Food Chemistry **46** (1993) 37–42

Effect of pH and heat treatment on the mutagenic activity of peanut beverage contaminated with aflatoxin B_1

Ismail Y. S. Rustom,[a] Miguel H. López-Leiva[a] & Baboo M. Nair[b]

[a] *Department of Food Engineering.* [b] *Department of Applied Nutrition, Chemical Center, University of Lund, PO Box 124, S- 221 00 Lund, Sweden*

(Received 4 March 1992; accepted 12 March 1992)

The effect of pH and heat treatment on the mutagenic activity (assayed by Ames test) of peanut beverage contaminated with aflatoxin B_1 (15, 30 and 45 μg/kg) was studied. Heating the beverage at 130°C for 20 s, 140°C for 5 s and 121°C for 15 min at pH 8·0 did not significantly ($p < 0.001$) reduce the mutagenic activity. Heating the beverage at 130°C for 20 s and 121°C for 15 min at pH 10·2, however, reduced the mutagenic activity by 73·3–83·0% (79·4 ± 5·3%) and 82·5–92·5% (86·6 ± 5·2%) respectively. Also heating the beverage at 130°C for 20 s and 121°C for 15 min at pH 5·0 reduced the mutagenic activity by 72·5–80·4% (75·2 ± 4·5%) and 70·4–74·7% (71·9 ± 2·4%) respectively. Changing the pH of the beverage from 8·0 to 10·2 or 5·0, without heating, did not significantly ($p < 0.001$) reduce the mutagenic activity.

INTRODUCTION

The problem of food and feed contamination with aflatoxins has received a great deal of attention during the last three decades as revealed by the large number of publications on various aspects of the subject (Dollear, 1969; Doyle *et al.*, 1982; Samarajeewa *et al.*, 1990).

Aflatoxins are secondary metabolites of the molds *Aspergillus flavus* and *A. parasiticus*. These molds are distributed world-wide in soil and air, and found to infest living or dead plants and animals (Diener & Davis, 1969).

Peanuts are infested by the molds in the field during and after harvest under poor storage conditions of temperature and relative humidity favorable for the growth of molds. Optimum conditions of growth for *A. flavus* and its production of aflatoxins is at 25–35°C and 85–95% relative humidity (Diener *et al.*, 1982).

Aflatoxins have been found to be involved in a number of human deaths in the Third World in connection with consumption of seriously contaminated foods. In 1974, consumption of corn contaminated with aflatoxin resulted in the deaths of 97 people in Western India (Tandon & Tandon, 1989). Aflatoxins are shown to be highly toxic, mutagenic and carcinogenic compounds with aflatoxin B_1 (AFB1) being the most potent and active one, followed by AFG1, AFB2 and AFG2 (Betina, 1989). The toxicity of aflatoxins was first recorded in England in 1960 following the outbreak of the turkey 'X' disease which resulted in the deaths of more than 100 000 turkeys. The cause of the disease was attributed to a feed, containing Brazilian peanut, which was heavily contaminated with *Aspergillus flavus*. Analysis of the feed revealed that a series of fluorescent compounds, later termed aflatoxins, were responsible for the outbreak (Sargent *et al.*, 1961). The toxicity of aflatoxins to experimental animals varies greatly depending on species and sex. Cytotoxic and immunosuppressive effects of aflatoxins have also been reported (Betina, 1989). Aflatoxins interfere with the synthesis of deoxyribonucleic acid (DNA) leading to development of mutations. AFB1 was reported to be highly mutagenic to strains of *Salmonella typhimurium* (Wong *et al.*, 1977; Matsushima *et al.*, 1980).

Epidemiological research in Africa and Asia revealed a strong positive correlation between the incidence of liver cancer and exposure to aflatoxins from contaminated foods (Peers & Linsell, 1973; Groopman *et al.*, 1988).

The contamination of peanuts with aflatoxins can be reduced, to some extent, by introducing good crop husbandry and suitable harvesting, drying and storage procedures. However, the contamination still remains a serious problem, and hence there is a need for an effective processing method for their inactivation.

