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# DECONTAMINATION AND DETOXIFICATION OF MYCOTOXINS

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Abstract - Decontamination and chemical detoxification are needed because preventive measures are not fully able to avoid contamination by mycotoxins. Criteria for safety study of decontaminated products have to be established. Few chemical methods are available on industrial scale, among those ammoniation and the mixture monomethylamine-calcium hydroxide treatments show greatest promise of short term application to oilseed cakes. Technical, economic and public health aspect of these treatments are considered. Other decontamination techniques are briefly reviewed.

## INTRODUCTION

The term of decontamination is more comprehensive than detoxification. We will adopt it for designing all the techniques employed to treat the contaminated products in order to remove, destroy or inactivate the contaminants toxins.

The preventive measures at the agricultural level, genetic means or improvement in agricultural practices constitute the best approach to minimize the contamination of raw agricultural commodities by fungi and their toxins.

Although appropriate preventive techniques exist and should be applied, the mycotoxins contamination is sometimes ineluctable, and we have to learn to live with (Ref. 1). It is obvious that with respect to the present situation in developing and developed countries, the preventive measures must be viewed as long term goal. In other respects, good the existing preventive measures are, they cannot be expected to succeed in all circumstances. For these reasons, we have a need for alternatives which can be adopted once it is discovered that the produce is contaminated.

The lack of decontamination treatments may lead to serious problems :

- The risk of health hazards for those who have no alternative that the consumption of contaminated products.

- The economic implication with potential loss of outlet, specially in several developing countries where the potential contaminated commodities contribute 60-70% to the national income.

- And last but not least, the reduced interest for low costed feed materiel source, it appears that there exists a threshold level of consumption below which a major feed constituent cannot maintain its importance in a given country. In France, we consider when this consumption level falls below 200.000 tons/year the product will be considered as marginal and will find in creasingly difficult to compete. This is the situation presently beetween groundnut inclined to aflatoxin contamination and soybeen cake. Some countries in Europe reported to be no longer using groundnut cake for cattle feed.

## DECONTAMINATION METHODS

The methods can be classified under three headings :

- Separation of the contaminated parts from the produce (physical process)

- Removal of mycotoxins

- Inactivation of mycotoxins by physical (heat, cooking, roasting), chemical and biological means.

Various decontamination methods have been described in the literature, however, very few are used so far. The decontamination has been mainly studied for contaminated products by aflatoxins. The other mycotoxins have received very little attention. The major concern of this paper is related to the decontamination of aflatoxin contaminated agricultural commodities.

## SPECIFIC DECONTAMINATION METHODS

Emphasis needs to be given especially to those decontamination processes which have been tried practically either the industrial scale or the farm level.

### Physical separation of the mycotoxins contaminated materials

Such approach seems attractive if it may allow the removal of mycotoxin infected commodities by either manual, mechanical or electrical means prior to processing. The physical removal of contaminated material involves separation of discoloured, fluorescent damaged and mouldy grains or seeds.

Electronic sorting is practised for shelled groundnuts scanned by a photoelectric cell on the basis of color. This sorting or hand picking may be successfully used after removing skin or testa by blanching, since the moulds often makes the skin adhere to the kernel. Very simple system for use at the farm level has been described, the kernels are previously steamed and only the contaminated kernels retain the skin, which permit to eliminate them. The ultra violet sorting have been employed for almonds, cottonseed, pecan, pistachio but this may not be economic. Some difficulties have to be arised with respect to this selection procedure.

It was pointed out that electronic sorting alone for aflatoxin contaminated products is inadequate and final hand-picking is necessary for efficient removal (Ref. 2). A segregation system by electronic sorting may be feasible for hand picked selected (HPS) groundnuts which are homogeneous as to variety and appearance (colour). This is economically favourable. However, separation is less likely to be possible for groundnuts normally available in developing countries for oil extraction, as the seeds are often heterogeneous and differently coloured, also the low output of the machine is not adequate.

Very recently electronic sorting based on selection by bichromatic infra red is attempted in Senegal, the results seem to show better resolution and suitable output (150 Kg/h./feeder). A segregation system of some sort would be used to separate out as fully as possible the mycotoxin contaminated portions from produce so that this quantity alone would need to be decontaminated.

