

Fungal and mycotoxin contamination of kokonte, a dried cassava product in Ghana

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## Summary

One hundred and twenty five households in 19 villages processing cassava were interviewed in Ghana. Kokonte (dried cassava pieces) was the most important product in 19% of households processing it. Most kokonte was produced from January to March. Mould growth during processing or storage was a problem during June and July, the rainy season. Most producers and market traders preferred non-mouldy kokonte, although many (59%) would consume a mouldy product. A price premium operated for non-mouldy kokonte. The most commonly isolated fungi were yeasts and *Cladosporium* spp., isolated from 44 out of 49 samples analysed. Other fungi isolated included *Aspergillus* spp. (20 samples), *Penicillium* spp. (15 samples) and *Fusarium* spp. (30 samples). Sterigmatocystin was detected in 10 samples at 0.17-1.67 mg/kg; patulin in 4 samples at 0.55-0.85 mg/kg; cyclopiazonic acid in 4 samples at 0.08-0.72 mg/kg; penicillic acid in 5 samples at 0.06-0.23 mg/kg; and tenuazonic acid in 3 samples at 0.02-0.34 mg/kg. Mycotoxin contamination of mouldy kokonte was a potential problem: there is therefore a need to improve kokonte processing to avoid mould growth.

## Introduction

Cassava is an important starchy root crop: it is eaten by a large number of people world-wide, and is widely used as an animal feed. It is a highly perishable commodity that requires processing to ensure stability during storage. One processing method used is drying, either by sun or by artificial means.

The important processed products in Sub-Saharan Africa are dried pieces and flours (NRI, 1992). In Ghana, dried cassava chips are called kokonte. Field observations and laboratory analyses at NRI have indicated that dried cassava products from certain parts of Africa are often visibly contaminated with fungi; this has also been reported by others (Clerk & Caurie, 1968; Essers & Nout, 1989; Jonsyn, 1989). A number of potentially mycotoxigenic storage fungi have been isolated from cassava, for example: *Aspergillus flavus* (Shank et al., 1972; Mota & Lourenço, 1974; Clerk & Caurie, 1968; Masimango et al., 1977; Bottalico et al., 1980), *Aspergillus ochraceus* (Masimango et al., 1977), *Aspergillus versicolor* (Clerk & Caurie, 1968), and *Penicillium* spp. (Clerk & Caurie, 1968; Mota & Lourenço, 1974). The potential mycotoxin risk associated with this contamination is not fully understood. Mycotoxin contamination of cassava has been documented (Bottalico et al., 1980; Sajise & Ilag, 1987; Constant et al., 1984; Brudzynski et al., 1977; Mota & Lourenço, 1974), although the findings have not always been subjected to confirmatory tests.

Scopoletin, a coumarin compound, can accumulate in cassava roots during post-harvest physiological deterioration (Wenham, 1995). It fluoresces in a similar way and has a similar R<sub>f</sub> to aflatoxin B<sub>1</sub> in some common Thin Layer Chromatography (TLC) systems. It is therefore possible that some reports of aflatoxin contamination of cassava chips may instead be due to the presence of scopoletin (Wheatley, 1984). Reports where the presence of aflatoxin has not been confirmed, or scopoletin not removed, should be treated with caution. Other mycotoxins have been reported, for example

zearalenone (Bottalico et al., 1980) and ochratoxin A (Soares & Rodriguez-Amaya, 1989).

Initial studies at NRI determined the capability of fungi isolated from cassava products to produce a range of mycotoxins (Westby et al., 1994). Fungi were isolated from cassava products from Ghana, Cote d'Ivoire, Uganda and Zaire, and tested for their ability to produce mycotoxins in sterile rice (a known good substrate) and sterile cassava. The most common potentially toxigenic fungi were of the genera *Fusarium*, *Penicillium* and, to a lesser extent, *Aspergillus*. Of 8 isolates of *Aspergillus* tested none produced aflatoxin on sterile cassava, whereas in rice 5 out of 7 isolates of *A. flavus* produced significant amounts of aflatoxin B<sub>1</sub>, and one isolate of *A. parasiticus* produced large amounts of aflatoxins B<sub>1</sub> and G<sub>1</sub>. Aflatoxin formation by *A. parasiticus* could be stimulated by the addition of extra nutrients (Westby et al., 1995). Isolates of *Penicillium* and *Fusarium* were generally toxigenic both on sterile rice and cassava.

The mycological and mycotoxin profiles of a limited number (26) of samples of cassava chips and flours from Uganda and Cote d'Ivoire were investigated in further work (Westby et al. 1994). *Penicillium* and *Fusarium* were the most common potentially toxigenic genera isolated. A wide range of mycotoxins was determined from the samples. The most common were: neosolaniol (8 samples, 0.18-3.11 mg/kg); patulin (7 samples, 0.11-1.61 mg/kg), cyclopiazonic acid (7 samples, 0.11-1.61 mg/kg), penicillic acid (4 samples, 0.07-3.60 mg/kg) and diacetoxyscirpenol (4 samples, 0.45-7.75 mg/kg). Aflatoxin was not detected in any of the samples.

The present study is a report on the findings of a field visit to Ghana in June 1993. The visit was undertaken to confirm observations about the potential of cassava to support mycotoxin formation. Particular attention was paid to correlating sample histories with mycological and mycotoxin contents in order to understand the causative factors of mycotoxin formation. The attitude of the population towards mouldy cassava consumption was determined using household and market trader questionnaires. Levels of consumption of dried cassava products were also determined. Kokonte is the dried cassava product in Ghana most subject to mould growth; the study was therefore concentrated on this product. Kokonte is prepared by peeling roots, chopping them into pieces and then usually sun-drying. Sun-drying can take a considerable amount of time. The visit took place during the rainy season in order for production and storage of kokonte to be observed when it is most likely for fungal contamination to take place.

## Materials and methods

### *Surveys*

The country was broadly divided into different agro-ecological zones (coastal, forest, transitional savannah), and villages were selected from each zone. Households within each village were selected at random and in all 125 households in 19 villages were interviewed. The distribution of villages was as follows: Ashanti Region (4 villages), Brong-Ahafo Region (4 villages), Central Region (2 villages), Greater Accra Region (3 villages), Eastern Region (1 village) and Volta Region (5 villages). Figure 1 gives the geographical

location of the villages surveyed. Forty-four market-trader interviews were conducted in urban centres in the Greater Accra Region (Accra), the Ashanti Region (Kumasi), the Brong-Ahafo Region (Techiman), the Volta Region (Ho), and the Eastern Region (Koforidua). Household and market trader questionnaires were used to obtain information on processing methods.

