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Overview on Technology of Degrading and Eliminating Mycotoxins in Agro-products and its Application

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Abstract Based on the perspective of risk control, this article introduces related technology of eliminating mycotoxins in agricultural products and the current situation of application, including traditional physical, chemical and biological methods as well as the contemporary situation of relatively advanced technology at home and abroad, which provides reference for the policy-making and technology application of mycotoxin control in agro-products in China.

Key words Mycotoxin, Risk, Elimination technology, Application

Mycotoxins are a kind of toxin produced by secondary metabolization of fungus, which are prevalent in agricultural products like maize, wheat, cereal, sorghum, nut and dairy products as well as forage. Compared with plant-derived and animal-derived toxins, mycotoxins have captured wider world attention. From the perspective of risk evaluation, it is "dose", not "toxicity", that determines final risks. Mycotoxins attract relatively high attention since the toxin affects a variety of agricultural products, involves a large amount of people and is hard to be eliminated. According to the report of FAO/WHO, 25% of crops were polluted by mycotoxins every year and nearly 2% of crops lose nutrition and economic value because of pollution in the world. The direct and indirect economic loss reaches 100 billion dollars^[1].

1 Comprehensive Strategies of mycotoxin risk prevention

Currently, comprehensive risk prevention and control of mycotoxins is greatly emphasized in the world (Fig. 1), including strategy of field and collection control in stage one, risk evaluation strategy in stage two and toxin degradation and elimination strategy in stage three, which aims to minimize the threat of toxin in agricultural products to humans and livestock, optimize the control cost and operation method as well as amplify the profit of agricultural production. Field and collection control and risk evaluation belong to risk "prevention" while toxin degradation and elimination belong to risk "elimination". "Prevention" aims to decrease risk while "elimination" aims to transfer risk. From the perspective of risk management, "prevention" is more active while "elimination" is more pertinent. This article mainly reviews the researches and application of mycotoxin risk elimination technologies in agricultural products at home and abroad.

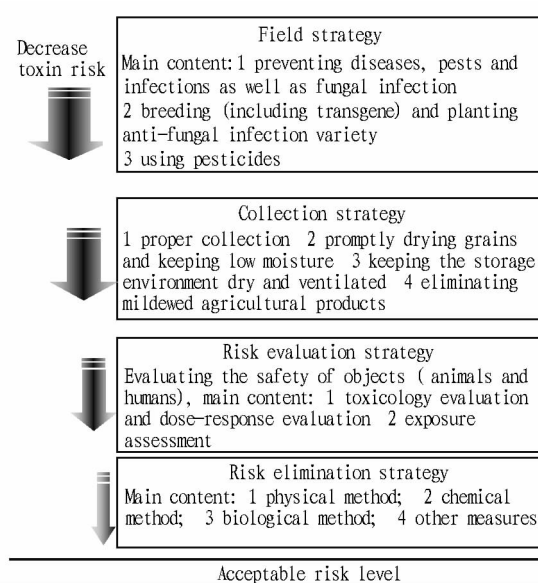


Fig. 1 Risk comprehensive control framework

2 Research progress of mycotoxin risk elimination technology

Currently, the traditional ways of degrading mycotoxin produced by food chain mainly involve physical, chemical and biological methods. With the sustained development of technology, some new degradation methods occur, including degradation technology integrating the above three methods, regulation based on diet and optimized degradation technology using transgenic technique. Degradation technology is generally defined and classified according to the "method", not "mechanism", therefore different kinds of degradation technologies may share the same mechanism.

2.1 Physical elimination technology Physical method means to kill fungus or degrade mycotoxin with physical means including washing, picking, absorption (activated carbon, clay, silicate and zeolite), heating (baking and pressure cooking) and radiation method (ultraviolet rays and γ -ray).

2.1.1 Washing and picking. Washing and picking are relatively easy to operate, but residual dirtiness and untimely drying may cause even worse toxin production. Besides, they are more suitable for grains like maize requiring wet milling and alkali treatment. Picking is relatively labor-consuming.