Encouraging tests on pneumatic decortication process (Ref. 3) based on the differential bursting of the shells have been employed in Senegal for unshelled peanuts, the effectiveness of this process depend on the moisture content of the husks which has to be constant and low 2,5 to 3,5%. This would seem impossible in Senegal.

With cereals contaminated with ergot, since the ergot seeds are lighter they could be removed either by flotation technique (Ref. 4), by suspending the grains in sodium chloride solution, or by air classification. Wet and dry milling of corn show that in the former aflatoxin was removed essentially in steepwater, fiber, starch is almost free (Ref. 5) and in the second, aflatoxin is divided between the different fractions, grits fraction contains the lowest (Ref. 6). Concerning zearalenone, in wet milling this toxin is concentrated in the fraction in the order of gluten soluble, fiber, germ (Ref. 7), the starch is devoid of $F_2$. $F_2$ is not bounded by gluten protein. In dry milling process, $F_2$ concentrates in the germ and feed fraction (Ref. 8).

### Removal of mycotoxins

Extraction of oils by polar solvents. This item concerns particularly those who are consuming the non refining oil. Through the use of caustic soda during refining of the oil, any aflatoxins present in the crude oil are completely eliminated.

A reduction of aflatoxin content of unrefined hydraulic pressed oil can be achieved by filtration. In pilot plant studies in India nearly complete removal has been obtained with a special adsorption filter unit which is said to be mounted easily in place of the conventional cotton cloth filter (Ref. 9)

Extraction of oilseed cakes by polar solvents. A number of proposed solvent systems (Ref. 10) have been studied mostly at the laboratory level. Attemps to apply them on an industrial scale have been made in U.S.A. and France, the mixture hexane-acetone-water (50-48.5-1.5) was considered for its effectiveness. However, the detoxification on industrial scale was not

comparable with that obtained in the laboratory (Ref. 11), needed additional investment is very substantial making an economic evaluation of the process essential and with acetone containing impurity (mesotyl oxide) a disagreable odor is imparted to the product due to reaction with sulfur aminoacids.

Other method has been reported by Japanese patent (Ref. 12) describing the removal of aflatoxins using extraction by a water-methoxymethane mixture, the process is still under study.

The principal advantages of eliminating mycotoxins through the extraction are :

- Preservation for food and some animal feed purposes of nutritional properties of proteins as they are not broken down.

- Removal of the toxin in its primary form, thus avoiding the possibility of hazard from a degradation products.

In conclusion, the removal of mycotoxins by solvents, although attractive is expensive and generally not acceptable.

## Inactivation of mycotoxins

Criteria for inactivation methods. To have a potential for industrial application, a decontamination process must be technically and economically viable and must meet the following criteria :

- destroys or inactivate the mycotoxin,

- does not produce or leave toxic or carcinogenic residues in the final product,

- ensures the human safety of resulting edible tissues and eggs,

- destroys if possible fungal spores and mycelia which could, under favourable conditions, proliferate and form new toxins,

- preserves the nutritive value and acceptability of the product,

- does not significantly alter important technological properties.

Physical inactivation.
Heat - Although aflatoxin is reduced in heat treated materials, cooking food, roasting peanuts (Ref. 13), corn which can help to minimize the hazards of this toxin, a serious threat remains for highly contaminated food and more research is required for this approach. Studies on the effect of aflatoxin during baking process (Ref. 14) show that the destruction of aflatoxin is incomplete.

Light - A simple method for inactivation of aflatoxin in oil is reported from India (Ref. 9) and consisting to exposure the oil in glass bottle to bright sunlight for at least one hour. Aflatoxin is reported to loose its toxicity. The effect of such exposure on quality of the oil may need to be considered because oxidative degradation of oil. It seems that neither ultra-violet or infra-red is implicated in this destruction.

Chemical inactivation.
Protocol for safety study on decontaminated material : To obtain the approval for using such treated materiel for feed, a protocol for safety study has been proposed in France ; it gives as indicative trial the research to be undertaken in application of previously presented criteria to contaminated materials.

This protocol (Ref. 15) established for aflatoxin, could be summarized as follows :

I. Effectiveness of decontamination process applied.

For this purpose the following steps are essential :

- Physico-chemical analysis for the mycotoxins normally associated with the product using official method.

