MYCOTOXINS IN GRAINS AND NUTS: II) DECONTAMINATION AND DETOXIFICATION METHODS

TAHILLARDA VE KURUYEMİŞLERDE MİKOTOKSİN BULAŞMIŞ ÜRÜNÜ VE MİKOTOKSİNİ AYIRMA YÖNTEMLERİ

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SUMMARY

Mycotoxins are worldwide important problem in term of public health, agriculture, and economics. Turkiye, with its high potential in agriculture, produces large amount of grains and nuts (e.g., hazelnut), besides dried fruits (e.g., dried figs, raisins) which are susceptible to mycotoxin contamination. In this study, detoxification and decontamination methods of mycotoxins in grains and nuts are reviewed.

ÖZET

Sağlık, tarım ve ekonomi açısından mikotoksinler dünya çapında önemli bir sorundur. Türkiye tarımdaki büyük potansiyeline koşut olarak büyük miktarlarda tahıl, kuruyemiş (fındık), kuru meyve (kuru incir, kuru kayısı, kuru üzüm) üretmektedir. Üretim, hasat, hasat sonrasındaki yetersiz ve yanlış uygulamalar gıdalarda mikotoksin oluşumu riskini arttırmaktadır. Bu derlemede makalede tahıllarda ve kuruyemişlerde mikotoksin bulaşmış ürünü ve mikotoksinleri ayırma yöntemleri tartışılmaktadır.

INTRODUCTION

Mycotoxins are worldwide important problem in term of public health, agriculture, and economics. Current inability in accomplishing preharvest control of mycotoxins has made postharvest control mandatory. Aflatoxin control now comprises segregation of contaminated lots, partial decontamination and prevention of further contamination by appropriate storage methods (Schmidt and Esser, 1985). Decontamination is physical removal of contaminate units, kernels or nuts. Detoxification is removal of toxin from the unit or on its destruction. However, these methods should be considered when diversion of food from human consumption to animal feed, or careful blending of contaminated food with good quality food to produce reduced overall level of the toxin to the limit that is accepted legally or considered sufficiently safe is not feasible.

Decontamination/Detoxification methods can be classified as: (i) Separation of the contaminated parts of the produce; (ii) Extraction of mycotoxin (iii) Inactivation of mycotoxins by physical (heat, cooking, roasting), and chemical, and biological means.

Decontamination and chemical detoxification methods must ensure inactivation of mycotoxin that will not result in the introduction of new toxic or

carcinogenic/mutagenic substances in the food or feed chain. Moreover the process must retain the nutritive value and acceptability of the product, does not significantly change important technological properties. Process must also destroys spores and mycelia that could, under favourable conditions, proliferate and form new toxins (Gnanasekharan and Chinnan, 1992).

Physical Separation of Mycotoxin Contaminated Materials

Fungal infection of any seed or grain changes colour, density or other physical properties that allows separation of such seeds or grains to minimise contamination.

Physical separation methods are simple, time and labour consuming when performed manually, and may be applicable to small lots of producer for smaller size grains.

Kernels that are inadvertently shelled by harvesting and handling processes must be removed by screening operation before the lot go to the sheller since aflatoxin contamination is usually is highest in those kernels. Moreover, since kernels in damaged pods contain more aflatoxin, they should be removed by sorting. Colour sorting is widely used for peanuts, coffee berries and other similar sized materials, either by using an electronic sorter, or manually or using both for efficient removal. Subsequent to shelling and sizing operations, the shelled kernels may be scanned with electronic sorters or hand picked to remove discolored and mouldy kernels that are not feasible if skin colours of good kernels are highly variable. If so, after blanching, testa and the skin are removed. Kernels that are retained skin after blanching are the contaminated ones, much be removed by electronic sorter or by hand picking. However, efficiency of electronic colour sorting and hand-picking are widely variable and only 72% of the aflatoxin contaminated lot can be removed. Handling is more selective, but labour and time consuming (Clavero et al., 1993). However, colour sorting will not separate contaminated peanuts that are visually acceptable, and cause a definite loss of non-contaminated peanuts with each pass (Dickens and Whitaker, 1975).