2.1.2 Heating. Pressure cooking is one of the most popular procedures of food disinfection. Compared with traditional methods, high-pressure cooking is poisonless and harmless, thus it is easy to be operated at home. Cetin Y^[2] treated maize with cooking and discovered that the content of Zearalenone decreased by 83%. Jalili M inoculated 5 mg/kg of fusarium mycotoxins to the flour of maize, wheat, soybean and rice and heated at three different temperatures of 160, 180 °C and 200 °C successively for 3, 6, 10, 15 and 20 min respectively. Toxin bioactivities of treated dough were analyzed with LC-MS/MS and the result showed that there was no obvious difference in degradation rate among different kinds of flour and degradation rate after thermal treatment ranged from 20% to 90%. Toxin was decreased to 2.89 ± 0.13 mg/kg after 3-minute heating at the temperature of 160 °C and was completely degraded after 20-minute heating at the temperature of 200 °C.

2.1.3 Adsorption. Adsorption means to decrease toxin content in agricultural products through adsorption. Huwig A.^[4] used the clay of sodium aluminosilicate hydrate and aluminium silicate to absorb ZEN while the effect was not obvious. However, the adsorption force of cetylpyridinium and montmorillonite modified from hexadecyl reached 0.108mg/kg. The degradation mechanism may be that the surface of clay has hydrophobic property and ZEN shows high affinity to hydrophobic substances. Avantaggiato G^[5] used colestyramine, a mix of bile acid and resin, and activated carbon as adsorbent of mycotoxins. The result showed that these two substances were excellent adsorbent of ZEN degradation and could be used as forage additive to prevent livestock from feeding ZEN and suffering intoxication. However, the cost of this kind of adsorbent is relatively high and the commercial application is not mature.

2.1.4 Radiation. Radiation means to degrade toxin in products by using rays, solar energy or microwave radiation. For example, Shahzad Z. I.^[6] used γ -rays with the dose of 2, 4 and 6 kGy to irradiate pepper infected with aflatoxin B (AFB). The result discovered that γ -ray irradiation with the dose of 6kGy can decrease five logarithmic series of AF. Besides, γ -ray irradiation with the dose of 6kGy can decrease one to two logarithmic series ($\alpha < 0.05$) of the total AFB1 in pepper surface and the whole pepper as well as AFB content. Furthermore, microwave irradiation can destroy monospore while solar energy can degrade AFB in maize.

2.2 Chemical elimination technology Chemical method means to add chemicals to destroy fungus and mycotoxins to eliminate risk, mainly including acetic acid (ethyl alcohol), ammonia or 3% -5% ammonium salt, calcium hydroxide, formaldehyde, hydrogen peroxide, methylamine, ozone, phosphoric acid, phosphine, sodium bicarbonate, sodium bisulfite and sodium hypochlorite.

2.2.1 Ammoniating. High pressure/ high temperature is mainly

used in feed-processing plant while pressure/ temperature is mainly used on the farm.

2.2.2 Ozone treatment. With ozone treatment, McKenzie K. S.^[7] discovered that ZEN degradation rate in maize 15S reached over 80% by using HPLC and no by-product was found.

2.2.3 Hydrogen peroxide processing. Abdulkadar A. H. W.^[8] adopted hydrogen peroxide process with concentration of 3% , 5% and 10% in maize polluted by ZEN. The result showed that ZEN degradation rate was in direct proportion to the concentration of hydrogen peroxide, time and reaction temperature. When 10% hydrogen peroxide reacted 16 hours at the temperature of 80 °C, the highest ZEN degradation rate reached 83.9% while the highest ZEN degradation rate amounted to 75% with the reaction time of 8 hours under the same condition.

2.2.4 Electrolyzed oxidizing water processing. Sudha S. M. K.^[9] used electrolyser of ion-exchange membrane to prepare acidic electrolyzed oxidizing water (AcEW) with pH < 3.0, oxidation reduction potential (ORP) > 1000 mV and high concentration chloride and adopted AcEW process in AFB1 polluted peanut to observe the effectiveness of toxin degradation. The result showed that 15 minutes after the polluted peanut immersed in AcEW solution (liquid - solid ratio V/ M was 5:1) at room temperature, AFB1 content in peanut decreased from 34.80 mg/kg to nearly 5 mg/kg and degradation rate reached 85%. At the temperature of 25°C or 45°C, elimination rate of AFB1 achieved 100%. Additionally, high concentration chloride was the main factor of eliminating AFB1 and perchloric acid would be more effective in eliminating AFB1 than hypochloric acid.

