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The effect of ozone gas on destruction and detoxification of aflatoxin

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Abstract

Aflatoxins are more significant than other fungal toxins due to carcinogenic effects and acute poisoning. In nature, four types of aflatoxin including B_1, B_2, G_1 and G_2 and also two types of metabolic products named M_1 and M_2 are found. These components can pollute livestock and human feeds like corn, sorghum, wheat, soya, cottonseed, peanut and nuts. To control aflatoxin, physical separation, deactivation by heat and microbes, radiography, solvent extraction and fermentation are used. Ozone is one of the eliminating compounds of aflatoxin that has capability tocarry out the process in three status of dry, watery and moist. Ozone can eliminate fungal toxins through reacting with 8 and 9 dual bond of furan ring in aflatoxin which means initial ozonation and subsequently rearrangement to derivations and then producing aldehydes, ketones and organic acids.

Keywords: Aflatoxin, Ozone, Deactivation methods

1.Introduction

Aflatoxins are toxins which are produced by some of fungus growing on livestock feed and foods and can cause aflatoxicosis disease in human and domestic animals. Different environmental factors are involved in producing aflatoxin. So, the severity of pollution depends on geographic location, agricultural technique, products' sensitivity before harvesting, process of foods' production and statusof products in the store. Aflatoxins are more important than other fungal toxins because of carcinogenic effects and acute poisoning [1].

Aflatoxins are a sort of mycotoxins which created by two types of molds named Aspergilus Flavous and Aspergilus Parasiticus. Based on studies, in nature, four types of aflatoxin including B_1, B_2, G_1 and G_2 and two kinds of metabolic products such as M_1 and M_2 are available that can pollute livestock and human feeds like corn, sorghum, wheat, soya, cottonseed, peanut and nuts.

Aflatoxin B_1, with molecular weight of 312 and $C_{17}H_{12}O_2$ formula, shows a relatively strong blue fluorescence against UV. B_1 consists of colorless crystals that hydrolyzed at the melting point of $268^{\circ C}$-$269^{\circ C}$. G_1, with 328 molecular weight and $C_{17}H_{12}O_7$ formula, shows green fluorescence against UV and its melting point is $244^{\circ C}$-$246^{\circ C}$.

B_2 and G_2 are available in nature with 314 and 330 molecular weight and $C_{17}H_{14}O_6$ and $C_{17}H_{14}O_7$ formula, respectively. Also, these aflatoxins stream blue and green fluorescence against UV and

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their melting point is 268°C-289°C and 240°C-247°C, respectively. B_2 and G_2 could be taken by careful hydrogenation of B_1 and G_1. If B_1 is eaten singly by animals or with other aflatoxins in livestock feed, it's converted to other aflatoxins in animals' tissues and secretions. M_1 and M_2 are considered as milk toxins and found in animals' milk.M_1 and M_2 are structurally derivations of 4-hydroxy aflatoxin B_1 and B_2. After being eaten B_1 by animal or after being injected directly to animal, 4-hydroxy aflatoxin is detectable in urea, stool, muscles, liver and kidney.

M_1 can tolerate the temperature of pasteurization. Conducted studies have proved the resistance of M_1 for milks which had been polluted naturally or artificially.This toxin endures the temperature of 64°C for two hours and can keep the own initial shape but its structural stability is reduced with increasing temperature. These aflatoxins are solid crystals and M_1 and M_2 have the melting point of 299°C and 293°C, respectively.

These toxins are found in milk, cheese, peanut, cottonseed, almond, condiments and fig and in various types of livestock and human feeds and also could be measured. Sometimes becauseof using polluted feed, egg and meat products are polluted by aflatoxin [2].

Some factors such as pollution of agricultural products before harvesting, putting the drying time of products off and high percentage of products' moisture, existing pests in vegetable and storehouse and also food storage conditions in the storehouse (for temperature and moisture) can create required field to grow fungus and produce aflatoxin. Moreover, other agents like stress caused by lack of water and drought, poor fertility of plants and their high aggregation in the farm, existence of weeds and increasing environment temperature play a role in proliferating molds and producing toxin [3].

If human daily receives aflatoxin B_1 less than 10 micrograms per kg of body weight for a long-term, he/she suffers from temporary effects. But if the amount of B_1 reaches to 50 micrograms, important epidemiologic and clinical effects will happen. Meanwhile, it could be said that augmentation of malnutrition about calorie and protein followed by B_1 and immune system suppression even occurred due to infection. People who expose to risk factors such as hepatitis, cirrhosis of the liver, aflatoxins, senility and hereditary factors, it is more likely to suffer from liver cancer [4]. Various factors are involved in making decision for regulating of aflatoxins' restriction including availability of toxins in aflatoxins, awareness of distribution of aflatoxin concentration in a large number of products, supervision laws and necessity amount of foods [5].