Density-based separation of sound peanut kernels is theoretically feasible but loss of peanuts is high and efficiency of the separation is highly variable. It is based on the observation that contaminated peanut kernels is usually less dense than sound ones. The process requires additional drying step after flotation treatment, so it has not been employed much in industrial scale (Clavero et al., 1993). However, it enables to remove fully mature and visually sound but contaminated kernels that can not be segregated by colour sorting (Gnanasekharan and Chinnan, 1992). Huff and Hagler (1980) stated that density based separation of contaminated corn from the sound ones is possible, but degree of aflatoxin contamination of corn can not be estimated by density segregation.

In wheat and millet, ergot contamination is frequently encountered and the ergot seeds are removed either by a flotation technique, by suspending the grains in sodium chloride solution, or by air classification. Since the ergot sclerotia are lighter than sound seeds they can be removed by any of these methods.

Separation of aflatoxin contaminated kernels from sound kernels by hydrogen peroxide treatment is under investigation, and gives promising results. It is based on the observation that aflatoxin contaminated kernels floats more rapidly than sound kernels when submerged in hydrogen peroxide reacting with the catalase produced by *A. parasiticus.* The reaction yields water, and oxygen bubbles on the surface of the kernels. As the level of catalase increases evolution of oxygen also increases, thus causing mould-infected kernels which may contain aflatoxins to float. Hydrogen peroxide concentrations of 0.075, 0.15, and 0.25% decreased aflatoxins 90% in the kernels within 1 min regardless of initial aflatoxin content. For peanut containing 50 ppb aflatoxin, 0.08 % hydrogen peroxide treatment for 0.7 min results in 85.5% reduction of aflatoxin in the original lot with a residual aflatoxin content of 5 ppb. (Clavero et al., 1993).

A bag, clump, batch or other unit of mouldy material may be easily set aside during storage, handling or processing, but it is very difficult to remove individual grains of this mouldy material after it has been mixed with non-mouldy grains. Therefore, any unit of material that has become mouldy or is suspected to contain mycotoxin must be removed and treated separately to remove the mouldy grains, or can be diverted to suitable non-food use that is much less costly than having to treat the entire lot of material. High-moisture material should also be set aside for drying and other special treatment. Small, shrivelled kernels, insect - damaged kernels and broken kernels that often contain high concentration of aflatoxin can be removed by sizing over screens and/or by aspiration.

Removal of Aflatoxin in Oils

Aflatoxin in crude peanut oil is in the suspended state, and can be removed by a suitable filter. The remaining toxin can be separated by adsorption on a suitable adsorbent. A filter adopted to use in the place of cloth filter can remove aflatoxin to an extent of 95-100 %. Peanut oil refinement is practised in USA (Jones, 1995).

Inactivation of Aflatoxin by Heat

If aflatoxin cannot be removed completely, the next approach is inactivation either by irreversible modification of the compound chemically, or by alteration of the active groups in the molecule. Solid aflatoxin B_1 is stable to dry heat up to its melting point of 260^{o}C; the thermal decomposition temperature is 269^{o}C with as high as 300^{o}C in certain foods. Table 1 shows the degradation of aflatoxins in foods under different heat treatment conditions. Presence of moisture in foods may enhance degradation while binding or association of aflatoxins with proteins may protect the toxin. Normal food processing and preparation conditions appear to cause, on the average 60% degradation under laboratory conditions. With edible oils (peanut oil, olive oil, and coconut oil) 200^{o}C is required for degradation (Samarajeewa et al., 1990). High temperature short time (900^{o}C/8s) processing of grains resulted in totally destruction of mould spores and 58% destruction of aflatoxin B_1 without affecting protein digestibility while all insects at all stages are killed at temperatures much

lower than those required for spore deactivation. The treatment is 50-100 times as effective as IR. treatment. But it is under investigation. *A. Flavus* spores in dry corn were found to very resistant to roasting, while 70% destruction of total aflatoxins is achieved by heat treatment of corn (Wilkins et al., 1992). The need for elevated temperatures and pressures for effective detoxification of contaminated foods makes heat treatment methods impractical. It is further enhanced with impairment of nutritional, organoleptic properties and generation of toxic pyrolsate at elevated temperatures.