Aflatoxins were partially removed from peanut meals by extraction with polar solvents (Dollear, 1969). Dry

Food Chemistry 0308-8146/92/$05.00

and oil roasting of contaminated peanut kernels under different conditions (121–204°C, 3–30 min) reduced the content of aflatoxins by 45–83% (Lee *et al.*, 1969). Cooking of unshelled peanuts in 5% NaCl solution at 116°C and 0·7 bar for 30 min resulted in a removal of aflatoxins ranging from 80% to non-detectable levels (Farah *et al.*, 1983). Roasting of contaminated peanuts in a microwave oven at 0·7 kW for 8·5 min destroyed 30–45% of AFB1 (Pluyer *et al.*, 1987). Exposure of peanut cake flour to sunlight in India for 6 h destroyed 50% of AFB1 added to the cake (Shantha & Murthy, 1981). A 14 h exposure to sunlight destroyed 90% and 77% of AFB1 added to full-fat and defatted peanut flakes respectively (Shantha *et al.*, 1986). Adsorption with bentonite at 30°C for 5 days removed 94–100% of AFB1 from a Sörensen buffer solution (Masimango *et al.*, 1978).

Ultra High Temperature (UHT) treatment is a technique used in the food industry to sterilize liquid food products. The product is heated at a temperature in the range of 130–150°C for a few seconds. Another sterilization treatment is to heat the product at 110–121°C for 10–40 min. In earlier investigations, we have studied and optimized the extraction process of peanuts with water for the production of a nutritious and safe peanut beverage (Rustom *et al.*, 1991*a*,*b*). One of the requirements concerning the nutritional quality and safety of food products is to be free from antinutritional factors especially those which possess mutagenic activity as aflatoxin. The purpose of the present work was to study the effect of pH and heat treatment, usually used for sterilization of foods, on the mutagenic activity of a peanut beverage contaminated with AFB1 at different concentrations.

MATERIALS AND METHODS

Peanuts

Raw whole peanuts (*Arachis hypogaea* L.), imported from Argentina and purchased from the local market in Lund, Sweden, were used for the preparation of peanut beverage.

Aflatoxin B_1 (AFB1)

AFB1 (A 6636) was obtained from Sigma Chemicals Co., St Louis, MO, USA. A solution of AFB1 was prepared by dissolving 10 mg AFB1 in 10 ml 99·5% ethanol. The required volume of this solution was added to 20 ml portions of peanut beverage to give concentrations of 15, 30, 45, 60 and 90 μg AFB1/kg beverage.

Preparation of the peanut beverage

Sound peanut kernels, after removal of their skin (testa), were ground twice to produce a fine meal using a kitchen meat mincer (Braun AG, Braun, Frankfurt, Germany). The meal (20 g) was extracted with 160 ml distilled water at pH 8·0 (adjusted with 1 M NaOH) for 30 min. The temperature of the mixture was kept constant at 50°C using a water bath (Julabo Paratherm II, Juchheim Labortechnik, Schwarzwald, Germany). The mixture was stirred continuously with a mechanical stirrer. The mixture was then filtered through a piece of muslin cloth, followed by filtration through a Munktell filter paper (no. 3). The filtrate was homogenized (Ultra-Turax, type TP 18/10, driven at 10 000 (rpm) for 5 min in order to obtain a representative sample.

Heat treatments

Ten different treatments, replicated twice, were carried out at each concentration of AFB1 (15, 30, 45, 60 and 90 μg/kg) in the contaminated beverage. The treatments are numbered consecutively with Arabic numerals from 1 to 10. Table 1 shows the values of pH of the beverage, temperature and time in each treatment. The pH was adjusted with either 1M NaOH or 1M HCl. In treatment 1 (control), treatment 9 and treatment 10, the contaminated beverage was not heated. In treatment 1, the pH of the beverage was maintained constant at 8·0. In treatment 9 and treatment 10, the pH was kept constant at 10·2 and 5·0, respectively, at room temperature (23 ± 2°C) for 4 h and thereafter adjusted to 8·0.

Treatment 2, treatment 3, treatment 5 and treatment 7 were UHT treatments in which 0·1 ml of the contaminated beverage was sealed in a disposable glass micro pipette (Blaubrand 100 μl, Brand, Wertheim, Germany). The sealed beverage was heated at the indicated temperature (Table 1) in an oil bath with an accuracy of ±0·5°C (Grant, Grant Instruments, Cambridge, UK), followed by immediate cooling in ice water.

In treatment 4, treatment 6 and treatment 8, the contaminated beverage (2 ml) was autoclaved (CERTO-Clav CVII/1600, Traun, Austria) in 10 ml glass tubes at 121°C and 1·4 bar for 15 min, followed by immediate cooling in ice water. In treatment 5 to treatment 8, the pH of the heated beverage was re-adjusted to 8·0 immediately after cooling. The treated beverages were stored at −18°C for further analysis.

