The quality of peanut samples was also evaluated in this research. No significant differences ($P > 0.05$) were found in the polyphenols, resveratrol, acid value (AV), and peroxide value (PV) between treated and untreated samples. The results suggested that ozonation was a promising method for aflatoxin detoxification in peanuts.

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1. Introduction

Aflatoxins are a group of natural compounds mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*. They have been found to be carcinogenic, teratogenic, and mutagenic to humans and animals (Hell & Mutegi, 2011; Li et al., 2010). Aflatoxin B₁ (AFB₁) is the most common and toxic among the aflatoxin group. In 1993, the International Agency for Research on Cancer (IARC) classified AFB₁ as a group I carcinogen (Alencar, Faroni, Soares, Silva, & Carvalho, 2012; Ding, Li, Bai, & Zhou, 2012; IARC, 2002). Aflatoxins are easily found in peanuts, corns, rice, and other agri-products, and they are considered inevitable in pre- and post-harvest crops. Biotoxins pose a serious threat to human health and greatly impact the food and feed industry. It is essential to bring aflatoxin contents of food and feedstuff to a safe level by developing detoxification technologies (Suzuki et al., 2002; Tripathi & Mishra, 2011; Velazhahan et al., 2010). Most of the reports on detoxification involve physical, chemical, and biological

methods (Banu & Muthumary, 2010; Chen, Liu, Xing, Liang, & Liu, 2010; Kabak, Dobson, & Var, 2006; Zhang, Xiong, Tatsumi, Li, & Liu, 2012). However, most of the current methods are not practical, due to time consumption, nutrition losses, or low detoxification efficiency.

Ozone is one of the most powerful sanitisers. It was affirmed as Generally Recognised As Safe (GRAS) in the United States and approved by the Food and Drug Administration (FDA) as an antimicrobial agent that can be directly applied in the food industry (Alencar, Faroni, Martins, Costa, & Cecon, 2011; Li et al., 2012; Tiwari et al., 2010). Previous studies have demonstrated that ozone is an effective agent to detoxify aflatoxins because the ozone-treated products exhibited much less toxicity than the untreated samples and showed no mutagenic activities or deleterious effects (Hideo, Yuji, Minoru, & Toshiyuki, 1988). Reports also show that detoxification efficiency increases with time and ozone concentration (Akbas & Ozdemir, 2006; Inan, Pala, & Doymaz, 2007). However, it is important to note that, as a chemical detoxification method, ozonation with low ozone concentration and short treatment time is required to mitigate the damage to peanut nutrition. In the present study, the effects of exposure time, ozone concentration, and moisture content on aflatoxin detoxification were investigated. Considering the importance of quality and nutrition components, we also evaluated the nutritional quality changes of

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peanuts by determining the polyphenols, resveratrol, AV, and PV. Polyphenols and resveratrol are considered as oxidised indicators for the first time in this research.

It is an urgent requirement that a commercially acceptable method for detoxifying aflatoxins be developed for application in the food industry. The purposes of the present study were to: (1) optimise the conditions for detoxifying aflatoxins in peanuts by gaseous ozonation and offer a practical method for ozonation detoxification, and (2) assess the peanut quality and nutrition changes caused by ozonation.

2. Materials and methods

2.1. Sample preparation

Peanuts were supplied by a peanut products company (Rizhao, Shandong, China) with an average aflatoxin content of 200 µg/kg detected by HPLC. The original moisture content of peanuts was 7.8%, and it was regulated by air-drying the peanuts or blending the peanuts with a calculated quantity of pure water. The moisture content was determined according to the Association of Official Agricultural Chemists (AOAC) method 934.06 (AOAC, 1996).

2.2. Detoxification treatments

Ozone gas was generated by an ozone generator (SOZ-YB-10G, Guangzhou Ozone Technology Co., Ltd., China). One kilogramme of peanuts was placed in a 30 l box, which was divided into two by a metal mesh (Fig. 1). The lower part is a 10 l gas chamber room, and the upper part is a 20 l reactor room. Peanuts were evenly spread over the metal mesh. Ozone gas from the generator was introduced into the chamber room, filtered through the peanuts in the reactor room, and finally passed through the vent hole on one side. The diameter of the outlet was greater than that of the inlet, ensuring that the exhausted ozone could be released successfully. The concentration of the injected ozone was regulated by controlling the proportion of the ozone output from the generator and determined by a ST04 portable many gas detection instrument (Zhengzhou Best Electronic Technology Co., Ltd., China). All experiments were performed at room temperature with relative humidity of 65–75%.