Heat treatment such as steam flasking (steam at 25-75 psi for 1-5 min), explosion cooking (dry steam at 33-43psi for 20-25 s), dry heat roasting (heating up to 128-149^{o}C), micronizing (infra-red heat at 149^{o}C for 20-50s) and popping (370-427^{o}C for 15-20s) are already applicable to food processing. Microwave treatment at high energy levels shows great potential for aflatoxin degradation as seen from the Table 2.

However, a serious problem remains from foods which even after cooking high concentration of aflatoxin and from those foods that are frequently contaminated with aflatoxin and are not subjected to proper heat treatment.

Inactivation of Aflatoxin by Light

Light has been successfully employed by destroys aflatoxin in unrefined peanut oil. Sunlight is the best agent for destruction compared to visible light, ultraviolet light, infrared light. Exposure to sunlight in a bottle destroys aflatoxin completely in one hour. However, it is not tried in industrial scale. Table 3 shows degradation of aflatoxins in foods on exposure to ultra-violet and/or visible radiation. Economical feasibility and flavour changes must be considered in the evaluation of the process (Samarajeewa et al., 1990).

Chemical Inactivation of Aflatoxins

Chemical degradation of aflatoxins is currently seemed to be more practical. Among many chemicals screened for their ability to detoxify pure aflatoxin B_1, chlorinating agents such as sodium hypochlorite, chlorite dioxide, gaseous chlorine; oxidising agents such as hydrogen peroxide, ozone and sodium bisulphite; and hydrolytic agents such as acids and alkalis appear to be effective.

Concern regarding the safety of chlorinated foods still exits. The presence of residual *chlorine* in the treated foods, production of modified fats and proteins that may be of unknown toxicity.

Hydrogen peroxide has been shown to inhibit to growth of alfatoxigenic fungi in synthetic media at concentrations of 0.3 and 0.5 % while at 0.03-0.05% it allows for fungal growth and enhances aflatoxin production. So, foods treated with hydrogen peroxide may still support fungal growth and aflatoxin production at a later stage if the treated foods are contaminated with fungi.

Ozone reported to reduce aflatoxin B_1 levels by 91% in cottonseed meal containing 22% moisture after treatment at 100^{o}C for 2 h while the reduction with peanut meal containing 30% moisture as only 78% after exposure to ozone for 1h. It

needs longer treatment duration and decreases protein efficiency ratio and available lysine. Therefore it is regarded as a less satisfactory method.

Bisulfite is low efficient for inactivation of aflatoxin B_1 and G_2. Moreover, there is possibility of regeneration of aflatoxin B_1 or the active toxic epoxide during metabolism of the treated samples.

Specific detoxification methods, which are tried in industrial scale, in particular those used for the treatment of oilseed cakes of peanut or cottonseed are designed for aflatoxins only. The others are extraction of oils and oilseeds cakes by polar solvents containing acetone, hexane and water; detoxification of oilseed cakes and corn using ammonia; detoxification of oil seed cakes using methylamine and calcium hydroxide; detoxification of oilseed protein isolates using hydrogen peroxide and elimination of aflatoxins using solvent extraction by a water-methoxymethane mixture, detoxification of peanut proteins using sodium hypochlorite (Gnanasekharan and Chinnan, 1992).

Ammonia is equally effective in a gaseous or aqueous phase in decontaminating aflatoxin in feeds. The treatment causes a 95% degradation of aflatoxins. The use of 5% ammonia, 10-20% moisture, and a temperature-time related combination is required for effective degradation of aflatoxins. Conditions for effective aflatoxin degradation by ammoniation are shown in Table 4. Ammoniation of animal feeds is carried out in France, Senegal, and Sudan Mexico, US, Brazil.. Alkali and acid based treatments needs more investigation. Results of acid experiments seem to be more promising. Ammonia and ozone treatments of grain appear to destroy several types of mycotoxins without any deleterious compounds or leaving any residues (Jones, 1995).

