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Use of Liquid Smoke to Prevent Aflatoxin Contamination of Coconut

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ABSTRACT. *Liquid smokes are used in the food industry to impart flavour, colour and preservative effects on foods. Smoke curing of coconut kernels is already reported to inhibit fungal growth and aflatoxin production. Effect of 'Zesti' liquid smoke (ZLS) on prevention of growth, and aflatoxin production by Aspergillus parasiticus NRRL 2999 on desiccated coconut and coconut-agar medium was examined under laboratory conditions.*

Desiccated coconut (20 g) in 500 ml conical flasks was steamed for 15 min and remoistened with ZLS at concentrations of 0.002-100% to get 20 and 30% final moisture content. Spores of Aspergillus parasiticus grown on potato-dextrose-agar slants for 10 days were suspended in 0.1% 'Tween 80', and 1 ml of this suspension was sprayed to each flask. ZLS concentrations of 0.1-100% were used to prepare Coconut-agar medium. Aspergillus parasiticus grown on potato-dextrose-agar slants was streaked on petri-dishes with coconut-agar medium. Incubation was done at 28°C in the dark and desiccated coconut samples were observed for fungal growth, colour changes and sporulation. Reverse of the petri-dishes was observed under UV light at 365 nm for violet-blue fluorescence.

'Zesti' liquid smoke suppressed the growth of Aspergillus parasiticus concentrations above 15% on desiccated coconut and above 3% in coconut-agar media.

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INTRODUCTION

Growth of toxigenic fungi on copra during storage is a problem of health and economic concern in coconut growing countries. Medium-high concentrations of aflatoxin B1 in coconut oil, poonac and copra have been reported in Sri Lanka (Samarajeewa and Arseculeratne, 1983) and other countries (Jones, 1976). The aflatoxins are secondary metabolites of *Aspergillus parasiticus/flavus* which grow readily on copra during storage at moisture concentrations above 12%.

Smoke curing of copra is reported to inhibit the fungal growth during storage probably due to some constituents in smoke (Arseculeratne *et al.*, 1976). It is not known whether the inhibitory effect is due to polycyclic aromatic hydrocarbons (PAH) deposited during smoking or due to other chemical constituents. Polycyclic aromatic hydrocarbons are carcinogenic and deposition of PAH may probably be as equally hazardous as aflatoxin contamination. The presence of PAH fluoranthene, Benzo(b) fluoranthene, Benzo(k) fluoranthene, Benzo(a) pyrene, Dibenzo(a,h) anthracene and Benzo(g,h,i) perylene are reported in copra, poonac and coconut oil. The reported mean concentration of total PAH in copra and coconut oil were 102 and 359 μg/kg, respectively. Almost all of sundried copra samples were free of PAH (Wijeratne, *et al.*, 1995). It is therefore important to find out methods to prevent fungal contamination avoiding use of smoke curing method, which could cause contamination by PAH.

Several liquid smoke preparations are available in the market for imparting acceptable flavours to foods. These liquid smokes are prepared by controlled combustion of wood and are reported to be free of PAH and safe as food additives. The application of liquid smoke could be done more uniformly than smoke curing. Some of the advantages associated with liquid smokes are their anti-oxidant activity, economical use, shorter processing time, easy cleaning up of smoke houses and reduced air pollution. Recent analysis showed absence of detectable concentrations of Benzo(a) pyrene in refined smoke solutions. Based on this analysis, the Council of Europe Committee of Experts on Flavouring Substances concluded that using smoke flavourings having a well known and safe composition, can minimize some of the health concerns that smoked foods can give rise to (Pszczola, 1995). United States Department of Agriculture and Food and Drugs Administration have approved 'Zesti' liquid smoke as a flavouring agent. In this study, ZLS was tested to examine its effect on preventing toxigenic fungal growth and aflatoxin production on desiccated coconut under laboratory conditions.

