J. Natn. Sci. Coun. Sri Lanka 1977 5 (1) : 1 -12

Degradation of Aflatoxins in Coconut Oil and Copra meal (poonac)

U. SAMARAJEEWA*, S. N. ARSECULERATNE AND C. H. S. R. BANDUNATHA
Department of Microbiology, Faculty of Medicine, University of Sri Lanka, Peradeniya Campus, Peradeniya, Sri Lanka.

(*Paper accepted : 12 October* 1976)

Abstract : Coconut is an excellent medium for the growth of aspergilli and for aflatoxin accumulation. Oil derived from mouldy coconut may show high levels of aflatoxin ; even commercial coconut oil from processed copra often shows low or medium levels of aflatoxin. On account of its use as an article of human food, the control of aflatoxin contamination in coconut oil is an urgent problem. The spontaneous drop of aflatoxin levels in stored oil, reported earlier, has been investigated and was found to be due to photodegradation by sunlight, probably through its content of ultraviolet light. Exposure of oil, with either high or low levels of aflatoxin, to sunlight (over 60 cal/cm^2) was found to significantly reduce the levels of aflatoxin. Commercial contaminated oil was decontaminated to aflatoxin levels of less than 30 ppb ; there was no change in the FFA content or colour. It is suggested that exposure to sunlight could form the basis of a cheap and efficient method of the industrial decontamination of aflatoxin contaminated coconut oil in tropical countries.

1. Introduction

The levels of relative humidity and environmental temperature in tropical countries are precisely the conditions which favour fungal colonisation of stored foods, especially those with more than 10% substrate moisture. This results in the accumulation of toxic fungal metabolites including aflatoxin. Coconut is one such source of a human and animal food which has been shown earlier to be an excellent medium for aflatoxin accumulation. The presence of aflatoxigenic strains of *Aspergillus flavus* and of aflatoxins in food products derived from coconut in Sri Lanka, was first reported in 1971.[2]

Aflatoxin was incriminated as the cause of 106 human deaths in an epidemic of poisoning in Western India due to the consumption of mouldy maize[14] and an outbreak of aflatoxicosis occurred among goats in Sri Lanka causing considerable economic losses to goat farming.[19] The cause of the outbreak was found to be aflatoxin which was present in a mouldy coconut-based food concentrate fed to the animals.

In the light of these observations, urgent measures are needed to prevent aflatoxin contamination, or to evolve cheap and effective methods for the detoxification of contaminated substrates, especially in poor tropical countries.

Prevention of fungal colonisation is the rational, cheapest and most efficient step in the control of aflatoxin contamination. The possible industrial use of smoke protection of solid substrates has been described elsewhere.[3] If fungal growth can be prevented during curing and storage, the question of detoxification does not arise.

* Present address :- Coconut Research Institute, Lunuwila

However, once the food items are contaminated, they should either be discarded, treated for removal of degradation of aflatoxin or diverted to the manufacture of non-edible products.

The contributions by various authors on the decontamination of aflatoxin-contaminated substrates are numerous and are reviewed by Dollear[9] and by Detroy, Lillehoj and Ciegler.[8] Removal of aflatoxins could be brought about either by the selective removal of the contaminated kernels by mechanical,[10, 13] electrical[4] or manual means. Once a batch of kernels or seeds containing a few contaminated ones is processed for oil, the aflatoxins pass into both the oil and pressed cake. At this stage, the toxin will be more uniformly distributed in the oil or cake and its destruction could be achieved only by chemical, thermal, radiation or microbiological treatment.

Chemical inactivation could be brought about by using gases such as chlorine, sulphur dioxide, ammonia, methyl amine, ozone, propylene oxide or by liquids such as hydrogen peroxide, sodium hydroxide, formaldehyde and acids. The extraction of the toxins by various combinations of solvents such as acetone, ethanol, hexane, isopropanol, methanol and water has also been described.[8] The chemical methods have the disadvantage of residual effects from the chemicals used and the production of various toxic compounds due to reactions between components of foods and the inactivating agents.[11] The biological value of the foods, too, could be reduced due to the action of the chemicals or solvents.