### *Samples*

Approximately 200 g of kokonte were collected from each household and market trader where possible. Product history was determined for each sample. The extent of mould growth was graded visually according to a four-point scale as follows: non-mouldy - no visible signs of mould growth; slightly mouldy - up to 10% of area covered with mould; moderately mouldy - up to 50% of area covered with mould; and very mouldy - profuse growth with over more than 50% of area covered. A total of 101 samples was collected and a representative sub-sample returned to NRI for mycological and mycotoxin analysis.

### *Mycological analysis*

Samples were aseptically ground in a Waring blender. A 30 g aliquot was mixed with 270 ml of 0.2% agar diluent and blended in a Colworth Stomacher for 2 minutes. A decimal dilution series was prepared in the same diluent. Sample aliquots (0.1 ml) were spread onto the surface of Dichloran Rose Bengal Chloramphenicol agar (DRBC) for non-xerophiles and Dichloran 18% Glycerol agar (DG18) plates for xerophiles and

incubated at 25°C for 5 days. Fungi were enumerated and assigned to genera.

#### *Mycotoxin analysis*

Selected ground samples from the mycological screen (30 g aliquot) were soaked in 30 ml of 0.1 M hydrochloric acid for 15 minutes. The mixture was then extracted twice in a Waring blender with separate 160 ml portions of acetone at high speed for 90 seconds. The extracts were filtered and evaporated to dryness, and redissolved separately in 20 ml of benzene and 20 ml of diethyl ether. Ether and benzene extracts were separately dissolved in 2 ml of methyl cyanide, 3-4 ml of iso-octane was added, and vortex-mixed. One ml of the methyl cyanide layer was drawn off and evaporated to dryness. The dry extracts were dissolved in 0.5 ml of a 9:1 benzene:methyl cyanide solvent for spotting on a High Performance Thin Layer Chromatography (HPTLC) plate. Methods of quantification by HPTLC are summarised in Table 1. Selective mycotoxin determinations were performed when species of the following fungi were isolated at more than  $10^4$  colony-forming units per gram (cfu/g): *A. flavus*, aflatoxin; *Penicillium* spp., sterigmatocystin, patulin, penicillic acid and cyclopiazonic acid; *Fusarium* spp., neosolaniol and T-2 toxin; and *Phoma sorghina*, tenuazonic acid.

#### Results

##### *Survey data*

A total of 125 household and 44 market questionnaires were completed and 101 samples of kokonte collected. Of these

samples, 49 were analysed for fungi, and 27 for selected mycotoxins.

Of the 125 households visited, 106 processed kokonte. Kokonte was the most important cassava product in 20 (19%) of these 106 households; the second most important in 51 (47%) households, usually after fufu (boiled, pounded cassava roots) or agbelima (fermented cassava paste), and third most important in 25 of 106 households (24%), usually after fufu and gari (roasted fermented grated cassava). Fufu was ranked the most important cassava product in 47% of households, compared with 19% for agbelima and kokonte, and 13% for gari. There were a number of small variations in the processing method for kokonte (Figure 2). However, the majority of producers peeled (97%) and washed (75%) roots.

There was marked seasonality in kokonte processing, with between 55 and 78 households producing it from January to March, and fewer than 10 households between the months of June to September (Figure 3a). Conversely, 60 households reported that they did not produce it in June and July.

One probable reason for the observed seasonality in kokonte processing was the incidence of mould growth during drying. Most households (92 and 80 respectively) reported that kokonte became mouldy if dried in June and July (Figure 3b). Most people (96%) said that they protected the product from rain during drying, but 89% of these said that it became mouldy during drying.

The mean minimum drying time during peak season production of kokonte was 6.7 days, with a mean maximum drying time of 11.5 days. There was a mean minimum drying time of 8.3 days and a mean maximum of 13.7 days during slack-season.



production. The peak production time coincides with the dry season (December-March) and the slack season correlates with the wet season.

Most households (89%) processing kokonte stored their product in the house, even if only for a short time. It was stored in a store room (22%), kitchen (30%) or elsewhere in the house (37%). Approximately 5% of households stored kokonte outside the house, either in a shed or simply covered over. Of those storing kokonte, 39% reported that it became mouldy during storage at some point in the year. As with the data on mould growth during processing, kokonte was reported to be most likely to become mouldy during storage in June and July (Figure 3c). There was no correlation between mouldiness during storage and the place where the product was stored. Mean storage time for peak season production was 12.7 weeks; that for slack season production was 10.6 weeks.

Overall, 21 different quality characteristics were ranked. Many households rated a white exterior or interior colour as most important (49% ranked it first, 11% ranked it second). A brittle or hard texture was second most important (9% gave it a first ranking, 24% second). This indicates the value most of the people interviewed placed on a non-mouldy, well-dried product. Other valued properties included certain cooking characteristics (tapioca quality, elasticity, sticky dough, fufu flavour: 10% of households ranked such characteristics first, 16% ranked them second).

Over half (59%) of those consuming kokonte would eat it if it were mouldy; however, only 6% of these preferred it mouldy. Of those who would eat mouldy kokonte, 45 out of 63 (71%) removed mould before cooking; 53% of these removed

mouldy pieces, 27% scraped mould off, 9% brushed it off, and 7% washed it off and redried pieces. The efficiency of these methods of removing mould growth on the removal of mycotoxin contamination has not been investigated.

The most common discolouration reported by those eating mouldy kokonte was a black colour. Microscopic examination of collected samples indicated that this was usually due to *Rhizopus*, *Cladosporium*, *Alternaria*, or other dematiaceous fungi. Green or greenish-black growth (6%) was probably *Cladosporium* in isolation. The pink growth reported by 6% of consumers was *Monilia*. Most of these moulds are not notably toxigenic, apart from *Alternaria* spp.

Quantities of kokonte consumed varied considerably. Some respondents ate kokonte only a few times a year in a small quantity ( a mean of 0.14 kg per person per meal) mixed with maize or plantain, whereas others ate it at least once a day, and at least 0.8 kg per person at each meal. The overall mean consumption was 0.36 kg per person per meal, consumed at 105 meals in a year, averaging 38.6 kg per person per year. For consumers who eat kokonte more than 100 times per year, consumption is 1-2 kg per person per month higher in May to August than in the preceding five months. In the period from May to August, the main maize crop has not been harvested and yams are very scarce; cassava, in the form of kokonte, represents an important carbohydrate source.