Table 1. pH, temperature and time for the treatments

Treatment	pH	Temperature (°C)	Time
1	8·0	—	—
2	8·0	130	20 (s)
3	8·0	140	5 (s)
4	8·0	121	15 (min)
5	10·2	130	20 (s)
6	10·2	121	15 (min)
7	5·0	130	20 (s)
8	5·0	121	15 (min)
9	10·2	—	—
10	5·0	—	—

Salmonella mutagenicity test

The Ames Salmonella mutagenicity test with metabolic activation (Maron & Ames, 1983) was used for the assay of the mutagenic activity of the beverages. Histidine-requiring, base-pair mutant tester strain TA100 of *Salmonella typhimurium* was a gift from Professor Bruce Ames, University of California, Berkeley, USA to the Department of Food Chemistry, University of Lund, Lund Sweden. Cultures of TA100 grown in Oxoid nutrient broth (No. 2) at 37°C for 16 h in a gyrorotary incubator (Stålprodukter, Uppsala, Sweden) were used throughout. The rat liver homogenate (S9 fraction) was prepared according to the procedure described by Maron and Ames (1983). The liver was obtained from male Sprague-Dawley rats (200 g body weight) induced with polychlorinated biphenyl mixture, Aroclor 1254. Fresh S9 mix (5% by volume) was prepared according to Maron and Ames (1983) for each run of Ames test.

In each plate, 0·1 ml of the test sample (containing 0, 15, 30, 45, 60 or 90 μg AFB1/kg beverage) was inoculated with 0·1 ml TA100 culture, 0·5 ml S9 mix and 2 ml top agar. The plates were incubated at 37°C for 48 h. For each treatment, three replicate plates were tested at each concentration of AFB1. Revertant colonies were counted using an automatic colony counter (Biotran II, New Brunswick Scientific Co., Edison, NJ, USA). The mutagenic activity was expressed as the number of revertant colonies per plate.

The plates containing 0 μg AFB1/kg beverage (i.e., uncontaminated beverage) were used to determine the number of spontaneous revertants.

Statistical analysis

Parametric statistical evaluation of the results was done by one-way analysis of variance (ANOVA) using STATGRAPHICS software. Pair-wise multiple comparison tests were performed adopting Scheffe's test at 95% confidence level.

RESULTS AND DISCUSSION

Figures 1–4 show the effect of different treatments on the mutagenic activity of the beverage containing aflatoxin B_1 (AFB1) at different concentrations. In general, the mutagenic activity increased when the concentration of AFB1 in the beverage was increased. This proportionality continued until a concentration of 45 μg AFB1/kg beverage was reached, after which the mutagenic activity was observed to decrease with increased concentration of AFB1. The observed decrease in the mutagenic activity, as indicated by a decrease in the number of revertant colonies per plate, was attributed to massive death of the bacteria due to intoxication by AFB1 at concentrations above 45 μg/kg. Maron and Ames (1983) reported that most mutagens are toxic to the bacteria tester strains at some concentration. Therefore,

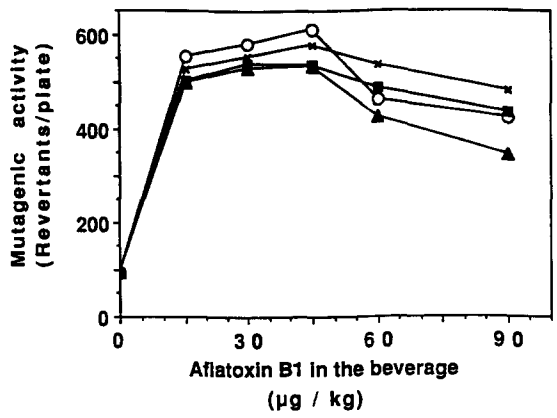

Fig. 1. Effect of pH and heat treatment on the mutagenic activity of peanut beverage contaminated with aflatoxin B_1. The mutagenic activity was assayed by Ames test in the presence of S9. Samples were heated at pH = 8·0 (adjusted with 1M NaOH). Heat treatments were replicated twice, and 3 replicate plates of each sample were assayed in Ames test. —○—, Control, no heat treatment; —▲—, 130°C for 20 s; —■—, 140°C for 5 s; —×—, 121°C for 15 min.

the number of revertant colonies per plate obtained at a concentration of AFB1 higher than 45 μg/kg was not considered since it might give a misleading measure of the mutagenic activity and hence a wrong interpretation of the results. The number of spontaneous revertants ranged from 96 to 104 (100 ± 3); therefore the point (0,100) represented the spontaneous reversion of the tester strain to histidine independence.