- Confirmatory biological test on one day-old duckling. When analysis indicates successful decontamination.

- Fate of toxin after treatment, condition of reversibility reaction.

- Assessment of general nutritive value. Composition of the amino-acid, protein efficient ratio for rat, chicken and duckling.

2. Detailed testing of animal feed.

This part will not be undertaken unless the effectiveness of the decontamination treatment has been established.

Composition :
- Analysis for residues of the material used for the treatment. Any transformation products should be identified if possible.

- Chemical analysis of the components of the feedstuff. Attention should be paid to components which are susceptible to quantitative or qualitative changes because of the decontamination process.

- Microbial counts of revivable germs.

Nutritive value :
It will be estimated through :
- Total feed efficiency studies to be carried out on at least two species of animals, rat and a farm animal.

- Specific measurement of the availability of compounds subject to modification as a result of the treatment.

Toxicity :
Absence of toxicity is demonstrated through acute and long term studies on animal species sensitive to the mycotoxins involved.

In the chronic toxicity, cancerogenic, teratogenic and all other effects are determined in the rat. Fertility and reproduction characteristics are followed up over two generations at least.

A study of transmitted toxicity should be done on duckling using milk from animal fed on a diet containing treated oilseed cake.

All the criteria have indeed narrowed the type of chemical agents likely to succeed in commercial scale decontamination process.

Oilseed cakes with ammonia : Most trials have been applied to oil cakes from aflatoxin contaminated groundnuts and cotton seed. At present, two plants are in operation in the U.S.A. for the treatment of cotton seed cake and cotton seed by ammoniation (US Pat. n° 3429709 : conditions being ammonia pressure of 48 pounds per square inch, 118°C, 30 minutes of contact time). Two other plants for oil cakes groundnuts (in Senegal and in Sudan) are building and will use French process (French Pat. n° 2184439 : conditions being oilseed cake at 12-15% moisture content, ammonia gaz pressure of 2 to 3 bars, 90°C, 15 to 30 minutes of reaction time).

The nutritional and toxicological evaluation of the treated product have been studied thoroughly. The available lysine value does not appear to be affected but cystine may be decreased to the extent of 15 to 30%, this could be corrected easily. In any case the decontamination reaction is irreversible. This process brings a gain of 1% soluble nitrogen. The change in the molecule of aflatoxin $B_1$ during ammoniation has been studied (Ref. 16) and leads to decomposition products ($D_1$ and 206 compound) (Fig. 1).

Nutritional trials, short and long term toxicological studies show no difference between control and treated material and led to the conclusion that the product is acceptable.

The cost of treatment represents a plus value of 3% on the cake value which does not touch two much on the net cost gains. The use of this decontamination is considered in France because all the obtained results respond to the exigences which we have precedently numbered.

Temporary approval has been given also in U.S.A. by F.D.A.

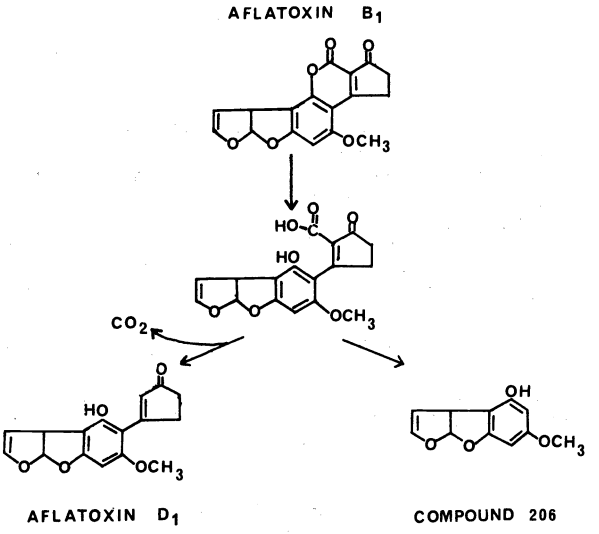


Fig. 1. The fate of aflatoxin $B_1$ after ammoniation

Aflatoxin contaminated corn with ammonia : This is based on use of either aqueous ammonia under atmospheric pressure or in the gaseous form over a period of several hours (corn 15-22% moisture content, 1% ammonia) (Ref. 17). The process does not raise technological problem, but the obtained corn is discoloured, thus could restricting its use to animal feed (Ref. 18).