Of kokonte producers, 73 out of 106 (69%) marketed the product, selling it to market traders (market 'queens'), direct to the public from their homes, or in the local market themselves. Most producers (93%) preferred to sell non-mouldy kokonte. The main reason for this was that purchasers

preferred non-mouldy kokonte (32% of respondents). Other reasons were that processors perceived that it was of better quality (25%), or found that it commanded a good price (10%).

A variety of reasons why producers made kokonte were given. The most common was that kokonte was made for sale, to earn money for the household (27%). Other reasons given were the using up of small or poor quality roots unsuitable for fufu or gari, or to utilise excess cassava in times of greater production.

Full processing histories were available for 71 samples collected. Of these, 52 (73%) were sun-dried, and 19 (27%) were dried over a fire. Tables 2 and 3 give drying and storage details for sun- and fire-dried samples respectively, compared with the visual assessments of mould. There was no correlation between average drying-time and mould category. Whether it rained during the drying period was more important. It had rained at least once during drying for 68% and 67% of samples in the moderately- and heavily-moulded categories, compared with 33% and 27% of samples in the "no visible mould growth" and "slightly-mouldy" categories. The humidity would also have been higher between periods of rain. For sun-dried samples, those falling into the "no visible mould" category were stored for longer than any other. Among fire dried samples, there were none in the "heavily moulded" category, in comparison with sun-dried kokonte, where 19% of the total were ranked in this category.

A white colour was the most important characteristic for 59% of commercial kokonte sellers in urban markets. Most traders (84%) stated that they sometimes sold mouldy kokonte, usually during May to July. Most traders (89%) said that some

consumers preferred mouldy kokonte, although 95% of traders preferred to sell non-mouldy kokonte. Comparisons of the price of mouldy and non-mouldy kokonte for 26 paired observations showed that only four traders accorded no price differential between types. The average cost of non-mouldy kokonte was 134.7 Cedis per kg., compared with 97.1 Cedis for mouldy kokonte.

Sun-drying took place: directly on the ground; on the ground on mats; by the roadside; on the roof of the house or an outbuilding; or on a platform of thatch or corrugated iron, approximately 1.5 m above the ground. Fire-dried kokonte was either dried outside on a platform 1.5 m above ground, or inside the house or an outbuilding on a platform approximately 1 m above ground.

#### *Mycological results*

Fungal counts of the 49 kokonte samples analysed were moderate to high, ranging from  $1.5 \times 10^4$  to  $8.1 \times 10^8$  cfu/g. Fire-dried kokonte gave counts of  $10$ - $10^2$  cfu/g less, on average, than sun-dried kokonte in the same mouldiness category (Table 4). This could be due to the effect of heat or smoke on fungal viability, or the discolouration of the chips by fire-drying giving them a more mouldy appearance.

There was a trend of increasing fungal counts with increasing visible mouldiness ratings up to mould category 3 (Table 4). However, some very visibly-mouldy samples gave only moderate counts, and some non-mouldy samples gave high counts. Yeasts were a major component of the fungal flora; their presence partially explains the high total fungal counts. *Cladosporium* was present in 44 samples, and in 40

samples at more than  $10^4$  cfu/g (Table 5). *Cladosporium* has an easily disrupted mycelium and relatively small areas contaminated with this fungus can give samples with high fungal counts. Both of these factors could explain the disparity between visible mouldiness rating and actual total fungal counts.

In some cases, the mouldiness rating relates directly to the proportion of root covered by *Rhizopus*, *Monilia* or *Cladosporium*, since these moulds are able to cover roots profusely. They were present at more than  $10^4$  cfu/g in 44 out of 49 samples (Table 5), and in 25 of the 28 samples with a mould rating of 3 or more. These moulds are of low toxigenicity, however, and consequently the degree of mouldiness of the product may not relate directly to serious mycotoxin contamination, or to any mycotoxin-related health risks. However, some kokonte overgrown by *Rhizopus* or *Cladosporium* were also found to have other potentially toxigenic fungi (*Aspergillus*, *Penicillium* or *Fusarium*) growing on the root pieces, although they were not as visible. Generally, toxigenic fungi were associated with the growth of *Rhizopus*, *Monilia* and *Cladosporium*, apart from one sample in which *Fusarium* was found at more than  $10^4$  cfu/g but from which *Rhizopus*, *Monilia* and *Cladosporium* were not isolated. The visible presence of *Rhizopus* or *Cladosporium* may therefore serve as an indicator of possible contamination with potentially toxigenic fungi.

Twenty nine samples had at least one toxigenic species at a count of more than  $10^4$  cfu/g. Of the potentially toxigenic fungi, *Fusarium* spp. were isolated from 30 out of 49 samples, but at counts of  $10^4$  cfu/g or more from 19 samples only.

Other fungi isolated at a level of  $10^4$  cfu/g or more from samples included: *Aspergillus flavus*, 3 samples; *Aspergillus ochraceus*, 2 samples; *Aspergillus versicolor*, 8 samples; and *Penicillium* spp., 9 samples.

#### *Mycotoxin determinations*

Twenty seven samples were analysed for at least one mycotoxin. On the basis of previous data (Westby et al., 1994), analyses were performed for the following toxins, where fungi of the relevant group were isolated at  $10^4$  cfu/g or more: *Penicillium* spp.: patulin, penicillic acid and cyclopiazonic acid (11 samples); *Fusarium* spp.: neosolaniol and T-2 toxin (18 samples); *Aspergillus flavus/parasiticus*: aflatoxin (2 samples); *Aspergillus versicolor*: sterigmatocystin (10 samples); and *Phoma sorghina*: tenuazonic acid (8 samples). Results are detailed in Table 6.

Neosolaniol and T-2 toxin were not found in any of the 18 samples analysed for these toxins. Aflatoxins were not found in either of the samples analysed for it, although sterigmatocystin, an aflatoxin precursor, was found, at 1.67 mg/kg in one of these samples (Table 6). Sterigmatocystin was the commonest mycotoxin detected, isolated from all 10 samples for which its analysis was carried out, at a mean of 0.54 mg/kg. Penicillic acid was isolated from five samples, at a mean of 0.14 mg/kg; patulin was isolated from four samples, at a mean of 0.68 mg/kg; cyclopiazonic acid from four samples, at a mean of 0.43 mg/kg; and tenuazonic acid was found in three samples, at a mean of 0.13 mg/kg.