2.2.5 Eugenol processing Komala V. V.^[10] adopted antifungal eugenol process in three different genotypes of sorghum (M35 - 1, C - 43 and LPJ) with AFB1. Eugenol with the concentration of 0.008 mg/kg can completely restrict the formation of aflatoxins. Sorghum genotypes with AFB infection ranked from the highest to the lowest as C - 43, LPJ and M35 - 1.

2.3 Biological elimination technology Application of biological method in toxin biodegradation will be the main trend in the future, which mainly uses microorganism or bacterial stains to compete with strains producing toxins or to be integrated with toxins in order to decrease toxicity or reduce ability of producing toxins, hence risk elimination.

2.3.1 ZEN degradation. Bennett J. W.^[11] reported that if maize is fermented to produce ethyl alcohol, ZEN will be in solid residuals after fermentation, not in the target product ethyl alcohol, which is the result of interaction between yeasts and mycotoxins. Megharaj M^[12] reported that mixed culture medium of various kinds of bacteria could completely degrade ZEN in the solution and no by-product similar to ZEN was found with HPLC and ELISA analysis. According to the report of Takahashi - Ando N.^[13], acid lactonase can transfer ZEN to the product with relatively low estrogenic effect. El - Nezami H.^[14] studied the interaction between lactobacillus strain in food (lactobacillus rhamnosus GG and LC705) and ZEN as well as its derivative a - ZEN.

The experiment proved that *Lactobacillus rhamnosus* strain LGG and LC705 can effectively combine ZEN and its derivative α -ZEN and the combination efficiency amounted to 55% (w/w). There was competitive inhibition between ZEN and α -ZEN in combination with *Lactobacillus* strain, which indicated that the two toxins shared the similar surface binding sites. Molnar O. ^[15] discovered a new yeast strain (*Sporotrichosis*) which can degrade ZEN and produce oxycarbide and other poisonless metabolites and no α -ZEN or β -ZEN was detected in the by-products. Cho K. J. ^[16] isolated and identified the subspecies of *Bacillus subtilis*. The bacterial strains were inoculated to liquid medium with 1 mg/kg of ZEN for 24 hours and 99% of ZEN could be degraded while over 95% of ZEN could be degraded in solid-state fermentation medium with 0.25 mg/kg of ZEN for 48 hours. Yi P. J. ^[17] separated *Bacillus licheniformis* from soil sample and the strain can degrade ZEN. After 36 hours of fermentation in LB medium with 2 mg/kg of ZEN, *Bacillus licheniformis* can degrade 95.8% of ZEN. After 36 hours of fermentation in corn flour medium polluted with ZEN, *Bacillus licheniformis* can degrade over 98% of ZEN. Besides, *Bacillus licheniformis* is nonhemolytic and does not generate endotoxins. It has high content of extracellular xylanase, cellulase and protease activity, therefore the bacterial strain can also increase the digestibility of nutrients in forage. With in vitro experiment, Samuel E. T. ^[18] discovered that both *Bacillus subtilis* 168 and *Bacillus natto* CICC 24640 can absorb and degrade ZEN. In liquid solution with ZEN concentration as 20 $\mu\text{g/L}$, the untreated cells, cells with high temperature sterilization (121°C, 20min) and cells with acid-treatment of these two strains can absorb over 55% of ZEN after 1.5 hours of fermentation under aerobic condition at the temperature of 37°C. The two strains can degrade 81% and 100% of ZEN respectively after 14 hours of fermentation under aerobic condition at the temperature of 30°C. Additionally, carbon dioxide was released during the degradation, which showed decarboxylation. Furthermore, ZEN degradation reaction of extract of *Bacillus natto* CICC 24640 changed with the variations of ZEN concentration, time, temperature and pH value. Mn^{2+} , Zn^{2+} , Ca^{2+} and Mg^{2+} can strengthen degradation reaction while Hg^{2+} , Cu^{2+} , Pb^{2+} and 1,10-phenanthroline would inhibit degradation. Related enzymes in degradation reaction was metalloproteinase, whose molecular weight ranged from 31 to 43 kDa.

