

Aflatoxin contamination of commercially available areca nut products in Sri Lanka: a research note

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Short Report

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Abstract

Objective

Areca nut, widely chewed in Sri Lanka, is an independent risk factor for oral potentially malignant disorders and oral cancer, but its contamination with aflatoxins has not been assessed in the domestic retail market. This study determined the levels of the four major aflatoxins (AFB1, AFB2, AFG1, AFG2) in four commercial areca nut products and assessed compliance with international maximum levels. The products were fresh nuts, sun-dried nuts (karunka), fermented nuts (mada-puwak) and sweetened areca nut (pani-puwak).

Results

Composite samples from three markets in the Kandy district were analysed by UHPLC with fluorescence detection after immunoaffinity column clean-up in an accredited laboratory. Only pani-puwak was contaminated (total aflatoxins 28.50 µg/kg; AFB1 23.47 µg/kg; AFG1 2.90 µg/kg; AFB2 2.05 µg/kg), exceeding European Union maximum levels for tree nuts (4 µg/kg total; 2 µg/kg AFB1) approximately 7- and 12-fold. The processing and storage of pani-puwak may favour fungal contamination, adding an under-recognised carcinogenic risk to a product already linked to oral cancer. Regulatory monitoring, improved processing and storage practices, and consumer awareness are recommended.

Introduction

Aflatoxins, produced mainly by *Aspergillus flavus* and *Aspergillus. parasiticus*, are potent carcinogens; aflatoxin B1 (AFB1) is an International Agency for Research on Cancer (IARC) Group 1 human carcinogen [1]. AFB1 requires metabolic activation: hepatic cytochrome P450 enzymes, principally CYP1A2 and CYP3A4, oxidise it to the highly reactive AFB1-8,9-epoxide, which binds the N7 position of guanine to form DNA adducts. These adducts produce characteristic G:C→T:A transversions at codon 249 of the *TP53* tumour-suppressor gene [2]. This mutation occurs in roughly half of hepatocellular carcinomas from high-exposure regions compared with about one-fifth in low-exposure regions [2]. Any newly identified AFB1 exposure route is therefore a public health concern rather than an incidental analytical finding. In Sri Lanka, aflatoxins have been reported in cereals, spices and nuts [3], however, data on areca nut (*Areca catechu*) remain limited despite, it's chewed by about 600 million people worldwide, predominantly in South and Southeast Asia [4]., are limited.

Sri Lanka ranks among the top ten countries for smokeless tobacco use; approximately 7% of the population chew areca nut, around 1% as commercially prepared products [5]. Areca nut use is independently associated with oral submucous fibrosis [6], other oral potentially malignant disorders and oral squamous cell carcinoma [7], and betel quid chewing with cancers of the oesophagus, liver, pancreas, larynx and lungs, particularly with concurrent smoking [8]. Arecoline, the principal areca alkaloid, generates reactive oxygen species, depletes antioxidant defences and is genotoxic and

hepatotoxic independently of fungal contamination [9]. Areca nut is consumed alone or as a component of betel quid. In Sri Lanka, it is sold in several commercial forms: fresh nuts, sun-dried nuts (karunka), fermented nuts (mada-puwak) and a sweetened preparation (pani-puwak). Pani-puwak, in which areca nut pieces are coated with sugar syrup and flavourings and sold in small sachets, is widely available in retail shops. These products differ in moisture, processing and storage, all of which influence fungal growth and aflatoxin production, yet an additional aflatoxin exposure route through these products has not been systematically evaluated.