In connection with the assessment of the mutagenic activity of substances in Ames test, Ames *et al.* (1975) recommend that a test compound should be evaluated as a positive mutagen when it induces a number of revertants that exceeds twice the number of spontaneous revertants. Accordingly, the treatment which reduced the number of revertants/plate, at a given concentration of AFB1, to 200 or less could be considered effective enough in reducing the mutagenic activity of the beverage to safe levels.

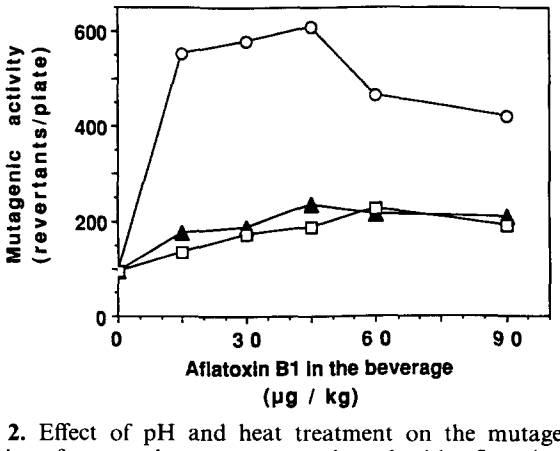

Fig. 2. Effect of pH and heat treatment on the mutagenic activity of peanut beverage contaminated with aflatoxin B_1. The mutagenic activity was assayed by Ames test in the presence of S9. Samples were heated at pH = 10·2 (adjusted with 1M NaOH). Heat treatments were replicated twice, and 3 replicate plates of each samples were assayed in Ames test. —○—, Control, no heat treatment; —▲—, 130°C for 20s; —□—, 121°C for 15 min.

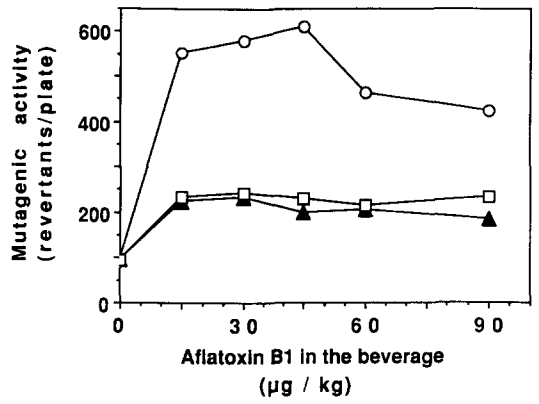

Fig. 3. Effect of pH and heat treatment on the mutagenic activity of peanut beverage contaminated with aflatoxin B_1. The mutagenic activity was assayed by Ames test in the presence of S9. Samples were heated at pH = 5·0 (adjusted with 1M HCl). Heat treatments were replicated twice, and 3 replicate plates of each sample were assayed in Ames test. —○—, Control, no heat treatment; —▲—, 130°C for 20 s; —□—, 121°C for 15 min.

Table 2. Comparison among effects of the treatments on mutagenic activity of aflatoxin B_1 in peanut beverage[a]

Treatment	Mutagenic activity (revertants/plate)[b]		
	Aflatoxin B_1 (µg/kg beverage)		
	15	30	45
1	552±25a	580±11a	610±4d
2	499±12a	528±19a	532±4c
3	508±20a	539±8a	534±6c
4	527±11a	533±17a	576±11cd
5	177±20b	187±12b	236±5ab
6	133±8b	173±12b	189±6a
7	223±11b	232±7b	200±8ab
8	233±18b	222±22b	229±4b
9	461±20ac	495±19a	521±24c
10	376±12c	526±21a	535±4c

[a] Means in the same column followed by the same letter are not significantly different by Scheffe's test at 95% confidence level.
[b] Values are the means ± SE, n=6.