2.3.2 AF degradation. Mohsen F. ^[19] separated *Bacillus subtilis* UTBSP1 from pistachio nuts and studied its degradability of aflatoxin B1. The content of AFB1 was determined with HPTLC, HPLC and LCeMS/MS and the result showed that the separated strain can degrade 85.66%–95% of AFB1. The degradation mechanism may lie in the fact that the strain can steadily express and secrete extracellular enzyme, which makes AFB1 lose active groups.

2.3.3 Deoxynivalenol (DON) degradation. Yoko I. ^[20] separated *Nocardia* bacteria from soil sample of wheat field, which was called WSN05-2. After 10 days of fermentation in the medium with 1000 mg/l of DON, the degradation rate of this strain reached

100%. With the analysis of MS, ¹H and ¹³C-NMR, the main metabolite of DON in culture supernatant was identified as 3-epi-dON3. The result first confirmed that *Nocardia* can degrade DON and the metabolite 3-epi-dON3 was discovered for the first time.

2.3.4 Ochratoxin (OTA) degradation. Peteria Z. J. ^[21] discovered that after 15 days of treatment at the temperature of 20°C, *Phaffia* yeast can degrade over 90% of OTA and the metabolite was OTAA. Besides, it was also found that chelate EDTA and 1,10-phenanthroline could inhibit *Phaffia* yeast from degrading OTA, which suggested that the active carboxypeptidase was a kind of metalloproteinase, similar to carboxypeptidase A, whose optimal temperature was over 30°C while the optimal temperature for *Phaffia* yeast cell growth was 20°C. Furthermore, both active *Phaffia* yeast cell and the dead *Phaffia* yeast cell after thermal treatment can greatly absorb 0.25 mg/kg of OTA.

2.4 Other elimination technologies

2.4.1 Integrated degradation technology. Hossein A. M. ^[22] studied the combined effect of γ -radiation of 0 kGy, 300 kGy, 600 kGy, 900 kGy as well as 1200 kGy and 1°C low-temperature on physico-chemical qualities of Hongyuanshuai Apple inoculated with *Penicillium* and the *penicillium* lesion. The result showed that mould stains on apples inoculated with *penicillium* at low temperature but without γ -radiation would still increase obviously after three months of storage. 900–1200 kGy of γ -radiation will obviously decrease apple phenolic content, antioxidant activity and water proportion, which may decrease the disease resistance of apples. However, the integration of 300–600 kGy of γ -radiation and cold storage was the best way. Besides, Jalili M. ^[23] studied the combined effect of sodium sulfite and different pressure as well as temperature on AFB1, AFB2, AFG1, AFG2 and OTAA in black pepper. The first treatment was 30 minutes of treatment in boiling water at atmospheric pressure while the second was 15 seconds of treatment at the temperature of 121°C under 1.5 bar high pressure to detect AF and OTA concentration with HPLC and fluorescence detection. The result showed that the elimination rate of high pressure and sodium sulfite treatment with the concentration of 2% on OTA, AFB1, AFB2, AFG1 and AFG2 was 96.0%, 96.1%, 77.7%, 100% and 100% respectively.

2.4.2 Field and postharvest comprehensive control. Akos M. ^[24] studied the effect of fungicide like mancozeb, captan and carbendazim, biological agent and plant extract on AEB infection in crops, such as maize and pepper, in field stage. The result showed that the inhibitory effect of mancozeb with the concentration of 0.3% was 91.1%, that of captan was 85.2%, that of the biological extracts of *Pseudomonas fluorescence* was 74.9%, that of carbendazim was 73% while that of *Trichoderma harzianum* was 70.4%. For pepper, the inhibitory effect of biological extracts of *Pseudomonas fluorescence* was 2% while that of captan was 1.6%.