## Discussion

Kokonte is an important cassava product in Ghana, but in the regions surveyed, it was rarely (19% of households) the most important. Its processing is very straightforward. The majority of processors are aware of mould growth in kokonte and a non-mouldy (white and hard) product is preferred. The seasonality of kokonte processing is a reflection of the attempts to avoid mould growth.

Although mouldy kokonte is not the preference of most of the households interviewed, a high proportion of them (59%) would still eat mouldy kokonte. If mouldy kokonte contains mycotoxins it is likely, therefore, that a significant proportion of the population would be exposed to them. The methods used to remove mould growth would not be expected to prevent the consumption of mycotoxins, although their effectiveness has not been tested. Visible mould growth is a selection criterion for consumers and, despite the fact that most of the visible mould growth was of low- or non-toxigenic fungi (such as *Rhizopus*, *Cladosporium* and *Monilia*), in many cases potentially toxigenic species were present.

The results of the mycotoxin analyses confirm previous observations (Bottalico et al., 1980; Sajise & Ilag, 1987; Constant et al., 1984; Brudzynski et al., 1977; Mota & Lourenço, 1974) that certain toxins can be associated with mouldy cassava products. The most common mycotoxins, sterigmatocystin, patulin and cyclopiazonic acid (Table 6), are produced mainly by species of *Penicillium*. They are of moderate toxicity, but were present at relatively high levels, which is a cause for concern.

Neither T-2 toxin nor neosolaniol were detected, although *Fusarium* spp. were the most common group of potentially toxigenic fungi enumerated (Table 5). Aflatoxins were not isolated from either of the samples containing *A. flavus*. These observations support earlier work (Westby et al., 1995) concerning the limited ability of *Aspergillus* spp. to produce aflatoxins on cassava. The two samples in this study both contained sterigmatocystin, an aflatoxin precursor. The source of this is uncertain, since *Penicillium* spp. are also able to produce the toxin, but the hypothesis that *A. flavus* can produce sterigmatocystin, but not aflatoxin, on cassava is worthy of further investigation. Other toxins present in the samples (Table 6) were penicillic acid and tenuazonic acid. This is the first report of penicillic acid, cyclopiazonic acid, sterigmatocystin and tenuazonic acid associated with cassava.

Twenty-seven of the 43 samples of kokonte analysed contained potentially toxigenic fungi at more than  $10^4$  cfu/g. There is a greater risk of mycotoxin contamination if fungal counts are above  $10^4$  cfu/g. Of the 27 samples, 15 contained at least one mycotoxin. It can therefore be concluded that there is a potential health problem from mycotoxin contamination of cassava during the rainy season (the time of this survey).

According to the data generated in this study, mould growth is worst when drying times are extended during the rainy season. This may be a consequence of the humidity of the air, or the fact that kokonte gets wet when it rains, although most processors (96%) did protect their product from the rain. Since kokonte is often produced from cassava roots,



that are of inferior quality for other products, and large pieces of root are dried, the quality of the product is further compromised.

In Ghana, there is a preference by consumers and retailers for kokonte that is not mouldy. This is reflected in the significant price differential between mouldy and non-mouldy kokonte. Bearing these factors in mind, there are clear incentives for producing a product that is not mouldy. This is also the most practical method of avoiding mycotoxin consumption by humans.

Mould growth can be avoided by relatively simple changes in processing. A drastic but effective method is not to produce kokonte in the rainy season. Increasing artificial drying during the rainy season is a possibility, but this is unlikely to be sustainable since more firewood is required. If kokonte is produced close to the house, rather than being dried on the farm, it can be covered or brought inside if it rains. It could be dried on slatted trays, or trays with a wire mesh base, to improve airflow and accelerate drying. Such trays could be brought under cover in the evenings, to reduce the effects of condensation and moisture uptake. The roots could be cut into smaller pieces and washed in clean water afterwards, which would allow faster drying of a cleaner product.

The implementation of simple changes to processing techniques can be difficult in the field. However, there are significant incentives in Ghana to produce a product that is not mouldy. The non-mouldy product is preferred for sale, it is preferred by consumers, and there is a large positive price differential between mouldy and non-mouldy kokonte.

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## Legends to Figures

Figure 1. Map of Ghana showing location of survey villages (+).

Figure 2. Flow diagram of kokonte production methods in Ghana. Numbers in boxes correspond to households processing kokonte at each step in the diagram. Numbers next to lines indicate number of households following a particular route.

Figure 3. Seasonal effects associated with kokonte production in Ghana. (a) Seasonality of maximum household production of kokonte. (b) Months in which kokonte becomes mouldy during drying. (c) Seasonality of mould growth on stored kokonte. Note: 94/106 householders said they stored kokonte of which 37 reported that it went mouldy during storage.

Table 1. Summary of high performance thin layer chromatography methods for mycotoxins

Mycotoxin	Solvent system <sup>1</sup>	Clean up	Quantification Wavelength Mode <sup>2</sup>	Derivative Method	Produced Wavelength & Mode <sup>3</sup>
Aflatoxin	CXA	Bidirectional	366 nm fluor	None	-
Patulin	TEFW	Bidirectional	280 nm absorb.	MBTH	554 nm absorb
Penicillic acid	TEFW	Bidirectional	220 nm absorb.	p-anisald.	365 nm fluor
Cyclopiazonic acid	TEFW	Bidirectional	250 nm absorb.	Erlichs	540 nm absorb
Neosolaniol	TEFW	Bidirectional	-	H <sub>2</sub> SO <sub>4</sub>	374 nm fluor
T-2 toxin	TEFW	None	-	H <sub>2</sub> SO <sub>4</sub>	374 nm fluor
Sterigmatocystin	TEFW	None	358 nm fluor	AlCl <sub>3</sub>	365 nm fluor
Tenuazonic acid	TEFW	Bidirectional	270 nm absorb.	p-anisald.	365 nm fluor

Footnote:

1: CXA : Chloroform/xylene/acetone (6:3:1)  
 TEFW: Toluene/ ethyl acetate/formic acid/water (1500:700:198:27)

2: Quantification wavelength. Where possible, samples were analysed without derivatisation first at the quantification wavelength, followed by derivatisation for confirmation

3: Derivative methods:

Erlichs Sprayed with 1% w/v p-dimethylaminobenzaldehyde in 1:10 concentrated hydrochloric acid/acetone. CPA gave a yellow spot after heating at 110°C for 10 minutes, scanned at 540 nm, absorbance mode.