2.3. Effects of different factors on detoxification

Experiments were performed on the peanut samples to discover the detoxification effects of factors such as the treatment time (10, 20, 30, 60, and 120 min), ozone concentrations (3.0, 4.5, 6.0, and 7.5 mg/l), and moisture contents of the peanuts (2, 5, and 8%). AFB₁, AFB₂, AFG₁, AFG₂, and the total aflatoxins were studied under various treatments.

2.4. Aflatoxin analysis

Aflatoxins in peanuts were extracted and cleaned up using the AOAC method 991.31 (AOAC, 2002). Aflatoxin immunoaffinity

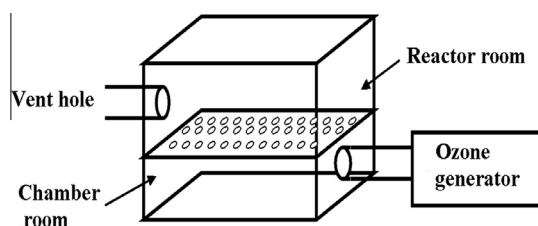


Fig. 1. Ozonation device for aflatoxin detoxification of peanut samples.

columns (IACs), with column capacity of 100 ng, were made, utilising the antibody-amino silica gel microparticle (Ma et al., 2013; Xiao et al., 2006). Quantitative analysis of aflatoxins was performed by Agilent 1220 HPLC, equipped with a fluorescence detector (FLD). A C18 analytical column (15 cm × 4.6 mm × 5 µm) and Romer Derivatisation Unit were used in the system.

2.5. Quality analysis

To study the ozone effect on the peanut quality, peanut samples from different purchase batches were treated under optimal ozonation conditions. Then, polyphenols, resveratrol, AV, and PV of samples were analysed. All the parameters were determined 48 h after ozone treatment; so was the untreated group.

Polyphenols were determined, using the Folin–Ciocalteu assay (ISO 14502-1, 2005; Stratil, Klejduš, & Kubáň, 2006). A Unicam UV 500 (Thermo, USA) was used to collect absorption spectra at 765 nm. A linear calibration graph was constructed with gallic acid concentrations of 5, 10, 20, 30, 40 µg/ml.

Resveratrol was analysed using an Agilent 1100 HPLC equipped with a diode array detector (DAD) with the target compound detected at 306 nm according to the method reported by Pereira, Câmara, Cacho, and Marques (2010).

The AV and PV were obtained from the crude oil of the peanut samples, which was prepared using a laboratory presser. The AV was determined using the titration method (AOCS Cd 3d-63, 1997). The PV was determined by the Acetic Acid–Chloroform method (AOAC 965.33, 2000; AOCS Cd 8-53, 2003) and calculated using formula (1):

$$PV = \frac{(V_1 - V_2) \times C \times 0.1269}{m} \times 100 \quad (1)$$

where V_1 is the volume (ml) of the Na₂S₂O₃ standard solution for the sample, V_2 is the volume (ml) of the Na₂S₂O₃ standard solution for the control check, C is the concentration (M) of the Na₂S₂O₃ standard solution, 0.1269 is grammes of iodine equivalent to 1 mmol of Na₂S₂O₃, and m is the mass (g) of the oil sample.

2.6. Statistical analysis

All experiments were replicated three times and one-way analysis of variance was used with a 5% probability. The significance analysis was carried out using the Statistical Analysis System (SAS) version 6.0 (2001).