2.4.3 Natural non-toxic substance using. Degradation technology and harmless degradation products are important index of evaluating mycotoxin degradation method. Meca G. ^[25] added natural

non-toxic substances like soluble dietary fiber β -1, 3-glucan, low-molecular chitosan, middle molecular chitosan, fructooligosaccharides, inulin and pectin with the concentration of 1% and 5% respectively in crispy bread. The bioavailability was measured with imitative gastrointestinal tract and the secondary metabolite, Beauveria (BEA), generated by fusarium in intestine was detected with LC-MS, which discovered that the average bioavailability of BEA ranged from 31.8% to 54.0%.

2.4.4 Risk assessment modeling Baert K.^[26] collected apples with three different kinds of HACCP treatment and measured the patulin contents at critical control points to formulate the quantitative risk assessment model of the whole industrial chain from apple picking to apple juice storage in order to further evaluate the degradation effectiveness of process control. The result showed that selection before processing could effectively decrease patulin in apple juice. Besides, selecting apples which were infected with patulin over 10 cm² area before processing could control the patulin concentration under 25 μ g/kg in 99.7% – 99.9% of apple juice. Furthermore, Heisl S.^[27] studied and reduced the dynamic enzymatic reaction during fumonisin B1 degradation with simulation method and quantitative analysis, which was deesterification at first and then deamination. The two genes of sphingomonadales in degrading fumonisin B1 were identified to be in the same gene cluster. The first protein-coding gene was similar to B carboxylesterase while the second homopolypeptide-coding gene stemmed from transaminase of type 3.

2.4.5 Dietary intervention. Meca G.^[28] studied and discovered that with reasonable dietary structure, autoimmunity and adjustment can partly degrade mycotoxins since the joint effect from choline, methionine, vitamin, protein, dietary fat, antioxidant and metabolic enzyme in body or food could form the inducer of degrading mycotoxins and promote toxin degradation.

2.4.6 Gene expression promoting. Petr K.^[29] discovered that arabidopsis thaliana, tobacco, wheat, barley and rice after genetic engineering could acetylate the third carbon atom of DON, therefore the genetically engineered wheat which can express DON acetylation activity can increase the resistance to wheat scab. However, the effect is to be improved. The reason why effect was not obvious may be that the genetically engineered wheat used Tri101 gene from fusarium which enzyme from fusarium graminearum had higher activity to DON. Furthermore, fungal acetyltransferase using the third carbon atom of fusarium toxin and plant glycosyltransferase were discovered to be the effective genes of scab resistance.

3 Conclusions and discussions

The potential danger and huge risk caused by mycotoxins to humans and livestock have captured growing world attention. The technology development and application may represent two main trends.

3.1 Mycotoxin elimination technology should be friendly and sustainable There are still many "black boxes" to be

opened in eliminating mycotoxin risk. Social and economic requirements bring great challenges for the elimination technology, which are mainly reflected on whether toxin degradation effect is obvious or not, whether degradation cost is low so as to be promoted and applied easily, what the degradation products are and whether the products have potential risks. For example, Hussein H. S.^[30] reported that rumen microbes could effectively degrade ZEN with the degradation rate reaching 90% – 100%. However, the main metabolite was a –ZEN which had more estrogenic effect than ZEN, so the degradation obviously increased toxin. Although the degradation effect was obvious, the degradation technique cannot be applied. Besides, the features of degradation inducing microorganism, degradation products and degradation mechanism are not clear enough, such as transgene dispute. If the degradation products and mechanism cannot be identified, the degradation method will not be feasible even though toxin degradation rate is largely improved.

3.2 Integration model should be developed "Integration technology" provides new thoughts for mycotoxin degradation, combining various controlling measures to eliminate mycotoxins. To be harmless to humans and animals and friendly to the environment is necessary in the future. Moreover, over emphasizing risk elimination will be overdependent on the back-end risk control and cause unnecessary economic loss to agricultural production. Therefore, properly utilizing the front-end "prevention" and back-end "control" in risk comprehensive control system can not only truly decrease and even eliminate the impact of mycotoxins on humans and livestock but also keep the sustainability of future development.

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