MATERIALS AND METHODS

Substrate

Desiccated coconut of fine grade was used as the substrate for growth of fungi and aflatoxin production in all experiments.

'Zesti' liquid smoke

Natural wood liquid smoke prepared by Hickory Specialties, Crossville, USA (ZLS) was used for the experiments. ZLS was diluted with sterile distilled water to final concentrations of 0.002, 0.006, 0.008, 0.01, 0.1, 1, 5, 10, 15, 20, 25, 50, 75 and 100% (undiluted).

Rehydration of desiccated coconut

Desiccated coconut (20 g) placed in 500 ml conical flasks was steamed for 15 min. ZLS at concentrations of 0.0, 0.002, 0.006, 0.008, 0.01, 0.1, 1.0, 1, 5, 10, 15, 20, 25, 50, 75, and 100% were used to rehydrate the desiccated coconut to 20 and 30% moisture.

Cultures

Aspergillus parasiticus NRRL 2999 grown in potato-dextrose-agar slants were suspended in 0.1% 'Tween 80' and 1 ml portions sprayed to each of the flasks containing rehydrated desiccated coconut. The flasks were incubated at 28°C in the dark and shaken once daily to facilitate uniform fungal growth. The culture flasks were observed up to 10 days for fungal growth, colour changes and sporulation. The experiment was terminated by steaming the flasks for 15 min and the contents were analyzed for aflatoxin.

Analysis of Aflatoxin

The contaminated desiccated coconut (10 g) was blended with 180 ml of 70% aqueous acetone in a Waring blender at high speed for 2 min. The filtrate was extracted with 10 ml of hexane to remove lipids. The aqueous acetone extract was re-extracted with three 30 ml portions of chloroform. The chloroform extracts were pooled, dried with 2 g of

anhydrous sodium sulphate and concentrated in a rotavapor under reduced pressure at 65°C to 5 ml. The concentrations of aflatoxin B1 in the extracts were estimated by spotting quantitatively on thin layer chromatographic (TLC) plates coated with Silica Gel 'G' (Merck) at 250 nm thickness. The TLC plates were developed using solvent systems methanol:chloroform (3:97) and acetone:chloroform (9:1) in lined and unlined tanks, respectively. The fluorescence was compared visually under UV light at (365 nm) with that of standard aflatoxin B1 samples of known concentrations (Samarajeewa and Arseculeratne, 1975).

Estimation of PAH in ZLS and in desiccated coconut

Oil from the desiccated coconut (10 g) was extracted in Soxhlet with hexane (250 ml) by refluxing for 4 h. Hexane was evaporated from the extract in a rotavapor under low pressure at 65°C followed by oven drying at 70°C for 2 h.

The extract of oil (0.2-0.3 g) was made up to 10 ml with hexane in a volumetric flask. The hexane solution (1 ml) was passed through a Sep-pak (Bond Elute LRC, Varian Inc.) cartridge conditioned by eluting with 2 ml of hexane. Polycyclic aromatic hydrocarbons were eluted with 3 ml of hexane:dichloromethane (3:1). The solvents were evaporated to dryness under a slow jet of nitrogen gas. The dry contents were dissolved in 500 ml of acetonitrile:water (3:1) for analysis by HPLC. Zesti liquid smoke was also analyzed for PAH after dilution with hexane (Southwell, 1993).

The HPLC system consisting of a Shimadzu model LC-6A machine containing a model SIL-6A injector, solvent delivery system (model LC-6A), a fluorescence detector (model RF-551) with 298 nm excitation filter and 439 nm emission filter were used. The reverse phase HPLC system, solvent program and peak integration parameters were controlled by Shimadzu SCL-6A system controller and CR-4A integrator. Samples were analyzed using Chrompack Chromspher PAH glass cartridge column (20 cm x 3 mm i.d.).