Physical separation, deactivation by heat and microbes, radiography, solvent extraction and fermentation are utilized to control aflatoxin. Among these methods, chemical methods are applied as a main strategy for neutralization. Recent researches show that existing nutrients like proteins, fats, vitamins, rare ingredients, additives such as antibiotics and preservatives cause decrease of poisonous effects of aflatoxin. So, new methods are studied to neutralize aflatoxin's effects in which inorganic absorbents (or chemical absorbents) are added to animal feed. One of these absorbents is HSCAS (Hydrated Sodium Calcium AluminoSilicate). HSCAS makes a conjunction with aflatoxin's molecules and causes this toxin becomes static. Thereby, HSCAS prevent absorption of these molecules by body [6] and [7].

2 Deactivation methods of aflatoxins

2.1 *Physical methods*

Aflatoxins' sensitivity against heat depends on environmental condition. Moisture in foods causes increase of decomposition percentage and elimination of aflatoxins. This action is performed through hydrolysis of lactone ring in effective moisture concentrations and temperature. Presence of proteins and other food components in the environment cause maintenance and consistence of aflatoxins in heated foods. So, heat penetration is decreased and aflatoxins are stabilized.

Nowadays, 100% of aflatoxin in edible oils is eliminated helping filters and filtration in different industries especially in oil industry. Centrifuge is able to eliminate 65% of aflatoxins in peanut oil and the rest of it (35%) can be removed by active soils and adsorption of aflatoxin.
Ionizing radiations like Gamma are mostly used to remove pathogenic microorganisms from different foods and any types of livestock feed. Gamma has more impact compared to visible radiation or UV because of its high penetration into solids and liquids than other radiations. But organic molecules with complex structure like aflatoxins are resistant against Gamma and indirect impact of Gamma causes decomposition of aflatoxins so that it can decompose water and results in releasing radicals and consequently required condition is made to destroy and decompose aflatoxins.

2.2 *Chemical methods*

Sodium chlorite is applied to eliminate aflatoxins from polluted surfaces as the main chemical substance. Foods' chlorination with sodium hypochlorite in 0.2, 1, 5 and 11% concentrations and hydrochloric acid 3% or gas 10% leads to decomposition of B1 in foods. The minimal concentration of sodium hypochlorite to decompose aflatoxin in foods completely is 8.8×10^{-3} mole in a two hours period. Controlling environment pH plays an effective role for better impact of sodium hypochlorite and Clare works as a dominant oxidizing under acidic conditions.
It should be noted that foods' chlorination has problems about removing aflatoxins from the aspect of safety and health of foods, because Clare in residual foods and in fats and proteins reshapes and eventually produces toxins. Hydrogen peroxide is a cheap substance that is available easily and has a high efficiency in decomposing fungal toxins. Also, it prevents the growth of fungus producing aflatoxin in artificial cultures and decomposes toxins completely in 0.5% concentration and pH=4 and/or 6% concentration and pH=9.5.
Sodium bisulfite has utilization in food industries as an additive. It's used in destruction of fungal toxins and causes deactivation of poisons in 0.5 and 1% concentration. 95% of aflatoxinsin livestock feed are decomposed by liquid or gas ammonia. If some factors like time, temperature and concentration are utilized properly, it has highly deconstructive impact. For example, food is needed to be heated 15-30 minutes in $80^{\circ C}$-$120^{\circ C}$ temperature and high pressure. Ammonia decreases toxicity with decarboxylation and hydrolysis of ketone ring of B_1 and converts it to D_1 [7] and [8].

2.3 *Biological and microbial methods*

HSCAS, is an acceptable absorbent that protects farm animals against aflatoxicosis. But it's not effective against other mycotoxins such as fumonisin, deoxynivalenol (DON) and ochratoxin A. So, organic components connect to mycotoxins using special chemical methods. These converted absorbents called organoclays. Microbial or enzymatic detoxification is another method to detoxify aflatoxins. This method is decomposition or structural deformation which leads to make nontoxic or less toxic products. In 1966, it was cleared that microorganisms such asyeasts, molds, molds' spore, actinomycetes, bacteria, mosses and algae can be used to remove mycotoxins. Flavobacteriumaurantiacum (B-184NRRJ) can cause the destruction of aflatoxin in culture and its detoxification has been proved in milk, oil, corn, peanut butter, bran and almond [9] to [13].
As a part of deactivation method was explained for aflatoxins, the use of ozone as an oxidant could be mentioned.
In nature, ozone is found in two forms. In stratosphere (about 10-15 km above planet) and in the earth, it exists as an earth protective layer against UV. The color of ozone is blue. Ozone consists of three atoms of oxygen and is found in nature cycle. It has high deoxidization ability. Ozone easily reacts with other molecules and changes their molecular structure. It's very unstable and if it doesn't face with molecules withoxidation ability, it reshapes again and is converted to oxygen [14] and [15].