RECOVERING AFLATOXIN-FREE PRODUCTS FROM CONTAMINATED PEANUT AND CORN

Although aflatoxin contamination can occur at significant levels at raw product, processing conditions such as screening and milling can reduce these levels. Peanut processing procedures such as steep tanks, coat splitting, sorting, and frying can reduce aflatoxins to non-detectable levels in finished products. Table 5 shows effectiveness of postharvest processing on aflatoxin levels.

Crude peanut oil retains only 15% of the aflatoxin, while the rest remains in the press-cake. Subsequent refining by filtering and photodegradetion under sunlight leads to peanut oil which aflatoxin is not detectable. The aflatoxin containing cake may be used as animal feeds if the concentration of the toxin is low, or must be used as a fertiliser. Alternatively, detoxification methods may be used, but not practised at the present because of cost (Anonymous, 1979). Peanut expeller processing retains 85% of the aflatoxin in the meal that can used in the animal feed mixes, having a permissible limit in the final mix (Anonymous, 1979).

For corn products, about 20 % is lost during baking, or boiling, 50% during frying, and even more during alkali processing (Jones, 1995). Subsequent to milling of corn, grits contained 10% of the aflatoxin concentration in the lot of whole kernels

from which they are produced, meal contained that of 13-16%, flour contained 30-70% depending on initial concentration (Anonymous, 1979).

Wet-milling of corn to produce starch, oil and other products results in aflatoxin-free starch and oil besides most other product while 80-90% of the aflatoxin is concentrated in the gluten feed fraction (steep-water, fibre and spent grain) which must be discarded or diverted to suitable uses (Anonymous, 1979).

UTILIZATION OF MOLDY OR MYCOTOXIN-CONTAMINATED PRODUCTS

Since it is not always possible to prevent moulding or mycotoxin contamination in agricultural products, alternative uses for the contaminated products are important to reduce economic loss to the producer and diversion of these products must be encouraged for acceptable uses. Peanuts infected with *A. flavus* is diverted to from edible market to oil stocks, and income of the producer is greatly reduced (Thai et al., 1990). Blending of toxin-contaminated material with toxin-free material reduces level of mycotoxin together with milling operation to acceptable levels for selected animals. However, production efficiency for some animals may be reduced by even low concentration of mycotoxin, and there is a risk that mycotoxin contamination may be transferred to some animal products used as food. Cows may transmit up to 3% of ingested mycotoxins to their milk as aflatoxin M_1. The risk involved, however, is high because, it is mainly consumed by children or young animals who are known to more susceptible to the effects of the toxin than adults or older animals. The ingested toxin, can also be transmitted to animal tissue (Anonymous, 1979).

Regulation and Control of Mycotoxins

Control of mycotoxins in foods are complex and difficult task. For most of mycotoxins information regarding toxicity, carcinogenicity and teratogenicity to humans, extent of contamination, stability of foods, population exposure and risk illness is required for regulatory guidelines, tolerances and seizure policies but they are lacking. Therefore safe tolerance levels have not been established for any of the mycotoxins. In the absence of the tolerances FDA has set what it considers to be practical limits for aflatoxins in foods and feeds. In the US the Food and Drug Administration has an action level of 20 ppb for aflatoxins in susceptible food and feed that are for dairy cow while for other feed it is 100 ppb. FDA has proposed lowering the action level to 15 ppb for peanut products (Bullerman, 1986). Reductions in permissible levels to 15 ppb would lower current liver cancer risk by 25%, 10 ppb by 50% and 5 ppb by 75% (Dichter, 1984). Current action level is given in Table 6. In many countries, tolerance levels for aflatoxins in foodstuffs are in the range of 5-50 ppb (Anonymous, 1993). However, consumer demand and the world export market for commodities susceptible to aflatoxin contamination are seeking a goal of aflatoxin free products by the year 2000 (Paster et al., 1992). But, there are no internationally accepted sampling plans. The results of the analysis is always dependent on the type

of sampling plan applied while it is somewhat dependent on the method of analysis. When one single aflatoxin contaminated nut presents a sample representative a lot, the entire lot may be rejected for human consumption. Such rejected consignments almost invariably end up in other markets posing health hazards elsewhere. The setting of internationally accepted tolerance levels for mycotoxins in food and feed is of global importance (Anonymous, 1993).