Table 1. (cont) Summary of high performance thin layer chromatography methods for mycotoxins

Footnote:

3. Methods for derivatives (cont)

AlCl<sub>3</sub> Sprayed with 20% w/v aluminium chloride in methanol and heated at 130°C for 10 minutes, the bright yellow spots of sterigmatocystin were read in fluorescence mode at 365 nm.

H<sub>2</sub>SO<sub>4</sub> Sprayed with 10% w/v sulphuric acid in methanol, heated at 130°C for 10 minutes, neosolaniol and T-2 toxin were read at 374 nm fluorescent mode.

MBTH Sprayed with freshly prepared 0.5% w/v 3-methyl-2-benzothiazolinone hydrazone hydrochloride in water. After heating at 140°C for ten minutes, patulin gave a yellow fluorescent spot read at 544 nm absorbance mode, moniliformin gave wine red spots read at 518 nm absorbance mode.

p-anisaldehyde. Sprayed with 0.5% p-anisaldehyde in 85 % methanol, 10 % glacial acetic acid, 5 % concentrated sulphuric acid, heated at 130°C for 8-20 minutes. Penicillic acid gave a light blue fluorescent spot, tenuazonic acid a green fluorescent spot which was scanned at 365 nm fluorescent mode.

Table 2<sub>3</sub> Drying and storage data for samples of sun-dried kokonte.

Mould category	Number of samples	Average drying time	Average storage time	Proportion samples rained on during drying	Times rained during drying	Number protected during drying
None	6	8 days	116 days	2/6 (33%)	3	2/2
Slight	11	6 days	12 days	3/11 (27%)	2	2/3
Moderate	22	10 days	45 days	15/22 (68%)	3	13/15
Heavy	9	9 days	36 days	6/9 (67%)	4	4/6

Footnote

- None = Non-mouldy
- Slight = Slightly mouldy (<10% of area covered)
- Moderate = Moderately mouldy (up to 50% of area covered)
- Heavy = Very mouldy (>50% of area covered)



Table 3. Drying and storage data for samples of fire-dried kokonte

Mould category	Number of samples	Average drying time	Average storage time
None	2	7 days	6 days
Slight	9	7 days	24 days
Moderate	8	8 days	13 days
Heavy	0	-	-

Footnote

- None = Non-mouldy
- Slight = Slightly mouldy (<10% of area covered)
- Moderate = Moderately mouldy (up to 50% of area covered)
- Heavy = Very mouldy (>50% of area covered)

Table 4. Comparison of visual mould rating with mean fungal count

Visual mould rating <sup>1</sup>	No. of samples in rating	Mean fungal count (cfu/g)	
		DRBC	DG18
1 F	2	$4.6 \times 10^5$	$9.5 \times 10^6$
1 S	2	$2.6 \times 10^6$	$1.5 \times 10^6$
2 F	5	$2.8 \times 10^5$	$4.4 \times 10^5$
2 S	12	$4.7 \times 10^7$	$1.8 \times 10^7$
3 F	9	$9.1 \times 10^6$	$9.2 \times 10^6$
3 S	14	$7.0 \times 10^7$	$1.4 \times 10^8$
4 F	1	$1.0 \times 10^5$	$7.0 \times 10^5$
4 S	4	$2.8 \times 10^6$	$3.2 \times 10^7$

Footnote

- 1: 1 = Non-mouldy  
 2 = Slightly mouldy (<10% of area covered)  
 3 = Moderately mouldy (up to 50% of area covered)  
 4 = Very mouldy (>50% of area covered)  
 S = Sun dried  
 F = Fire dried
- 2: DRBC Agar - Dichloran Rose Bengal Chloramphenicol Agar, a general isolation medium favouring the growth of non-xerophilic fungi.  
 DG18 Agar - a medium favouring the growth of xerophilic fungi.

Table 5. Distribution of counts for fungal genera amongst samples collected (49 were analysed).

Fungi isolated	No. of samples with counts in the range:				
	$<10^2$	$>10^2$	$10^3-10^4$	$10^5-10^6$	$10^7-10^8$
<b>Field type fungi</b>					
<i>Alternaria</i> spp.	42	1	3	3	0
<i>Cladosporium</i> spp.	5	0	4	29	11
<i>Colletotrichum</i> spp.	48	0	0	1	0
<i>Drechslera</i> spp.	45	0	2	2	0
<i>Fusarium</i> spp.	19	3	8	19	0
<i>Monilia</i> spp.	22	8	7	12	0
<i>Nigrospora oryzae</i>	36	6	4	3	0
<i>Phoma sorghina</i>	40	0	1	8	0
<i>Geotrichum</i> spp.	33	0	4	11	1
<i>Aureobasidium</i> spp.	43	0	0	6	0
Other yeasts	5	1	1	24	18
<i>Mucor</i> spp.	45	1	3	0	0
<i>Rhizopus</i> spp.	17	0	11	20	1
<b>Storage type fungi</b>					
<i>Aspergillus niger</i>	38	5	5	1	0
<i>Aspergillus flavus</i>	36	3	7	3	0
<i>Aspergillus wentii</i>	47	1	0	1	0
<i>Aspergillus candidus</i>	48	0	0	1	0
<i>Aspergillus ochraceus</i>	45	0	2	2	0
<i>Aspergillus versicolor</i>	38	1	2	8	0
<i>Eurotium</i> spp.	28	2	3	16	0
<i>Penicillium</i> spp.	34	1	5	9	0
<i>Paecilomyces variotii</i>	48	0	0	1	0
<i>Walleimia sebi</i>	36	0	2	11	0

Table 6. Mycotoxin analyses on kokonte samples

Sample Number	Visual mould rating	Mycotoxins detected (mg/kg) <sup>3</sup>									
		Afla	Pat	Pen	CPA	Neo	T-2	Ster	Ten		
247	3S	-	0.55	0.12	*	*	*	-	-	-	
133	4S	-	*	0.20	*	-	-	1.06	-	-	
124	3S	-	*	*	*	*	-	0.52	-	-	
514	3S	-	-	-	-	*	-	0.46	-	-	
222	2S	-	-	-	-	-	-	0.52	-	0.34	
455	2S	-	*	*	*	*	-	-	-	-	
313	3S	*	0.57	*	0.08	-	-	0.13	-	-	
314	2S	-	-	-	-	-	-	0.48	-	0.02	
412	3F	-	*	*	0.72	*	-	0.19	-	-	
136	2S	-	0.85	0.06	0.47	-	-	-	-	-	
621	3F	-	*	*	0.45	*	-	-	-	-	
211	3S	-	*	*	*	-	-	-	-	*	
123	2S	-	-	-	-	*	-	-	-	*	
14EC	3F	-	-	-	-	-	-	-	-	*	

Footnote:

1: Sample number refers to laboratory sample codes.