Thermal treatment involves roasting or cooking of the substrate before consumption. Aflatoxins are stable to high degrees of dry heat. Roasting[15] and cooking[16] bring about only partial degradation of aflatoxins. Even with autoclaving at 15 psi[5, 7] variable results were obtained with regard to the efficiency of heat detoxification. A further disadvantage could be that some vitamins and amino acids in the substrates are destroyed during the heating.

Among the radiation methods, high doses of radiation were found to be necessary to bring about detoxification.[17] At such levels of radiation, the quality of foods could be adversely affected.[12] Ultraviolet radiation has been successfully used in detoxification of aflatoxins. Aibara and Yamagishi[1] have shown that the products of ultraviolet degradation of aflatoxins are less toxic than aflatoxins themselves.

By screening a large number of micro-organisms, Ciegler *et al.*[6] showed that *Flavobacterium aurantiacum* could remove aflatoxin B_1 in solution ; they successfully applied it to milk and butter. However, the possibility of other effects such as lipolysis due to these organisms was not ruled out.

Some of the methods described above, adapted for the removal or inactivation of toxins, involve the use of costly equipment and chemicals. On the other hand, the methods could cause a loss of nutritive value of the treated food, alterations of flavour and aroma and the presence of residual chemicals. Newly formed by-products of treatment may be of unknown characteristics and potentially hazardous.

Among the various coconut-based food items, copra, coconut oil and poonac were the major items which were found to contain aflatoxins. Fungal colonisation occurs on stored kernels with more than about 10% moisture and the accumulated aflatoxins pass into oil and poonac during processing. No fungal growth occurs in coconut oil but colonisation in poonac occurs only under exceptional conditions of very high moisture and even then only very low aflatoxin concentrations are produced. In a copra heap, only a few kernels will usually be mouldy. The kernels are handled individually by workers during curing, storage and transportation to the crushers and hence they could easily be separated manually, because the fungal growth is visible. Since the contamination of oil and poonac is due to the admixture of a few contaminated kernels, the cheapest and easiest method of control would be the manual separation of mouldy kernels.

The most effective method for detoxifying contaminated oil is chemical refining which removes aflatoxins with pigments. This method, however, is expensive and hence cannot be used for all the oil produced in this country. The chemical removal of aflatoxins, which is the only effective method applicable to poonac, is a costly process.

It has been reported earlier[2] that a spontaneous fall of the aflatoxin content occurs in coconut oil during prolonged storage in the laboratory. This phenomenon was studied as a possible basis for a cheap and effective method for the decontamination of oil and poonac. This paper reports that the degradation of aflatoxin in oil under storage was found to be due to sunlight and that experimentally contaminated oil as well as contaminated commercial oil was effectively decontaminated by exposure to sunlight.

2. Materials and Methods

2.1. Oil

2.1.1. *Preparation of samples :*

Freshly grated coconut was inoculated with a highly aflatoxigenic culture of *Aspergillus parasiticus* NRRL 2999 and the highly toxic oil expelled from the culture was added to commercial coconut oil to produce samples of oil containing 10 ppm to 20 ppm of aflatoxin B_1.

2.1.2. *Degradation under storage*

Replicate portions of the oil mixtures were treated as follows :—

(*a*) storage at —85°C in total darkness,

(*b*) storage at room temperature (25°C ± 2°C) in total darkness,

(*c*) heated to 70°C (dry heat) and stored at room temperature in total darkness,

(*d*) storage at room temperature in diffuse sunlight (inside a room close to a window excluding direct sunlight).

Samples were withdrawn for assay at 10 day intervals, for 4 months.

2.1.3. *Degradation by sunlight*

Coconut oil samples containing different aflatoxin levels, either experimentally produced or purchased from commercial sources, were exposed in the form of thin layers (1½ cm or 4 cm) for different periods, to various intensities of sunlight. The amount of sunlight falling within the duration of the exposure, was calculated from readings on a Robitzsch type actinogram.

The 'quality' of the oil before and after treatment was estimated by its free fatty acid (FFA) values and colour.