Coconut-agar media containing liquid smoke

Desiccated coconut (500 g) was blended for 2 min at high speed in a Waring blender with ZLS diluted to the concentrations of 0.0, 0.01, 0.1, 1, 2, 3, 5, 25, 50, 75 and 100% (undiluted) with sterile distilled water. The resulting slurry was filtered through a piece of cloth. The filtrate was

adjusted to pH 4.6, whenever necessary, using sodium hydroxide and was boiled with 30 g of agar. The media were sterilized at 121°C for 20 min and poured into sterile petri-dishes (Lin and Dianese, 1976).

Aspergillus parasiticus NRRL 2999 spores grown on Potato-dextrose agar were streaked onto Coconut-agar media containing ZLS and incubated at 28°C in the dark. The petri-dishes were observed daily for growth of fungi and the reverse of the petri-dishes was examined under UV light at 365 nm for violet-blue fluorescence indicating probable production of aflatoxin B1.

Analysis of coconut-agar media for aflatoxin

Agar medium (10 g) was blended with 10 ml of water and 30 ml of chloroform in a mini Waring blender at high speed for 2 min. The blend was filtered through a sintered glass funnel and centrifuged at 3000 rpm for 2 min. Chloroform (10 ml) was separated from lower liquid phase, dried in anhydrous sodium sulphate (0.5 g) and concentrated to 1 or 5 ml in a rotavapor under reduced pressure at 65°C. The aflatoxin was estimated on TLC as described above (Lin and Dianese, 1976).

RESULTS AND DISCUSSION

Composition of desiccated coconut

Desiccated coconut contained 2.5% moisture, was free of PAH, had a pH of 7 in 25% aqueous solution and showed the following particle size analysis

more than 1180 μ	= 72%
500 - 1180 μ	= 24%
250 - 500 μ	= 4%

Effect of ZLS on aflatoxin production

ZLS suppressed the growth of fungus in desiccated coconut moistened with the diluted ZLS at 20% and 30% moisture concentrations. No fungal growth was observed in desiccated coconut at ZLS concentrations at and above 15%. The aflatoxin concentrations dropped from 100,000 ppb to 1000 ppb with the use of 0.01% ZLS preparations and dropped further with increased concentrations of ZLS (Figure 1).