2: Visual mould ratings are as in Table 4.

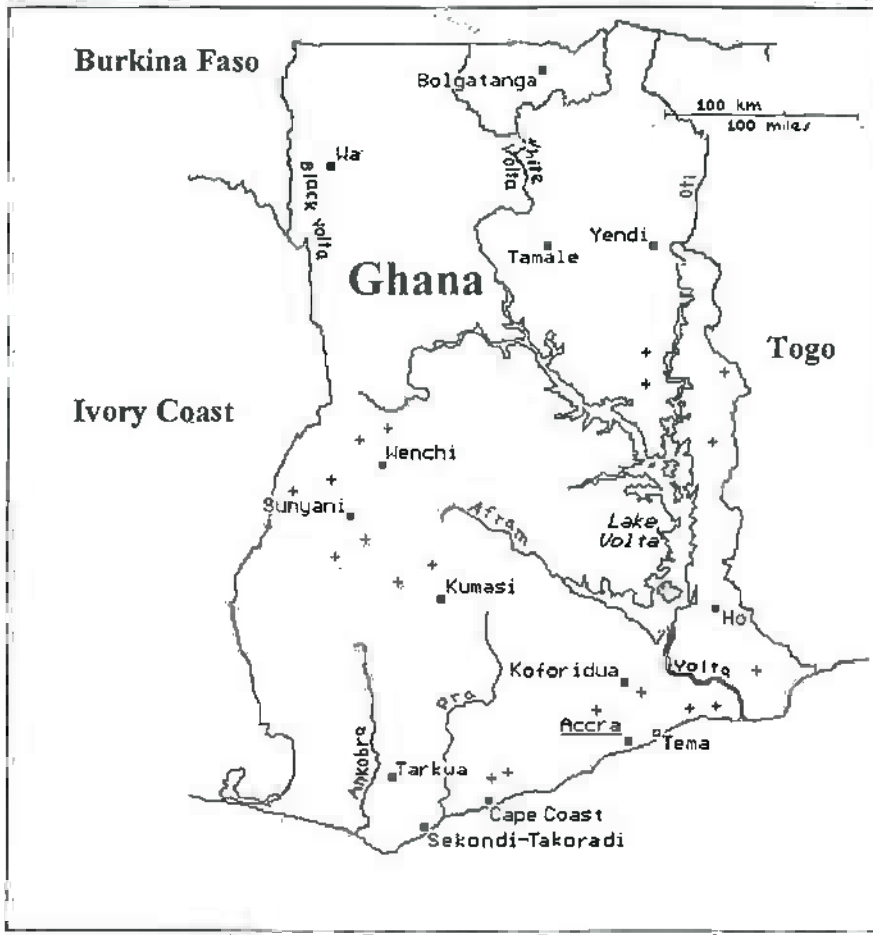
3: Afla are aflatoxins B<sup>1</sup>, B<sup>2</sup>, G<sup>1</sup> and G<sup>2</sup>, recorded as total aflatoxins. Pat, Pen, CPA are the *Penicillium* toxins patulin, penicillic acid and cyclopiazonic acid. Neo and T-2 are the *Fusarium* toxins Neosolaniol and T-2 toxin. Ster is Sterigmatocystin, from *Aspergillus versicolor*. Ten is Tenuazonic acid, from *Phoma sorghina*. '-' indicates that no analysis was performed for this toxin because the fungi able to produce this toxin were either absent, or present at less than 10<sup>4</sup> cfu/g. \* indicates that the toxin was analysed for, but was below the limit of detection.

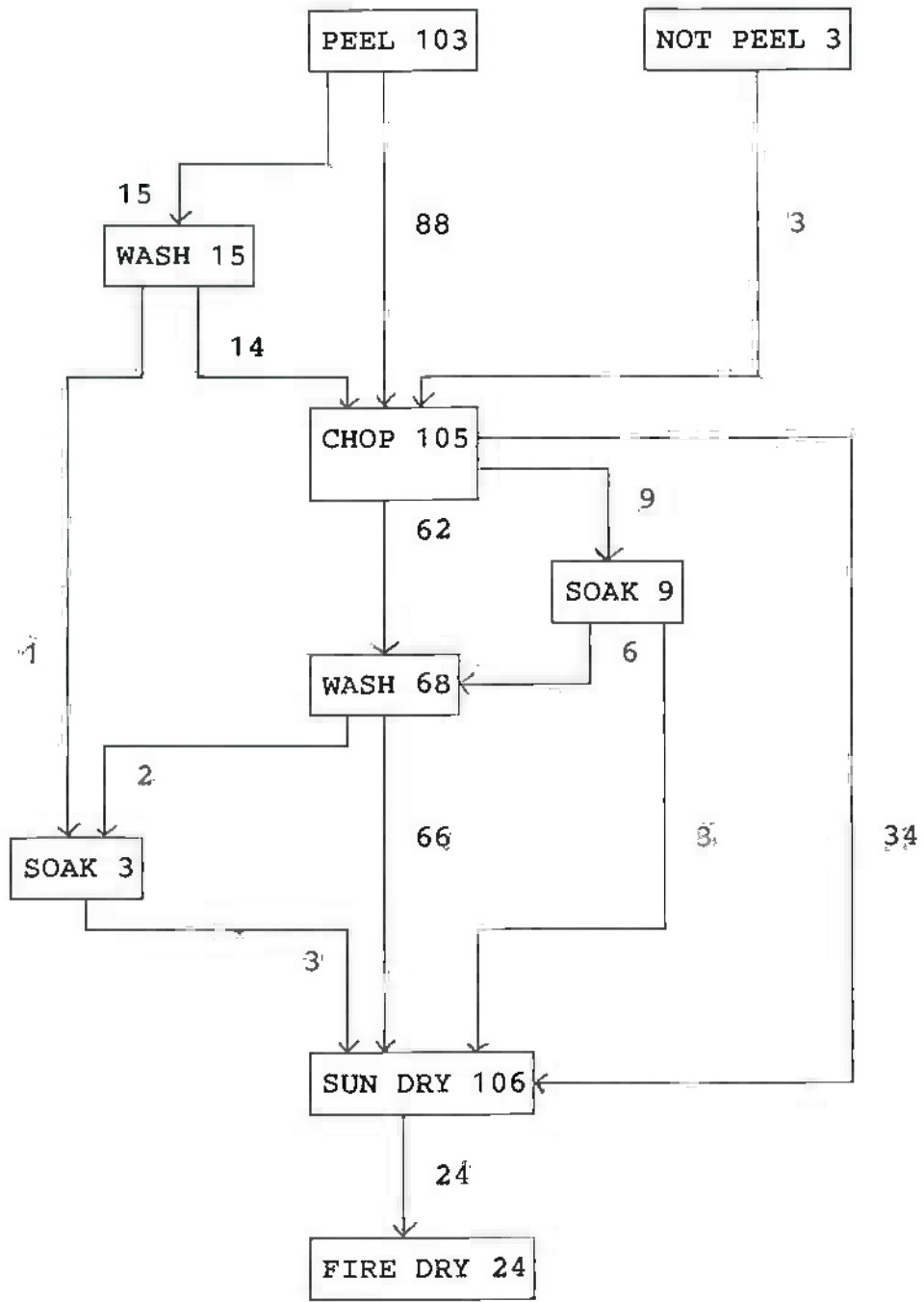
Table 6. (cont) Mycotoxin analyses on kokonte samples