2.1.4. *Penetration of sunlight into thick layers of oil*

Coconut oil layers of 30 cm thickness in glass cylinders with the sides completely covered with black paper, were exposed to direct sunlight so that the light fell through the top of the layer of oil. Samples withdrawn from the top, middle and bottom were assayed for aflatoxin.

2.1.5. *Effect of dry heat :*

Samples of oil were (*a*) maintained at 100°C or (*b*) heated to 300°C until they showed discolouration.

2.1.6. *Effect of steam.*

Steam was passed into coconut oil maintained at 100°C in a boiling water bath. Any water of condensation was accounted for in the assay.

2.2. Poonac

2.2.1. *Effect of sunlight.*

Powdered samples of poonac were exposed to direct sunlight in the form of a layer ½ cm in thickness, for varying periods.

2.2.2. *Methods of assay*

All samples were extracted by the aqueous acetone procedure[18] and aflatoxins were estimated on thin layer chromatograms (silica gel 'G Merck') with standards of aflatoxins B_1 and G_1 in chloroform run in methanol : chloroform (3 : 97) and acetone : chloroform (1 : 9). Samples were prepared and assayed in duplicate and mean values from typical experiments are presented.

2.3. Fungal strain and inocula

Samples of grated, fresh coconut were inoculated with *A. parasiticus* NRRL 2999 spores from a 2 week old culture on potato dextrose agar ('Difco') suspended in 0.1 % 'Tween 80'. Cultures were shaken manually on alternate days and were steamed on the 7th day before extraction of oil.

3. Results

3.1. Oil

Of the four sets of coconut oil mixtures stored under different conditions only the samples exposed to diffuse sunlight at room temperature, showed a drop in aflatoxin levels to below 0.03 ppm (Figure I). The aflatoxin levels in the other samples showed no significant change.

On exposure of replicates of the same mixtures to direct sunlight in the form of layers of 1 cm and 4 cm in thickness, for different periods, a drop in aflatoxin levels was observed after 1 h to 2 h exposure at midday. Very low aflatoxin values were recorded after exposure to about 100 calories/square cm (cal/cm^2) of sunlight (Figure II).

With naturally contaminated commercial coconut oil samples containing about 0.1 ppm of aflatoxin B_1, degradation of aflatoxin to levels below 0.03 ppm was possible on exposure to 40 cal/cm^2 of sunlight in the form of a layer 4 cm in thickness (Figure III).

In contrast to thin layers, thicker (30 cm) layers of oil showed no reduction in aflatoxin levels, after exposure to sunlight for similar periods.

No changes in aflatoxin levels were observed in coconut oil subjected to dry heat or steam at 100°C (Table 1). Although there was degradation of aflatoxin on dry heating at 300°C, the loss was partial and the treated oil yet contained aflatoxin above permissible levels (Table 2).