3. Results and discussion

3.1. Effect of treatment time on detoxification

The effect of time on the detoxification is presented in Fig. 2. Ozone concentration of 7.5 mg/l and peanut moisture content of 5% were applied in treatments. The detoxification rate of the total aflatoxins increased with time if the treatment time was not more than 30 min. When the reaction time was 30 min, the highest detoxification rates of the total aflatoxins and AFB₁ were obtained, these being 66.9% and 65.0%, respectively. However, no distinct trend ($P > 0.05$) was observed if the reaction time was more than 30 min. According to Alencar's research (Alencar, Faroni, Martins, et al., 2011), the saturation time and residual concentration of gaseous ozone demonstrated that gaseous ozone reached a constant concentration after a certain period of time. Hence, the reaction between ozone and aflatoxins would reach a balance after a period of time. We chose 30 min as the best exposure time in this research.

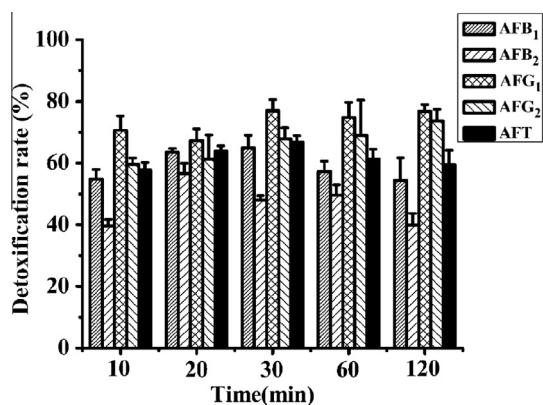


Fig. 2. Detoxification rates of aflatoxins in peanuts at different treatment times. The experiments were conducted with an ozone concentration of 6.0 mg/l and moisture content of 5%.

3.2. Effect of ozone concentrations on detoxification

Fig. 3 shows the detoxification rates at ozone concentrations of 3.0, 4.5, 6.0, and 7.5 mg/l. Other experimental conditions were optimised as follows: treatment time was 30 min and moisture content was 5%. The results indicated that the detoxification efficiency was proportional to the ozone concentration ($P < 0.05$). The highest detoxification rates of the total aflatoxins and AFB₁ were obtained at an ozone concentration of 7.5 mg/l, these being 64.0% and 62.1%, respectively. However, no significant difference was found between 6.0 mg/l and 7.5 mg/l, which indicated that the detoxification would finally reach a balance after the concentration increased to a certain value. We selected 6.0 mg/l as the best concentration for detoxification.

3.3. Effect of the moisture content on detoxification

The moisture content is a key factor in the process of gaseous ozone adsorption on the peanut surface. An increase in the moisture content facilitates ozone adsorption by the peanut surface (Alencar et al., 2012; Tiwari et al., 2010). As for peanut products companies, the moisture content of raw materials purchased from suppliers is below 9%, which is the safety threshold for controlling the growth of *Aspergillus* and other fungi (Codex Standard, 1995). After blanched peanut kernels are processed, the moisture content generally ranges from 4.5% to 5.5%, and the kernels are further dried with the moisture content changed to 2% before peanut

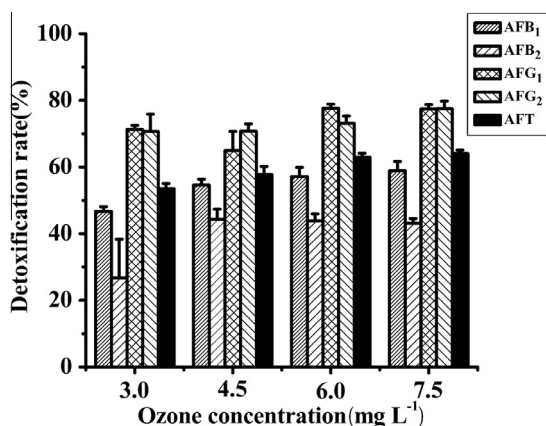


Fig. 3. Detoxification rates of aflatoxins in peanuts at different ozone concentrations. The experiments were conducted for 30 min with a moisture content of 5%.

butter is made. In this study, the moisture contents of 2%, 5%, and 8% were investigated.

Experiments were performed for 30 min with an ozone concentration of 6.0 mg/l (Fig. 4). The highest detoxification rates of the total aflatoxins and AFB₁ were 65.8% and 65.9%, respectively. However, no significant difference was found between moisture contents of 5% and 8%. We suggest that peanuts with low moisture content were insensitive to ozone.