Table 2 lists the results of the multiple comparison tests. The statistical analysis of the results of the mutagenic activity using parametric ANOVA was valid in this experiment because data populations of the mutagenic activity were almost normally distributed and had constant variance, two requirements for the validation of application of parametric statistical analysis (Klienbaum *et al.*, 1988).

Heat treatments of the beverage at pH 8·0 were not effective in reducing the mutagenic activity of the beverage (Fig. 1). The number of revertants per plate at all concentrations of AFB1, obtained after these treatments, were not significantly different ($p < 0·001$) from those obtained in the control where the beverage was not heated. Also the multiple comparison tests (Table 2) revealed non-significant ($p < 0·001$) differences among the effects of these treatments on the mutagenic activity in the beverage at AFB1 concentration of 15, 30 and 45 µg/kg.

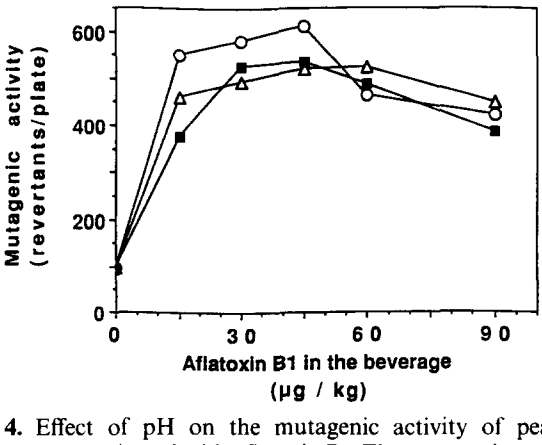

Fig. 4. Effect of pH on the mutagenic activity of peanut beverage contaminated with aflatoxin B_1. The mutagenic activity was assayed by Ames test in the presence of S9. The pH of the samples was kept constant (at 5·0 or 10·2) at 23°C ± 2°C for 4 h followed by adjustment to 8·0. Treatments were replicated twice, and 3 replicate plates of each sample were assayed in Ames test. —○—, pH = 8·0; —Δ—, pH = 10·2; —■—, pH = 5·0.

AFB1 has two reactive sites for mutagenic and toxicological activity. The first site is at the double bond of the terminal furan ring. The adducts formed by AFB1-DNA and AFB1-protein at this site alter the biochemical functions of these macromolecules leading to impairment of the necessary biochemical activities in the living cell. The second site is the lactone ring (Betina, 1989). Therefore, an effective treatment for inactivation of the toxin could be aimed at either opening the double bond in the furan ring, or cleaving the lactone ring.

Figure 2 shows that treatment 5 (pH 10·2, 130°C, 20 s) and treatment 6 (pH 10·2, 121°C, 15 min) reduced the mutagenic activity of the beverage significantly ($p < 0·001$). The mutagenic activity was reduced by 73·3–83·0% (79·4 ± 5·3%) and 82·5–92·5% (86·6 ± 5·2%) in treatment 5 and treatment 6 respectively. The beverage processed by treatment 5 was 3·8–5·9 (5·1 ± 1·1) times less mutagenic, and that processed by treatment 6 was 5·7–13·3 (8·5 ± 4·2) times less mutagenic than the unheated beverage in the control (treatment 1). Treatment 5 and treatment 6 resulted in a similar ($p < 0·001$) reduction in the mutagenic activity at AFB1 concentrations of 15, 30 and 45 µg/kg, as revealed by the result of the multiple comparison tests (Table 2). These concentrations of AFB1 were far above the tolerance level of 5 µg AFB1 in a kg of foodstuff assigned by many countries (Van Egmond, 1989). Both treatments reduced the mutagenic activity of the beverage at AFB1 concentration of 15 and 30 µg/kg to safe levels, since the number of revertants per plate obtained after treating the beverages did not exceed twice the number of spontaneous revertants (Ames *et al.*, 1975). However, at AFB1 concentration of 45 µg/kg only treatment 6 reduced the mutagenic activity to a safe level while treatment 5 resulted in a number of revertants per plate (236) which slightly exceeded twice the number of the spontaneous revertants. Treatment with aqueous solutions of alkali such as NaOH, NH_4OH and $Ca(OH)_2$ significantly inactivated aflatoxin in water (Draughon

& Childs, 1982), a corn snack product (Camou-Arriola & Price, 1989) and coconut meal (Mercado *et al.*, 1991). Moreover, it has been reported that alkalies catalyse the conversion of AFB1 to AFD1 through hydrolysis of the lactone ring in AFB1 (Coomes *et al.*, 1966; Lee & Cucullu, 1978). Therefore, the inactivation caused by treatment 5 and treatment 6 in this experiment may be attributed to a partial conversion of AFB1 to AFD1, catalyzed by NaOH used to adjust the pH of the beverage to 10·2. Lee *et al.* (1981) found AFD1 to be 450 times less mutagenic than AFB1 in Ames test.