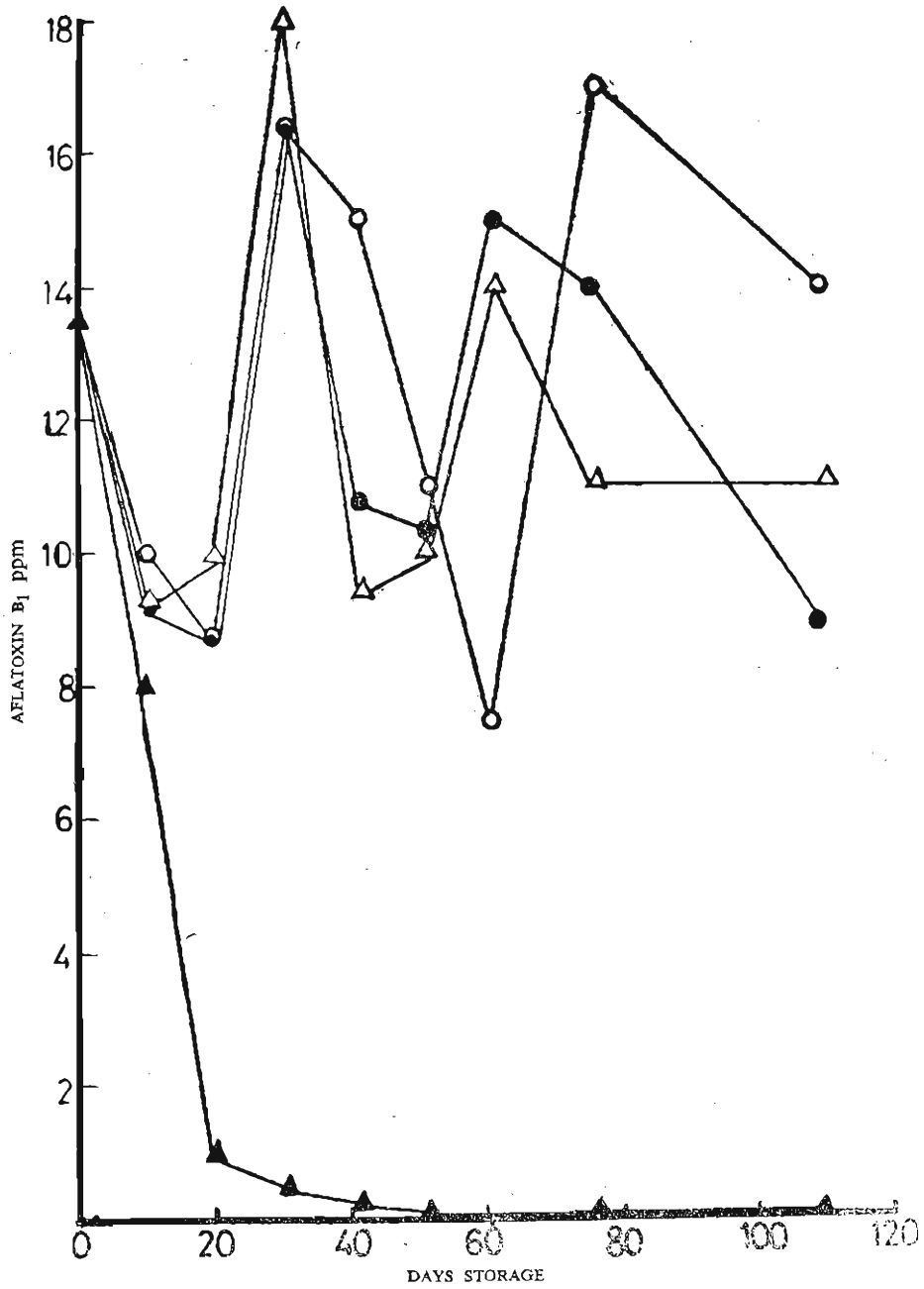

Figure I. Effect of storage at —85°C in the dark ● — ● ; storage at 25°C ± 2°C in the dark o—o; storage at 25°C ± 2°C in the dark after heating at 70°C for ½ hr Δ—Δ; and storage at 25°C ± 2°C in diffuse sunlight ▲—▲; on the aflatoxin B_1 content of experimentally contaminated coconut oil. Aflatoxin content in parts per million (ppm).