In conclusion, the optimal conditions for detoxification were an ozone concentration of 6.0 mg/l, treatment time of 30 min, and peanut moisture content of 5%, under which conditions the detoxification rates of the total aflatoxins and AFB₁ were 65.8% and 65.9%, respectively. Chromatograms of the untreated samples and treated samples are shown in Fig. 5.

The mechanism of the detoxification is speculated to be that ozone could attack the C8-C9 double bonds in aflatoxin molecules. AFB₁ and AFG₁ had dihydrofuran rings in their structures, and the C8-C9 double bonds were easily attacked by ozone and broken down into organic acids, aldehydes, and ketones. AFB₂ and AFG₂ had no C8-C9 double bonds in their structures, and other unsaturated bonds were less reactive than the C8-C9 double bonds. So AFB₂ and AFG₂ were more resistant to ozonation than were AFB₁ and AFG₁. However, it is important to note that, compared with previous studies, the results showed that both AFB₂ and AFG₂ were relatively sensitive, and G-aflatoxins were more sensitive than B-aflatoxins. We speculate that a better detoxification rate was obtained mainly because of the matrix effect from various active materials in the peanuts.

3.4. Peanuts quality analysis

Polyphenols are common constituents in plant products and important antioxidants in food, which are contained, in large amounts, in peanut seed coats (Shem-Tov et al., 2012). Resveratrol, a naturally occurring polyphenol, has been reported as a phytoalexin against fungal infections in grapes, red wine, and peanuts. Polyphenols, including resveratrol, play a role in the prevention of degenerative diseases, particularly cardiovascular diseases and cancers with their antioxidative properties (Chukwumah, Walker, & Verghese, 2009; Jang et al., 1997; Sgambato et al., 2001). In this research, polyphenols were used as indicators to evaluate the degree of oxidation by ozone. It was apparent that the observed polyphenols and resveratrol contents (Table 1) showed no significant difference between treated and untreated groups ($P > 0.05$), which indicated that polyphenols and resveratrol were not affected by

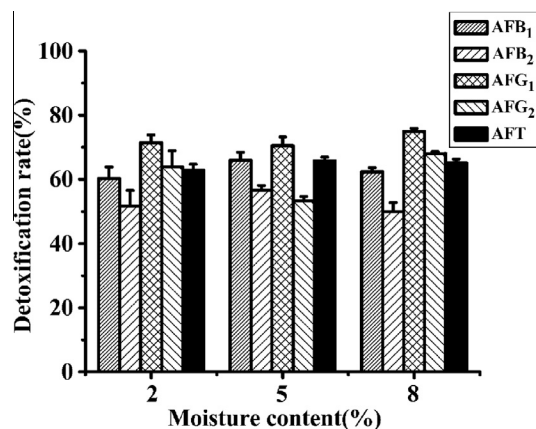


Fig. 4. Detoxification rates of aflatoxins in peanuts with different moisture contents. The experiments were conducted for 30 min with an ozone concentration of 6.0 mg/l.

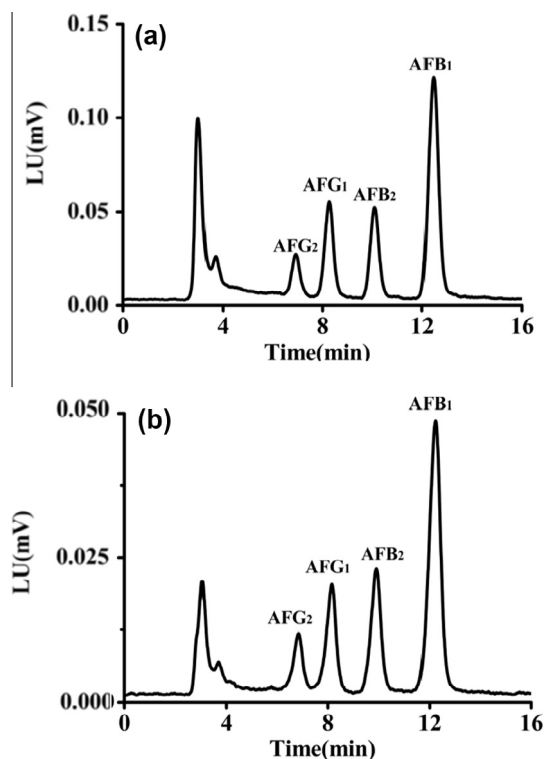


Fig. 5. HPLC chromatograms of aflatoxins in untreated peanut sample (a) and treated peanut sample (b).