Treatment 7 (pH 5·0, 130°C, 20 s) and treatment 8 (pH 5·0, 121°C, 15 min) reduced the mutagenic activity by 72·5–80·4% (75·2 ± 4·5%) and 70·4–74·7% (71·9 ± 2·4%), respectively (Fig. 3). According to the result of the multiple comparison tests (Table 2), treatment 7 and treatment 8 resulted in a similar ($p < 0.001$) reduction in the mutagenic activity at AFB1 concentrations of 15, 30 and 45 μg/kg. The beverage processed by treatment 7 was 3·6–5·1 (4·1 ± 0·8) times less mutagenic, and that processed by treatment 8 was 3·4–4·0 (3·6 ± 0·3) times less mutagenic than the unheated beverage in the control (treatment 1). At AFB1 concentrations of 15, 30 and 45 μg/kg, the number of revertants per plate obtained after each of the two treatments was slightly greater than twice the number of the spontaneous revertants. AFB1 and AFG1 were completely destroyed during the production of hydrolyzed vegetable protein from defatted peanut meal hydrolyzed with 3 M HCl at 120°C for 9 h (Williams & Dutton, 1988). Strong acids have been reported to catalyze the hydration of AFB1 at the terminal furan ring to yield AFB2a (Ciegler & Peterson, 1968; Pohland *et al.*, 1968; Pons *et al.*, 1972). Therefore, the inactivation caused by treatment 7 and treatment 8 may be attributed to partial conversion of AFB1 to AFB2a, catalyzed by HCl used to adjust the pH of the beverage to 5·0. AFB2a was shown to be 1000 times less mutagenic than AFB1 (Wong & Hsieh, 1976).

Changing the pH of the beverage from 8·0 to 5·0 in treatment 7 and treatment 8 resulted in coagulation of peanut proteins in the beverage. These protein coagula were not soluble even after neutralizing the pH of the beverage to 8·0.

Figure 4 shows the effect of changing the pH of the beverage from 8·0 to either 10·2 (treatment 9) or 5·0 (treatment 10) at 23°C ± 2°C on the mutagenic activity of the beverage. These treatments did not significantly ($p < 0.001$) reduce the mutagenic activity as compared with the control (treatment 1) in which the pH was held constant at 8·0. This observation emphasized the role of heat in reducing the mutagenic activity in beverages processed by treatment 5, treatment 6, treatment 7 and treatment 8.

Aflatoxins are sensitive to moist-heat, and the moisture content of a contaminated food is a critical factor for the destruction of the toxins by heat. The rate of degradation of aflatoxins increases with an increase in moisture content of the heated food. Mann *et al.* (1967) observed that heating a cottonseed meal containing 30% moisture at 100°C for 1 h degraded 74·8% of aflatoxins ($B_1 + B_2$) in the meal, whereas, only 32·7% of the toxins were destroyed after heating a meal containing 6·6% moisture under the same conditions. Therefore, in addition to the effect of the heat treatment at pH 5·0 and pH 10·2, the high moisture content of the beverage (93%) might also have contributed to the reduction in the mutagenic activity observed in beverages processed by treatment 5, treatment 6, treatment 7 and treatment 8.

About 85% of known or suspected carcinogens manifested themselves as mutagens in Ames test (Ames & McCann, 1981). Therefore, the reduction in the mutagenic activity achieved will probably be reflected in reduced carcinogenicity in beverages heated following treatment 5, treatment 6, treatment 7 and treatment 8.

CONCLUSIONS

Among the various methods of heat treatment studied in this work, treatment 5 (pH 10·2, 130°C, 20 s) appears to be promising and suitable for reduction of the mutagenic activity of the beverage contaminated with AFB1 to a safe level.

The effect of the treatments on the mutagenic activity of samples of the beverage contaminated with AFB1 to a level higher than 45 μg/kg was not studied as the Ames test failed to provide accurate results due to the direct toxic effect of aflatoxin on the bacteria.