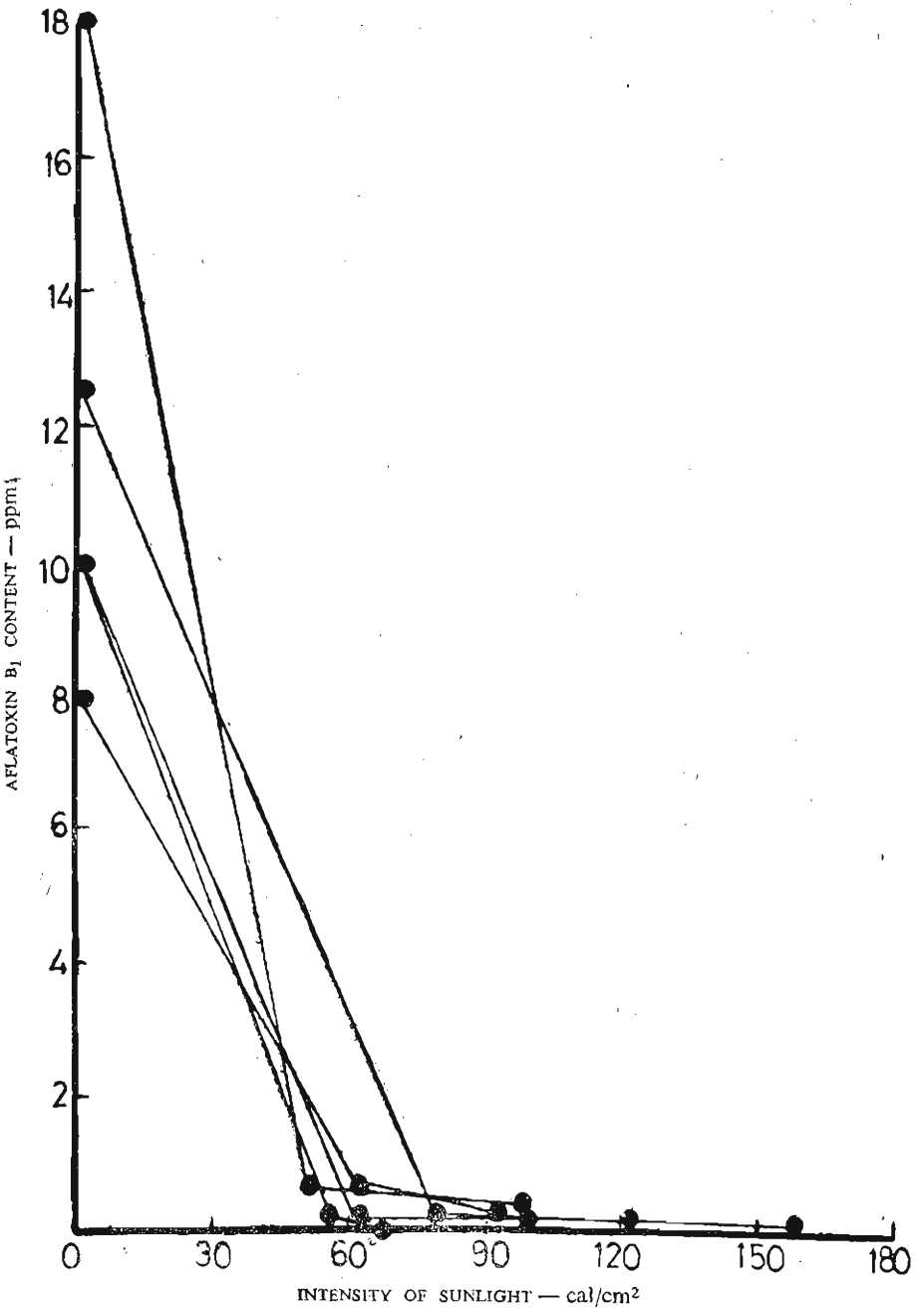

Figure II. Effect of exposure to sunlight, on the aflatoxin B_1 content of experimentally contaminated coconut oil containing high levels of toxin. ● — ● = replicate samples.