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Table 1:
Degradation of Aflatoxins in foods under different heat treatment conditions (Samarajeewa et al., 1990)

Treatment	Degradation(%)	Food
Heat at 120 °C, 10 min	50	peanut oil (crude)
Heat up to 150 °C	negligible	peanut oil (unrefined)
Heat up to 250 °C	partial	peanut oil
Heat at 180-215 °C, 10 min	41	coconut oil
Heat up to 200 °C	little	olive oil
Heat up to 250 °C	65	olive oil
Heat at 120 C, 20 min	small	aqueous solution
Dry heat at 105 °C	35-59	peanuts
Cooking at 100 °C, 2 h, 30% moisture	66	peanut meal
Cooking at 100 °C, 2 h	80	cotton seed meal
Normal cooking	49	rice
Pressure cooking at 120 °C	73	rice
Pressure cooking, excess water	82	rice
Pressure parboiling, 20 psi, 10 min	100	paddy
Cooking	72-86	brewer's mash
Alkaline cooking	partly	tortillas
Tortilla preparation	40	corn
Tortilla	46	corn
Boiling	28	corn meal grit
Autoclaving at 120 °C, 4 h	95	peanut meal
Autoclaving at 120 °C, 30 h	9-39	fruits and spices
Autoclaving at 120 °C, 60 h	>50	fruits and species
Autoclaving at 1.5 atm, 30/60/90 min	72,96,100	peanuts
Oven drying at 60 °C, 60 h	22-77	fruits and species
Baking bread	60-90	wheat flour
Baking at 120 °C, 30 min	80	wheat flour
Baking of muffin	13	corn meal
Preparation of tortillas	70	corn
Frying	33-53	corn meal grit
Frying in veg. oils at 190 °C, 6 min	60	pecans
Oil roasting at 325-345 F, 3-7 min	65	peanuts
Roasting at 145-165 °C	40-81	corn
Roasting at 190 °C, 15 min	80	pecans
Roasting at 190 °C, 15 min	60	pecan meal
Roasting at 204 °C	41-63	peanuts
Roasting at 150 °C, 30 min	50-83	artificially contaminated peanuts
Roasting at 150 °C, 30 min	30-45	artificially contaminated peanuts
Dry roasting at 250-400 °F, 5-30 min	58-79	peanut
Dry roasting at 191 °C, 30 min	60-90	pecans
Microwave roasting, 6 kw, 4 min	95	peanuts
Microwave roasting, 1.6 kw, 16 min	95	peanuts
Microwave roasting, 0.7 kw, 8.5 min	30-45	artificially contaminated peanuts
Microwave roasting, 0.7 kw, 8.5 min	48-61	peanuts

Table 2:
Degradation of aflatoxins by gamma radiation (Samarajeewa et al., 1990)

Dose (Mrad)	Degradation (%)	Substrate
7, 15, 30	none	on TLC plate
>30	partial	pn TLC plate
0.25-1	partial	aqueous solution
>1	total	aqueous solution
2.5	none	peanut meal
3	none	rice, 8, 16, & 32% moisture
0.25-0.50	0-50%	bread; dried slurry
0.64	none	peanuts
2 with 5% H_2O_2	50-75%	peanuts
2-5	partial	benzene: acetonitrite
0.1-1	75-100%	peanut meal
5-10	100%	peanut meal
0.5	95%	dimethyly sulfoxide water (9:1)

Table 3:
Degradation of aflatoxins in foods on exposure to ultra-violet and/or visible radiation (Samarajeewa et al., 1990)

Treatment	Degradation (%)	Food
UV radiation, 8 h	0	peanut meal
UV radiation, 2 h	40-45	peanut oil
Flourescent tube, 1 h	partial	on TCL plate
Flourescent tube, 1 h	partial	coconut oil
Tube light, up to 60 h	up to 45	species, dry fruits
Incandescent bulb, 1 h	partial	on TLC plate
Mercury tungsten bulb	63-93	rice
Sunlight, 15 min	100	peanut oil
Sunlight, 30 min	>75	coconut oil
Sunlight, 40 min	95	olive oil
Sunlight, 6 h	83	casein
Sunlight, 6 h	50	peanut cake
Sunlight, 3.5 h, 5 mm layer	negligible	copra meal
Sunlight	partial	tropical foods
Sunlight, 14 h, 0.5 mm layer	90	peanut flakes with fat
Sunlight, 14 h, 0.5 mm layer	77	peanut flakes without fat
Sunlight, 14 h, 0.5 mm layer	50	naturally contaminated peanut