Table 1. Ozone characteristics

Chemical name	Ozone
Shape	Colorless and sharp smell
Molecular formula	O_3
Registration N°.	10025-15-6
Molecular weight	48.0 g/mol
Concentration (gas)	2.144 kg/m^3
Liquid (NTP)	1574 kg/m^3
Solid	1728 kg/m^3 (-183°C)
Solving in water	4.9 ml/l (0°C)
Boiling point	-111.9°C (1 atm)
Melting point	-192.5°C (1 atm)

Ozone passes 50% faster than Clare from cellular membrane and eliminates a various number of bacteria 3000 times faster than Clare. Even in low concentration, it has high disinfection power and doesn't produce any byproduct when converting to oxygen [16].
Ozone can react with 8 and 9 dual bond of furan ring in aflatoxin. It means that it causes destruction by initial ozonation and rearrangement to derivations (monozonide) and production of aldehydes, ketones and organic acids. To do this action, three methods are available including drying, watery and moist process.
In drying method, ozone enters into the glass reactor directly and after putting sample into the reactor, oxygen cylinder faucet and flow are opened quickly. So, oxygen and ozone are injected to the reactor from the bottom and upper side of generator, respectively. Watery process is the second method in which water is added and sample becomes float. During enrichment, vapor ozone is used directly to be entered into the water and some reactions occur between watery ozone and aflatoxin. In moist process, there is a water container between generator and reactor of ozone. Vapor ozone which was produced in the first step enters into the water and then sample is injected to the ozone reactor. In the following, the reaction between vapor ozone and aflatoxin is performed.
The destructive effect of ozone is done by these three methods. Moist process has highest destructive effect and lowest impact is related to drying method. In watery process, ozone is widely used in food industries (i.e. for stored grains). But it's not suitable for storage media. It seems that water or steam has a main role in reacting between ozone and aflatoxin.

$$O_3 + H_2O \rightarrow H_3O^+ + OH^- \quad (1)$$
$$H_3O + OH^- \rightarrow 2H_2O \quad (2)$$
$$O_3 + H_2O^{\cdot} \rightarrow OH^{\cdot} + 2O_2 \quad (3)$$
$$HO^{\cdot} + H_2O^+ \rightarrow H_2O + O_2 \quad (4)$$

In this reaction, free radicals like (OH) show the ability of powerful oxidation of ozone. So, the oxidation ability of ozone has been greatly increased from evaporated water. In conducted studies about destroying aflatoxinsby ozone, it was concluded that ozone follows of creegie mechanism using electrochemical oxidation of water in medium acidic environment so that it initiates a reaction by attacking to C8-C9 band in aflatoxin and after initial formation (ozonide) and rearrangement (molozonide), it's converted to types of carbonyl components such as aldehydes, ketones and organic acids. Finally, reaction is directed toward byproducts including CO_2 and H_2O. One way to deactivate aflatoxin by ozone is reacting ozone with initial shape of N-oxide amine in aflatoxin and with losing second hydrogen of third carbon (C_3), it can produce ketone including 3-keto [14], [17] and [18].

Ozone was used for date fruit with 1, 3 and 5 ppm concentrations in 15, 30, 45 and 60 minutes and a significant decrease was observed in Staphylococcus aureus and E. coli [19]. Ozone even decreased the amount of polyphenols and oxidative products in peanut's skin. This action was performed by HPLC and MS devices [20]. To decrease microbial charge and eliminate Salmonella, Listeria monocytogenes and E. coli from edible fungi, 2.8 and 5.3 mg/l ozone concentrations were used for 60 minutes and the amount of bacteria declined 3.61, 2.8 and 3.41 logarithmic phase [21].
Spore (Penicillium expansum) is eliminated due to continuous contact with ozone, while its colony is very resistant on packing surface and isn't eliminated because of continuous contact with the air containing ozone 0.72 ppm for five months [22]. The inhibitory effect of ozone has been determined on decay of orange, strawberry and peach because of Penicillium, Botrytis cinerea and Monilinia fruticola, respectively [23].
Now, green mold's spore has become resistant to fungicides. Thereby, ozone is utilized for slowing spore production in injured fruits. So, decay is prevented during packing. Controlling ozone's sporulation has been observed in citrus which are kept in cold storehouse in $50^{\circ F}$ temperature and/or less. Controlling sporulation was seen in 0.06 ppm ozone concentration. However, its penetration with 0.01 ppm concentration or less doesn't lead to control sporulation in oranges inside the plastic packs or fibrous boxes [24].
In conducted study on fig containing B_1, two types of ozone (gas and liquid) were used and the results showed that the destruction of toxin is raised with increasing exposing time in ozone condition [25]. In a study about the destruction of aflatoxin in pistachio, 5, 7 and 9 mg/l ozone concentrations were used in the temperature of $20^{\circ C}$ and 70% relative humidity for 140 and 240 minutes and eventually liquid chromatography with high efficiency was applied for evaluating [26].

3. CONCLUSION

In conducted studies, ozone has a high oxidative power (50 times stronger than Clare) and also has impact on various spectrums of microorganisms including fungus that are main producer of mycotoxins especially aflatoxins. Destructive effect of ozone is performed by three methods that moist process has highest destructive impact and drying process has lowest effect. Watery ozone is widely used in food industries including stored grains but it's not suitable for storage environment.

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