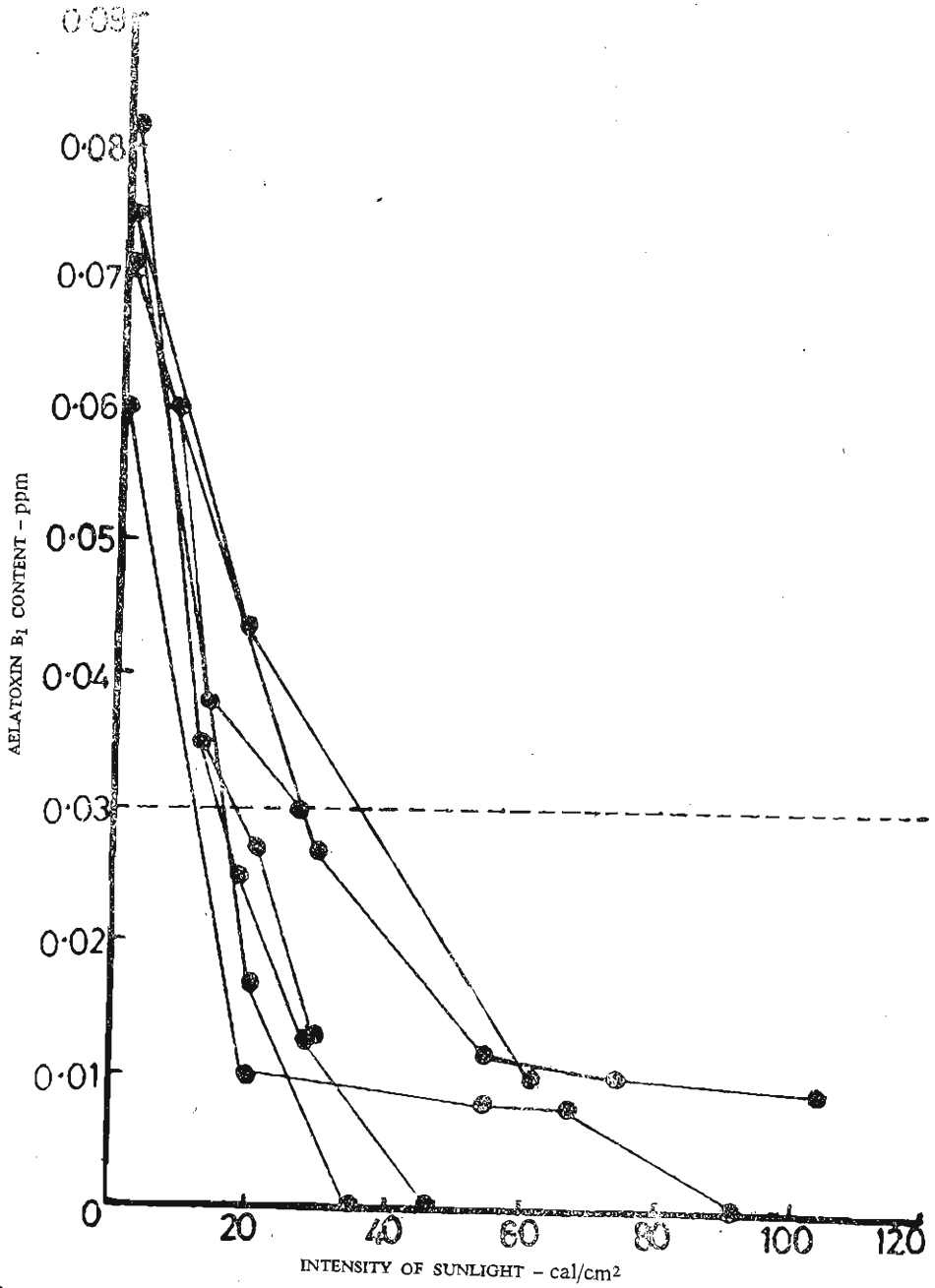

Figure III. Effect of exposure to sunlight, on the aflatoxin B_1 content of commercial coconut oil containing low levels of toxin. ●—● = replicate samples.

TABLE 1. The effect of dry heat at 100° C and steam at 100°C on the content of aflatoxin in experimentally contaminated coconut oil. Duration of heating 1h.

Experiment number	*Aflatoxin B_1 (ppm)*			*Aflatoxin G_1 (ppm)*		
	before treatment	*dry heated*	*steam heated*	*before treatment*	*dry heated*	*steam heated*
1	5.0	4.4	2.5	1.0	0.9	0.9
2	1.3	1.0	1.3	0.8	1.0	1.0
3	0.9	0.9	0.7	0.2	0.1	0.1

TABLE 2. The effect of dry heat on the content of aflatoxins in coconut oil.

Treatment	*aflatoxin content (ppm)*			
	B_1		G_1	
	before treatment	*after treatment*	*before treatment*	*after treatment*
10 min at 180°C to 215°C	8.5	5.0	4.3	1.5
10 min at 300°C	0.20	0.06	0.05	0.03

After photodegradation of aflatoxins, no new fluorescent compounds were observed in oil which was almost totally detoxified. On two occasions when partial decontamination occurred, a reddish purple compound of the same R_f as aflatoxin B_1 together with a violet blue compound of a slightly higher R_f were seen on TLC run in methanol : chloroform.

3.2. Poonac

No significant changes in aflatoxin levels were seen on exposure of powdered poonac to direct sunlight for upto $3\frac{1}{2}$ h, in a layer of 5 mm thickness.