Table 4:
Conditions for effective aflatoxin degradation by ammoniation (Samarajeewa et al., 1990)

Ammonia concentration	Moisture (%)	Pressure	Temperature (°C)	Duration	Substrate	Aflatoxin initial ppb	Aflatoxin % loss
5%[1]	20		145	3 h	corn	270	93
anhydrous	15	45 psi	93	30 min	cottonseed meal	334	>99
anhydrous	10	30 psi	82	30 min	cottonseed meal	340	>95
4%	14	40 psi	100	30 min	cottonseed meal	4000	>99
anhydrous	12.5	45 psi	93	15 min	cottonseed meal	350	>99
anhydrous	10	45 psi	93	15 min	peanuts	121	>99
	10-15	20 psi	93-121	1 h	peanuts meal	709	97
6.70%	15	43 psi			peanuts meal	111	>99
anhydrous	15	3 bar	95	30 min	expeller cake	600	>99
gas		3 bar	80	15 min	peanuts meal	1530	95
gas		3 bar	80	15 min	peanuts meal	1140	95
gas	8	0.5-2 bar	80	15 min	peanuts meal		>99
2.50%	20	(extrusion cooking)	85		peanuts meal		40-87
1%	10-15	amb[2]	5-95	1 h	peanuts cake	300	93
4%	17	26 psi	118	1 h	peanuts meal	1977	98
4%	dry	40 psi	100	30 min	silica-gel H	40000	99
1.50%	17.5	amb	49	12 d	corn	160	98
gas (recycled)	17.6	amb	25	14 d	corn	1000	>99
1.5	11	amb	amb	179 d	corn	896	96
1.50%	15	amb	amb	13 d	corn	750	>99
1.1	11	amb	amb	7 m	corn	90	>99
0.50%	15	amb	38	3 d	corn	600	97
1.5% (aq)	20	amb	amb	21 d	cottonseed-whole	1900	>99
2%	12.5	amb	43	15 d	cottonseed	800	98
1.50%	17	amb	amb	21 d	cottonseed	400	96
5%	20	amb	amb	5 d	peanuts meal	2500	>99
3% (aq)	15	amb	50	5 d	peanuts meal	970	98
5%	20	< 1bar	amb	10 d	peanuts cake		79
7%	17	amb	100	1 h	peanuts meal	1000	95
5%	20	amb	amb	10 d	maize		97
NH4OH		amb	20	7 d	cottonseed		62
NH4OH		amb	100	1 h	cottonseed		>99

[1] Percentages refer to grams per 100g substrate added in the form of aqueous ammonia or ammonium hydroxide.
[2]Ambient pressure or temperature.

Table 5:
Effectiveness of posharvest aflatoxin management strategies at the processing level[1] (Anonymous, 1993)

Technology	Aflatoxin Level (ppb)	Reduction (%)	Cumulative Reduction (%)
Farmers stock	217	--	--
Belt separator	140	35	35
Shelling plant[2]	100	29	86
Color sorting[2]	30	70	86
Gravity table[2]	25	16	88
Blanching/color sorting	2.2	91	99
Re-color sorting	1.6	27	99.3

[1] Results from the processing of 40 ton lot of contaminated peanuts.
[2] Data based on medium category peanut only.

Table 6:
Current aflatoxin action levels (Park, 1993)

Food or feed	Action level (ppb)
Human foods (except milk)	20
Milk	0.5
Animal feeds (except as listed below)	20
Cottonseed meal (used for mature beef, swine, and poultry rations)	300
Corn for breeding beef cattle, breeding swine, or mature poultry	100
Corn for finishing swine	200
Corn for feedlot beef cattle	300