In addition to the effectiveness in reducing the mutagenic activity, treatment 5 had no adverse effect on texture, color and flavor of the beverage in comparison with those treatments in which the beverage was autoclaved at 121°C for 15 min. The autoclaved beverage developed a pronounced cooked flavour which is an undesirable property.

ACKNOWLEDGEMENTS

The authors wish to thank Kerstin Skog, Department of Food Chemistry, Chemical Center, University of Lund, Lund, Sweden, for help with the Ames test, and Inger Lindwall, Department of Food Engineering, Chemical Center, University of Lund, Lund, Sweden, for technical assistance.

REFERENCES

Ames, B. N. & McCann, J. (1981). Validation of the Salmonella test. *Cancer Research*, **41**, 4192–6.

Ames, B. N., McCann, J. & Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutation Research*, **31**, 347–63.

Betina, V. (1989). Mycotoxins: Chemical, biological and environmental aspects. In *Bioactive Molecules*, vol. 9 ed. V. Betina. Elsevier Science Publishers, Amsterdam, pp. 42–150.

Camou-Arriola, J. P. & Price, R. L. (1989). Destruction of aflatoxin and reduction of mutagenicity of naturally-

contaminated corn during production of a corn snack. *J. Food Protect.*, **52**, 814–17.

Ciegler, A. & Peterson, R. E. (1968). Aflatoxin detoxification: hydroxydihydro-aflatoxin B_1. *Appl. Microbiol.*, **16**, 665–6.

Coomes, T. J., Crowther, P. C., Feuell, A. J. & Francis, B. J. (1966). Experimental detoxification of groundnut meals containing aflatoxin. *Nature*, **209**, 406–7.

Diener, U. L. & Davis, N. D. (1969). Aflatoxin formation by *Aspergillus flavus*. In *Aflatoxin*, ed. L. A. Goldblatt. Academic Press, New York, pp. 13–54.

Diener, U. L., Pettit, R. E. & Cole, R. J. (1982). Aflatoxins and other mycotoxins in peanuts. In *Peanut Science and Technology*, ed. H. E. Pattee & C. T. Young. American Peanut Research and Education Society, Inc., Yoakum, Texas, chapter 13, pp. 486–519.

Dollear, F. G. (1969). Detoxification of aflatoxins in foods and feeds. In *Aflatoxin*, ed. L. A. Goldblatt. Academic Press, New York, pp. 359–91.

Doyle, M. P., Applebaum, R. S., Brackett, R. E. & Marth, E. H. (1982). Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. *J. Food Protect.*, **45**, 964–71.

Draughon, F. A. & Childs, E. A. (1982). Chemical and biological evaluation of aflatoxin after treatment with sodium hypochlorite, sodium hydroxide and ammonium hydroxide. *J. Food Protect.*, **45**, 703–6.

Farah, Z., Martins, M. J. R. & Bachmann, M. R. (1983). Removal of aflatoxin in raw unshelled peanuts by a traditional salt boiling process practised in the north east of Brazil. *Lebensmit Wiss u. Technol.*, **16**, 122–4.

Groopman, J. D., Cain, L. G. & Kensler, T. W. (1988). Aflatoxin exposure in human populations: measurements and relationship to cancer. *CRC Critical Reviews in Toxicology*, **19**, 113–45.

Kleinbaum, G. K., Kupper, L. L. & Muller, K. E. (1988). *Applied Regression Analysis and Other Multivariable Methods*, 2nd edn, PWS-KENT, Boston, USA, chapter 17, pp. 341–86.

Lee, L. S. & Cucullu, A. F. (1978). Conversion of aflatoxin B_1 to aflatoxin D_1 in ammoniated peanut and cottonseed meals. *J. Agric. Food Chem.*, **26**, 881–4.

Lee, L. S., Cucullu, A. F., Franz, A. O. & Pons, W. A., Jr (1969). Destruction of aflatoxins in peanuts during dry and oil roasting. *J. Agric. Food Chem.*, **17**, 451–3.

Lee, L. S., Dunn, J. J., DeLucca, A. J. & Ciegler, A. (1981). Role of lactone ring of aflatoxin B1 in toxicity and mutagenicity. *Experientia*, **37**, 16–17.

Mann, G. E., Codifer, L. P. & Dollear, F. G. (1967). Effect of heat on aflatoxins in oilseed meals. *J. Agric. Food Chem.*, **15**, 1090–2.