4. Discussion

Of the various laboratory methods described in the literature for the degradation of aflatoxins in agricultural products, only chemical treatment appears to have been successful. However, the disadvantages of these methods are (1) the removal or conversion into non-availablity of essential components in foods, for example, lysine,[20] (2) chemical reactions leading to the formation of secondary toxic products.[11] There is no literature on the successful adaptation of any of these methods, on an industrial scale for the detoxification of contaminated agricultural products.

Although aflatoxins have been the subject of very extensive research and in spite of the fact that photodegradation of aflatoxin was known since their discovery, no work has, as far as we are aware, been done to utilise natural photodegradation as the basis of a detoxification method for aflatoxins in oil. This is probably due to the fact that most of this research has been done in temperate countries where sunlight is of low intensity and is not uniform throughout the year. On the other hand, the ready availability of chemicals and technology may have diverted research interests to the use of chemicals in detoxification of aflatoxins. Under tropical conditions, the sunlight falling during the day is uniform and of high intensity over a large part of the year. A method which uses sunlight would be inexpensive and practicable with minimal technical complexity, features which would be of advantage in poor, tropical countries such as ours.

Our results suggest that the decontamination of coconut oil was due to photoactivity rather than to aflatoxin degrading enzymes. Aflatoxin is well known to be degraded by ultraviolet light, and it may be this component in sunlight that was responsible for the degradation of aflatoxin in the oil. The direct heating effect of sunlight, causing a rise of temperature by about 10°C to 15°C was probably a negligible factor in this degradation, since even much higher degrees of dry heat were ineffective.

The absence of a change in the FFA content and colour of the exposed oil indicates that exposure to sunlight could be an acceptable method in industrial practice. In the application of this method under field conditions, the amount of sunlight available throughout the year and the penetration of light into thick layers of oil in tanks are important factors. Our results indicate that a sunlight intensity of approximately 60 cal/cm^2 was sufficient to bring down the aflatoxin levels to below 0.03 ppm (the maximum permissible level for aflatoxins in human foods proposed by the Protein Advisory Group of the WHO/FAO/UNICEF). The sunlight data recorded for the year 1973 at Peradeniya is shown in Table 3.

TABLE 3. Solar radiation data for Peradeniya in 1973.

	cal/cm^2
mean per day	353.7
range per day	92*b*—532
mean values recorded at different hours of the daytime.	
8.00 am — 9.00 am	40
9.00 am — 10.00 am	50
10.00 am — 11.00 am	60
11.00 am — 12 noon	90
12 noon — 1.00 pm	100
1.00 pm — 2.00 pm	80

b = on a rainy day.

Most of the coconut mills in Sri Lanka, are situated in the Negombo and Colombo districts which experience less rainfall than Peradeniya. Hence the duration of sunlight in these mills will be greater than in Peradeniya. Thus, it may be possible to make use of sunlight for the detoxification of aflatoxin in coconut oil under industrial conditions in the mills in these areas.

Since oil is stored in overhead tanks for several days after processing, it may be convenient to use a system which permits the oil to flow under gravity into storage tanks, along narrow transparent glass pipes or in shallow layers in trays, while being exposed to sunlight, for about one hour in transit. This method will involve only the initial cost of setting up the flow system. Further work will however be necessary for the adaptation of this method under field conditions.

Although no fluorescent products were detected after photodegradation of aflatoxins in coconut oil, further work will be needed on the toxicity of the treated oils, since the properties of photodegraded oils have not hitherto been investigated.

Since coconut oil is used for the frying of foods it was of interest to know of the fate of aflatoxins in oil at high temperatures. The stability of aflatoxins in oil at 100°C with only partial degradation even at 300°C, at which temperature the oil becomes discoloured, indicated that contaminated oil is unsuitable for cooking even at high temperatures.

With poonac particles, efficient degradation of aflatoxins was not achieved by exposure to sunlight ; this was probably because the light did not penetrate the particles. Exposure to ultraviolet light was also found to be unsuccessful in reducing the toxicity of aflatoxin contaminated peanut meal.[11] Methods involving autoclaving were not attempted since the food values of such heated products may have been altered by the heat treatment, as has been described of groundnuts.[20] However, the efficiency of steam treatment with powdered copra as practised in certain mills, before oil extraction, merits study.