The mechanism of inactivation has been studied (Ref. 19). Aflatoxin $B_1$ combines with protein macromolecules and thereby becomes inactive.

Toxicological evaluation with rainbow trout of treated corn was found that was not significantly different from that with the untreated non contaminated sample (Ref. 20). One of these authors manifested recently (Ref. 21) some doubt on control of aflatoxin by ammoniation, he founds that the trouts fed by milk from cows fed by contaminated cotton seed oil previously treated by ammoniation developed liver cancer, it may be asked whether in the tested milk it remains not detectable aflatoxin (the used ammoniation destroys partially aflatoxins) and toxic metabolite from cotton seed could play synergic role in this carcinogenic effect. Furthermore, the different behaviour of aflatoxin after ammoniation in contaminated corn and cotton seed is to be considered for explanation of this discrepancy. Rainbow trout test needs to be conducted for contaminated peanut meal treated with ammonia.

Ammoniation has also advantage in killing spoilage molds in corn (Ref. 22).

Decontamination of corn by ammonia is still under nutritional evaluation in U.S.A. and under F.D.A. approval.

Oilseed cake by monomethylamine and calcium hydroxide mixture : Decontamination trials have been carried out on an industrial scale (Pat. CH 566-110). The process involves the simultaneous use of lime milk Ca(OH) 2% and an aqueous solution of methylamine 0,5% of the weight of the cake. Both investment and maintenance costs are reasonable (Ref. 23). With very slight modifications, the equipment may be installed in a standard vegetable oil factory. It seems that the mechanism of destruction of aflatoxin $B_1$ is similar to that of ammoniation, opening of the lactone ring and further decarboxylation could take place.

The first evaluation of toxicological and nutritional data appears promising and led to the conclusion that the procedure effectively decontaminated the oilseed cake.

Oilseed protein isolates with hydrogen peroxide : This process used in India (British Pat 1.117.573) consists of the treatment of isolated groundnut protein for human food with hydrogen peroxide. This method is utilised on commercial scale, and daily production is reported to be 2-5 tones of toxin free protein. The cost of the treatment represents about 15% of the cost of the isolated oilseed cake proteins.

Other method of decontamination of protein groundnut using sodium hypochlorite has been

studied. Trials on a industrial scale have not yet been undertaken.

Biological. One approach towards denaturation of myxotoxins could be realised,is the ability of microorganisms to denature or to transform mycotoxins. So far, the results of research in this area is not optimistic. The attempts undertaken with bacteria (Ref. 24), ensiling conditions (Ref. 25), are not promising and are either not efficient or not practically feasible. Trial on decontamination of aflatoxin-peanut meal by composting (Ref. 26) appear promising and need to be considered seriously at farm level. Some mycotoxins may destroyed in conventional food processing, ochratoxin and patulin are metabolised by yeast (Ref. 27) and disappeared in beer and fermented apple juice, patulin and penicillic in other circonstances are inactivated in presence of sulfhydryl group (Ref. 28), they are complexed by thiol groups present in food (meat, flour). PR toxin has been proved instable in cheeses, amino acids are likely the responsible for its destruction (Ref. 29).

When circonstances do not permit the decontamination, the use of contaminated products in animal feed must be considered but with much care. Although the ratio of mycotoxins consumed to mycotoxins in tissues or milk is low, the risk is increased for milk because it is often consumed by children and young animals known to be relatively susceptible to the mycotoxins effects. Proper dilution of the contaminated material in the ration will lower the risk.

It may be asked why the mankind was not decimated in the past by mycotoxins, which they were not apprehended and therefore not destroyed ? first, because the nature spares no expense and makes the things wonderful, very few mycotoxins are natural contaminants (produced under natural conditions) comparing with the numerous toxic metabolites produced by fungi under artificial conditions. Secondly, food is generally consumed after processing and reduction of mycotoxins will take place.

## CONCLUSION

The major concern of decontamination methods is related to aflatoxin. At present, that is no proven process for the removal or destruction of other mycotoxins at pilot scale.

The decontamination should constitute a priority. We can regret the relatively rarity of studies in respect to the interest and the deriving consequence.

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