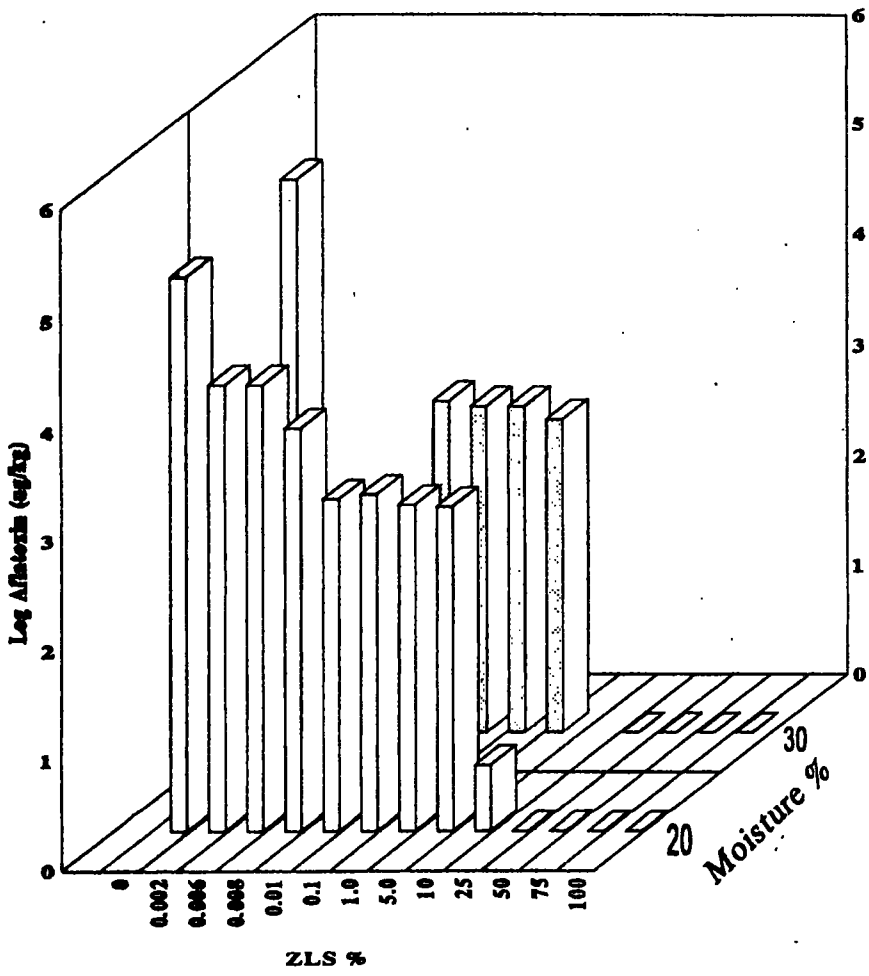

Figure 1. Protective Effect of Zesti Liquid Smoke (ZLS) on Aflatoxin Production in Relation to Moisture.

Aspergillus parasiticus did not grow in the coconut-agar medium containing more than 3% aqueous ZLS. Aflatoxin B1 or no blue fluorescence was observed on the reverse of the plates of coconut-agar medium containing more than 2% ZLS. Estimation of aflatoxin B1 confirmed the visual observation on the reverse of petri-dishes (Table 1). Although constituents in liquid smoke are suggested to carry preservative effects, the role of liquid smoke on suppressing growth of *Aspergillus parasiticus* and aflatoxin production are not reported in the literature up to now. This observation therefore makes an important contribution towards protection of coconut kernels from aflatoxin contamination. Although some chemicals are reported to prevent aflatoxin production on foods (Zaika and

Buchanan, 1987), the use of 'Zesti' smoke liquid carries a distinct advantage over the use of chemicals; because liquid smoke preparations are considered 'natural' and already approved for using in food.

Table 1. **Growth of *Aspergillus parasiticus NRRL 2999* and production of aflatoxin B1 in 7 days on coconut-agar medium prepared using different concentrations of 'Zesti' liquid smoke (ZLS).**

ZLS conc. (%)	Aflatoxin B1 (μg/g)	Culture characteristics	
		Growth	Fluorescence
0.0	78	Complete plate	Full plate
0.01	70	6.5 cm thick	7.5 cm band
0.1	27	7.5 cm band	7.5 cm band
1.0	23	7.0 cm thin	7.5 cm band
2.0	4	6.0 cm band	absent
3.0	nd	no growth	absent
5.0	nd	no growth	absent
25.0	nd	no growth	absent
50.0	nd	no growth	absent
75.0	nd	no growth	absent
100.0	nd	no growth	absent

nd = not detectable

Estimation of PAH

ZLS did not contain any detectable concentrations of Fluoranthene, Pyrene, Benzo(a) anthracene, Benzo(b) fluoranthene, Benzo(k) fluoranthene, Benzo(a) pyrene, Dibenzo(a,h) anthracene and Benzo(g,h,i) perylene indicating that it is safe to use ZLS as an agent to protect coconut kernels. The minimum detection levels for the above PAH s were 0.5, 0.2, 3.6, 2.1, 0.4, 2.8, 1.8 and 3.5 ug/kg respectively.

'Zesti' liquid smoke had a pH of 2.4. It is not yet known whether the protective effect is due to pH or other constituents in liquid-smoke or a combination of both.

CONCLUSIONS

'Zesti' liquid smoke may be used to protect coconut kernels from contamination of *Aspergillus parasiticus* and aflatoxin production. The protective effect on coconut kernels is not due to the polycyclic aromatic hydrocarbons because 'Zesti' liquid smoke, which does not contain PAH, inhibits fungal growth and aflatoxin production.

ACKNOWLEDGEMENTS

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