Maron, D. M. & Ames, B. N. (1983). Revised methods for the Salmonella mutagenicity test. *Mutation Research*, **113**, 173–215.

Masimango, N., Remacle, J. & Ramaut, J. L. (1978). The role of adsorption in the elimination of aflatoxin B_1 from contaminated media. *Europ. J. Appl. Microbiol. Biotechnol.*, **6**, 101–5.

Matsushima, T., Sugimura, T., Nagao, M., Yahagi, T., Shirai, A. & Sawamura, M. (1980). Factors modulating mutagenicity in microbial tests. In *Short-Term Test Systems for Detecting Carcinogens*, ed. K. H. Norpoth & R. C. Garner. Springer-Verlag, Berlin, pp. 273–85.

Mercado, C. J., Real, M. P. N. & Del Rosario, R. R. (1991). Chemical detoxification of aflatoxin-containing Copra. *J. Food. Sci.*, **56**, 733–5.

Peers, F. G. & Linsell, C. A. (1973). Dietary aflatoxins and liver cancer—a population based study in Kenya. *Brit. J. Cancer*, **27**, 473–84.

Pluyer, H. R., Ahmed, E. M. & Wei, C. I. (1987). Destruction of aflatoxin on peanuts by oven- and microwave-roasting. *J. Food Protect.*, **50**, 504–8.

Pohland, A. E., Cushmac, M. E. & Andrellos, P. J. (1968). Aflatoxin B_1 hemiacetal. *J. Assoc. Anal. Chem.*, **51**, 907–17.

Pons, W. A., Jr, Cucullu, A. F., Lee, L. S., Janssen, H. J. & Goldblatt, L. A. (1972). Kinetic study of acid-catalyzed conversion of aflatoxin B_1 and G_1 to B_{2a} and G_{2a}. *J. Amer. Oil Chem. Soc.*, **49**, 124–8.

Rustom, I. Y. S., López-Leiva, M. H. & Nair, B. M. (1991*a*). A study of factors affecting extraction of peanut (*Arachis hypogaea* L.) solids with water. *Food Chem.*, **42**, 153–65.

Rustom, I. Y. S., López-Leiva, M. H. & Nair, B. M. (1991*b*). Optimization of extraction of peanut proteins with water by response surface methodology. *J. Food Sci.*, **56**, 1660–3.

Samarajeewa, U., Sen, A. C., Cohen, M. D. & Wei, C. I. (1990). Detoxification of aflatoxins in foods and feeds by physical and chemical methods. *J. Food Protect.*, **53**, 489–501.

Sargent, K., Sheridan, A., O'Kelly, J. & Carnaghan, R. B. A. (1961). Toxicity associated with certain samples of groundnuts. *Nature*, **192**, 1095–7.

Shantha, T. & Murthy, V. S. (1981). Use of sunlight to partially detoxify groundnut cake flour and casein contaminated with aflatoxin B_1. *J. Assoc. Off. Anal. Chem.*, **64**, 291–3.

Shantha, T., Murthy, V. S., Rat, E. R. & Prema, V. (1986). Detoxification of groundnut seeds by urea and sunlight. *J. Food Safety*, **7**, 225–31.

Tandon, H. D. & Tandon, B. N. (1989). Pathology of liver in an outbreak of aflatoxicosis in man with a report on the follow up. In *Mycotoxins and Phycotoxins*, ed. S. Natori, K. Hashimoto & Y. Ueno. Elsevier Science Publishers, Amsterdam, pp. 99–107.

Van Egmond, H. P. (1989). Current situation on regulation for mycotoxins. In *Mycotoxins and Phycotoxins*, ed. S. Natori, K. Hashimoto & Y. Ueno. Elsevier Science Publishers, Amsterdam, pp. 249–56.

Williams, K. R. & Dutton, M. F. (1988). Destruction of aflatoxin during the production of hydrolysed vegetable protein. *J. Food Protect.*, **51**, 887–91.

Wong, J. J. & Hsieh, D. P. H. (1976). Mutagenicity of aflatoxins related to their metabolism and carcinogenic potential. *Proc. Nat. Acad. Sci., USA*, **73**, 2241–4.

Wong, J. J., Singh, R. & Hsieh, D. P. H. (1977). Mutagenicity of fungal metabolites related to aflatoxin biosynthesis. *Mutation Research*, **44**, 447–50.