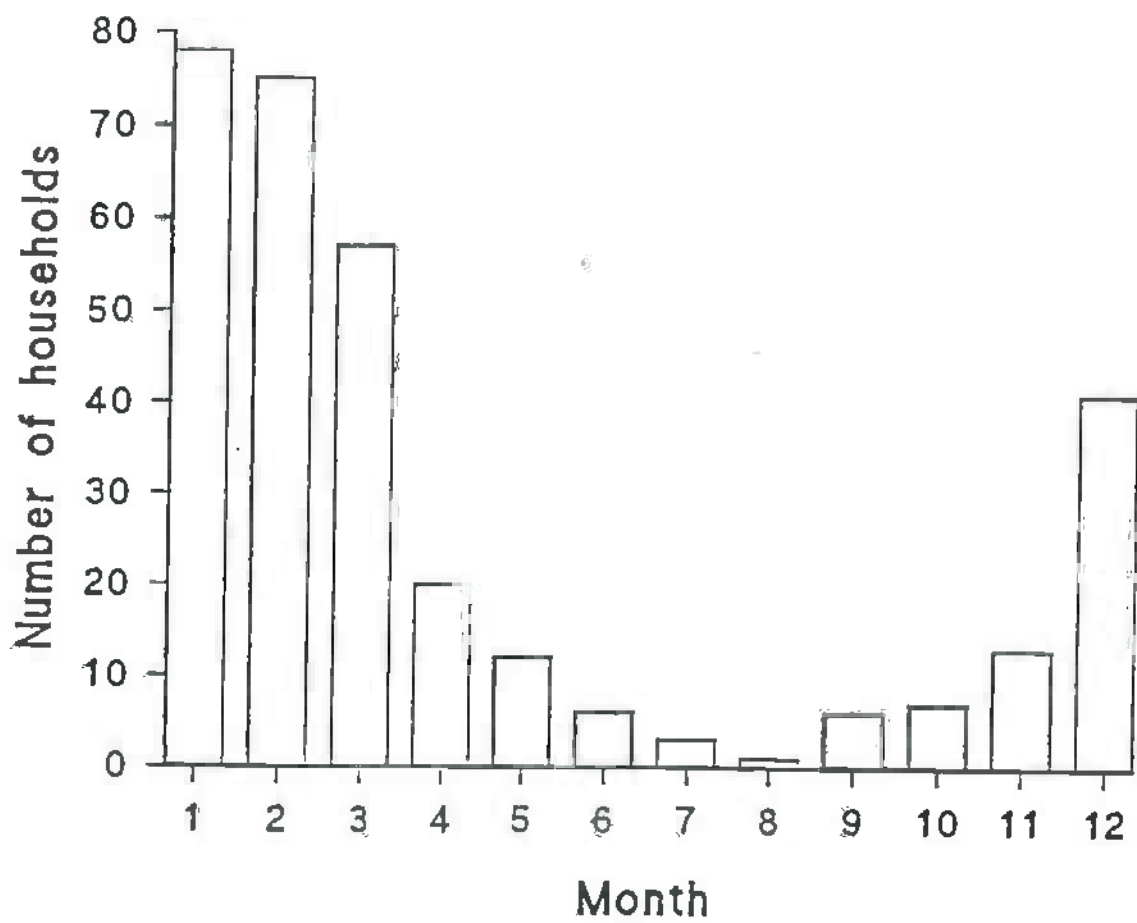
Sample Number	Visual mould rating <sup>2</sup>	Afla	Pat	Pen	CPA	Neo	T-2	Ster	Ten
		Mycotoxins detected (mg/kg) <sup>3</sup>							
442	3F	*	-	-	-	-	-	1.67	-
61E1	4S	-	*	0.11	*	*	*	-	-
212	2S	-	-	-	-	*	*	-	*
414	3F	-	-	-	-	*	*	-	-
14E1	3S	-	-	-	-	-	-	-	*
233	3S	-	-	-	-	*	*	-	-
14EB	3S	-	-	-	-	*	*	-	-
45E1	4S	-	-	-	-	*	*	0.17	*
232	4F	-	-	-	-	*	*	-	-
13E2	2S	-	-	-	-	*	*	-	*
451	3S	-	-	-	-	*	*	-	-
217	3F	-	0.76	0.23	*	*	*	0.25	-
221	3F	-	-	-	-	-	-	-	0.03

Footnote:

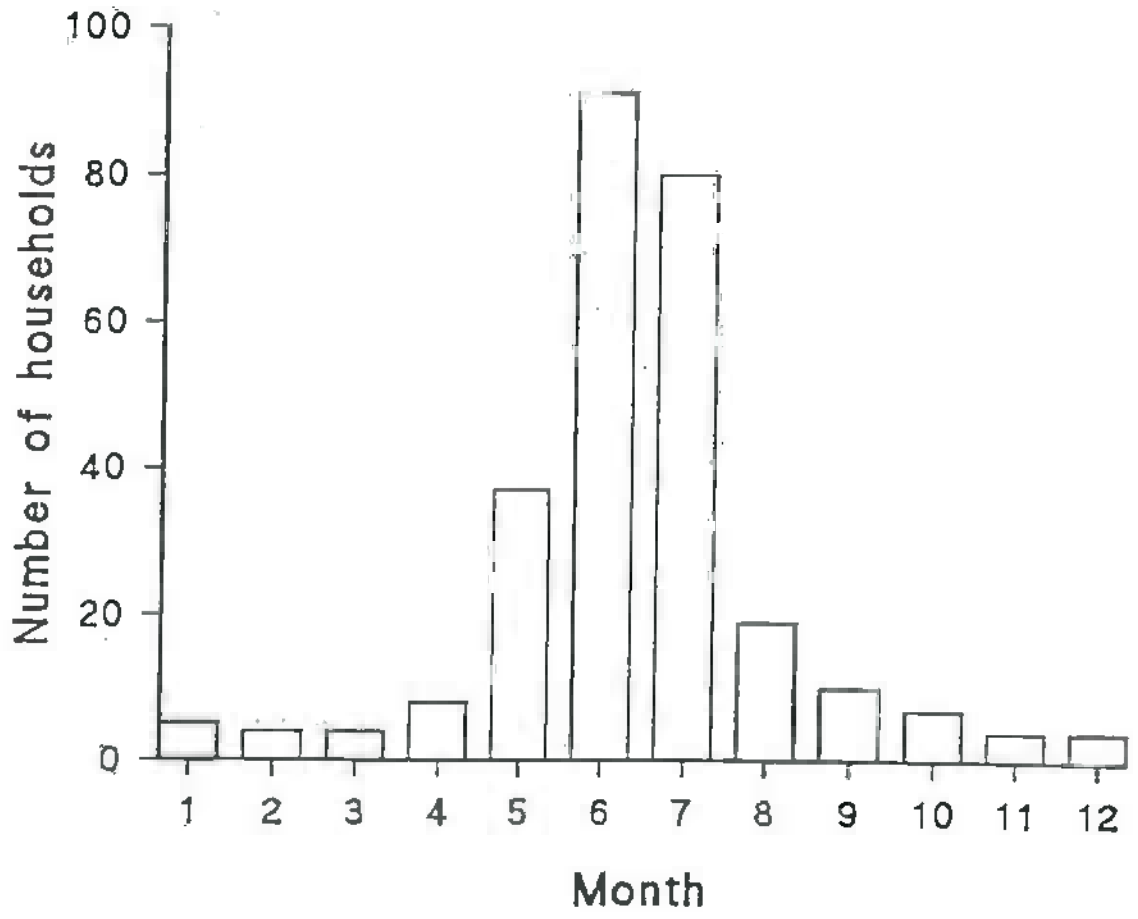
- 1: Sample number refers to laboratory sample codes.
- 2: Visual mould ratings are as in Table 4.
- 3: Afla are aflatoxins B<sup>1</sup>, B<sup>2</sup>, G<sup>1</sup> and G<sup>2</sup>, recorded as total aflatoxins. Pat, Pen, CPA are the *Penicillium* toxins patulin, penicillic acid and cyclopiazonic acid. Neo and T-2 are the *Fusarium* toxins Neosolaniol and T-2 toxin. Ster is Sterigmatocystin, from *Aspergillus versicolor*. Ten is Tenuazonic acid, from *Phoma sorghina*. '-' indicates that no analysis was performed for this toxin because the fungi able to produce this toxin were either absent, or present at less than 10<sup>4</sup> cfu/g. \* indicates that the toxin was analysed for, but was below the limit of detection.











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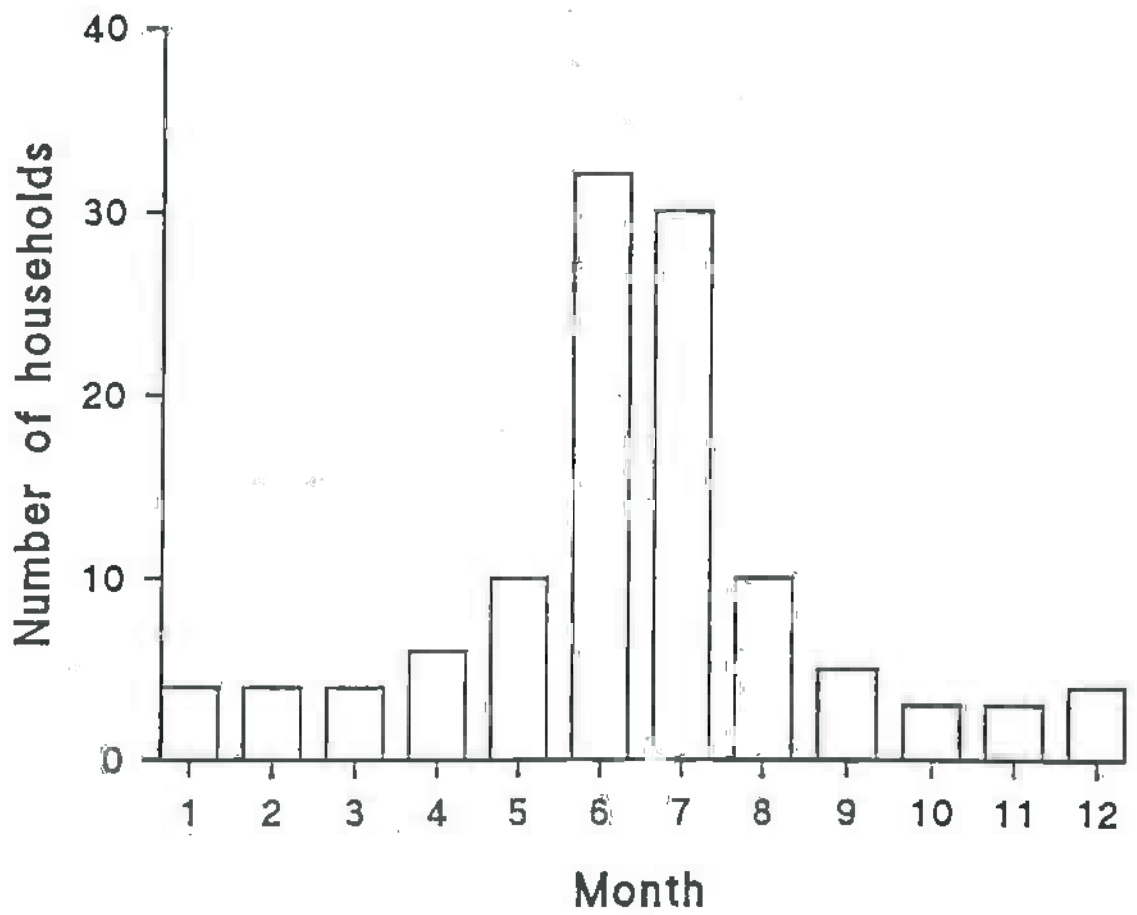


Fig. 6