ozonation under the optimal conditions. We conclude that the degree of oxidation, using this method, was acceptable.

Peanut is a high oil content product (50–55%), and 80% of the peanut oil is unsaturated fatty acids, which are susceptible to oxidation (Nepote, Mestrallet, & Grosso, 2006; Olmedo, Nepote, Mestrallet, & Grosso, 2008). AV and PV are two common parameters for illustrating the peanut oil quality and assessing the oxidation extent (Rao et al., 2009; Shantha & Eric, 1994). The acceptable values of AV and PV in peanut oil are 3 mg/g and 0.25%, respectively (Chinese Standard 2716, 2005). When peanuts are treated with a high concentration ozone for long time, the fatty acid is oxidised rapidly, and the AV and PV would increase (Byun, Yook, & Kwon, 1997). In this study, we estimated the oxidation degree by analysing the AV and PV. It is clear that no significant difference ($P > 0.05$) was observed between treated and untreated groups (Table 1). The results demonstrate that the peanuts treated under the optimal conditions were stable in the AV and PV, which were in compliance with the detoxifying effects of peanuts with the moisture content of 8%, ozone gas at concentrations of up to 21 mg/l, and

Table 1
Polyphenols, resveratrol, AV, and PV of peanut sample.

Sample	Oxidised indicator ^a			
	Polyphenols (%)	Resveratrol ($\mu\text{g/g}$)	AV (mg/l)	PV (%)
Untreated-1 ^b	17.4	0.488	0.376	0.010
Treated-1	16.9	0.465	0.369	0.010
Untreated-2	17.8	0.492	0.387	0.009
Treated-2	17.5	0.490	0.400	0.009
Untreated-3	18.2	0.517	0.376	0.010
Treated-3	18.6	0.520	0.375	0.009

^a Results are represented as means ($n = 3$).

^b Untreated-1, 2 & 3 were peanut samples from different purchase batches, then detoxified under the optimal condition as treated-1, 2 & 3, respectively.

detoxification time of up to 96 h (Alencar, Faroni, Soares, Carvalho, & Pereira, 2011).

4. Conclusions

Ozonation is considered as one of the potential methods for detoxification since the method is easy to use for large-scale samples. It is also an important fungicide and commonly applied in the preservation of fruits and vegetables. After being introduced to peanut samples, ozone will not only detoxify aflatoxins, but also inhibit the growth of *A. flavus* and *A. parasiticus*, which will further control the aflatoxins in peanuts.

The results show that ozonation is an effective way to detoxify aflatoxins. G-aflatoxins were more sensitive than B-aflatoxins. Under the optimal conditions, the detoxification rates of the total aflatoxins and AFB₁ were 65.8% and 65.9%, respectively. No significant variations were observed for the polyphenols, resveratrol, AV and PV between treated and untreated groups. Alencar et al. (2012) exposed the peanut samples at 21 mg/l for 96 h, which achieved 30% and 25% detoxification of total aflatoxins and AFB₁, respectively (Alencar et al., 2012). Proctor, Ahmedna, Kumar, and Goktepe (2004) obtained a relatively high detoxification for AFB₁ of $77 \pm 2\%$ in the spiked samples (Proctor et al., 2004). Compared with the methods in the literature, the optimised method achieved better detoxifying effects at low ozone concentration and short time with naturally contaminated peanut, and was a simple, rapid and economic detoxification method for peanut samples. Our further research aims to improve detoxification by combining ozonation with a catalyst or UV irradiation. In addition, the mechanism for detoxifying aflatoxins, especially in the end-products, will be elucidated.

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