Further experiments in the field and at industrial oil processing mills will be needed to define the exact conditions under which degradation of aflatoxin in oil could be achieved, in industrial practice.

Acknowledgements

We wish to thank the Coconut Processing Board, the Coconut Research Board and the National Science Council of Sri Lanka, for research grants. Our thanks are also due to the climatology division of the Central Agricultural Research Institute, Gannoruwa, for providing the solar radiation data and to Dr. R.A. Kulatunga of the Coconut Processing Board, for the assessment of the quality of the oil samples.

References

1. AIBARA, K. & YAMAGISHI, S. (1968). In *Proc. of the 1st US-Japan Conference on Toxic Micro-organisms, Honolulu, Hawaii*, US Dept. of the Interior and UJNR panels on Toxic Micro-organisms, Washington. pp. 211-221.
2. ARSECULERATNE S. N. & DE SILVA, L. M. (1971). *Cey. J. med. Sci.*, **20** : 60.
3. ARSECULERATNE, S. N., SAMARAJEEWA, U. & WELIANGA, L. V. (1976), *J. appl. Bact.*, **41**: 223-233..
4. ASHWORTH, L. J. (Jr.), MCMEANS, J. L., PYLE, J. L., BROWN, C. M., OSGOOD, J. W. & PONTON R. E. (1968), *Phytopathology*, **58** : 102-107.
5. BLOUNT, W. P. (1961) *Turkeys (J. Brit. Turkey Federation)*, **9** : 55-58.
6. CIEGLER, A., LILLEHOJ, E. B., PETERSON, R. E. & HALL, H. H. (1966). *Appl. Microbiol.*, **14**: 934-939.
7. COOMES, T. J., CROWTHER, P. C., FENNELL, A. J. & FRANCIS, B.J., (1966), *Nature. Lond.* **209**: 406-407.
8. DETROY, R. W., LILLEHOJ, E. B., & CIEGLER, A. (1971) In *Microbial Toxins*, Ed. Ciegler A., KADIS, S. & AJL, S. J., New York, Acad. Press, p. 33.
9. DOLLEAR, F. G. (1969), In *Aflatoxin*, Ed. Goldblatt, L. A., New York, Acad. Press, p. 360.
10. DOLLEAR, F. G. & GARDNER, H. K. (1966). *Proc. 4th Nat. Pea. Res. Conf.*, Tifton, Georgia, July 14-15, pp. 72-81.
11. FEUELL, A. J., (1966). *Trop. Sci.*, **8** : 61.
12. FRANK, H. K. & GRUNEWALD, J. H. (1971) *Food Irradiation*, **11** : 15.
13. GOLDBLATT, L. A. (1968), *Econ. Bot.* **22** : 51-62.
14. KRISHNAMACHARI, K. A. V. R., BHAT, R. V., NAGARAJAN, V. & TILAK, T. B. G., (1975) *Lancet*, **1**: 1060.
15. LEE, L. S., CUCULLU, A. F., & GOLDBLATT, L. A. (1968), *Food Technol.*, **22**: 1131-1134.
16. MANN, G. E., CODIFER, L. P. (Jr) & GARDNER, H. K. (1970), *J. Amer. Oil Chem. Soc. m* **47** : 173.
17. MIYAKE, K., AIBARA, K. & MIURA, T. (1967), *Rept. Panel Microbiol. Probl. Food Preserv. Irradiat.*, Vienna, 1966., IAEA, Vienna, p. 57.
18. PONS, W. A. Jr., CUCULLU, A. F., LEE, L. S., FRANZ, A. O. & GOLDBLATT, L. A. (1966). *J. Assoc. Offic. Anal. Chemists*, **49**: 554-562.
19. SAMARAJEEWA, U., ARSECULERATNE, S. N. & TENNEKOON, G. E. (1975), *Res. vet. Sci.*, **19** (3): 269-277.
20. WOODHAM, A. A. & DAWSON, R. (1966). *Proc. Nutr. Soc., (Engl., Scot)*. **25** (8).