Surveys of raw areca nut from India and Taiwan reported aflatoxins at 3.5–26.2 µg/kg, with about 40% of samples exceeding 5 µg/kg [7], and a surveillance study of betel nut consignments imported into Pakistan found that all Sri Lankan-origin samples, together with most Indian- and Indonesian-origin samples, were contaminated, some at several hundred µg/kg [10]. However, that study examined export consignments rather than retail products and did not distinguish processed forms. Data on the products actually purchased by Sri Lankan consumers are therefore lacking. To our knowledge, this is the first survey of aflatoxins across areca nut product categories in the Sri Lankan retail market. This study aimed to determine AFB1, AFB2, AFG1 and AFG2 concentrations in these products and to assess compliance with international maximum levels.

Methods

Sampling

This study used a targeted market-survey design. Because aflatoxins are distributed heterogeneously within a lot (“hot-spots”), the Codex Alimentarius recommends that laboratory samples be constituted from multiple incremental samples rather than a single sub-sample [11, 12], and this principle was followed. Twenty nuts (or 20 retail units of pani-puwak) of each product were purchased from each of three markets in the Kandy district (Kandy central, Katugastota and Pilimathalawa) and pooled into one composite sample per product (Fig. 1). Samples were transported in sealed, labelled polyethylene bags at ambient temperature and stored in tightly sealed containers until analysis.

Extraction and clean-up

Analyses were performed in an ISO/IEC 17025-accredited laboratory following the principles of AOAC Official Methods 999.07 and 2005.08 [13]. HPLC-grade solvents, deionized water and certified standards of AFB1 (2000 µg/L), AFB2 (500 µg/L), AFG1 (2000 µg/L) and AFG2 (500 µg/L) (Fermentek Ltd., Jerusalem, Israel) were used. Nuts were de-husked, ground and sieved through a 500 µm mesh to obtain a homogeneous test portion. A 25.00 (±0.05) g portion was blended with 2.50 (±0.05) g NaCl and 100 mL of methanol: water (80:20, v/v) and filtered; 10 mL of filtrate was diluted with 40 mL of deionized water, filtered and passed through an immunoaffinity column (AflaTest®, VICAM, USA), which was washed according to the laboratory’s validated procedure. Aflatoxins were eluted with 1 mL of methanol followed by 1 mL of deionized water.

HPLC-FLD analysis

Aflatoxins were determined on a Shimadzu UHPLC-FLD system (Shimadzu Nexera X2, Kyoto, Japan) with fluorescence detection (excitation 360 nm, emission 450 nm) according to laboratory procedure HPLC/FSQAL/SOP/006. Separation was achieved on a reversed-phase Phenomenex Luna C18 column (4.6 × 250 mm, 5 µm; Phenomenex Inc., Torrance, CA, USA) maintained at 40 °C, using a gradient mobile phase of water::acetonitrile:methanol (60:24:16, v/v/v) filtered through 0.20 µm nylon membranes and delivered at 1.0 mL/min. The injection volume was 10 µL and the run time 12 min. Post-column photochemical derivatisation (PHRED™, Aura Industries Inc., NY, USA) was used to enhance the fluorescence of AFB1 and AFG1. Data were processed using LabSolutions Software. Aflatoxins were identified by retention time against certified standards and quantified using external calibration. Two positive controls fortified at 2 µg/kg were included in each analytical sequence to monitor recovery. The average recovery of the method for all four AFs within the required 80-120% range.

Data handling

As no specific maximum level exists for areca nut, results were compared with European Union (EU) limits for tree nuts intended for direct human consumption (4 µg/kg total aflatoxins; 2 µg/kg AFB1) [14], the most closely related commodity category, and with the Sri Lankan national limit for foods [15, 16]. With one composite sample per product, this was a screening-level survey and results are reported descriptively without statistical comparison. Values below the limit of detection (AFB1: 0.03 µg/kg, AFB2: 0.01 µg/kg, AFG1: 0.02 µg/kg and, AFG2: 0.01 µg/kg) were treated as non-detects, and total aflatoxins were calculated as the sum of the four quantified analogues.

Results

Of the four products analysed, only pani-puwak contained detectable aflatoxins (Table 1). Total aflatoxins were 28.50 µg/kg, comprising AFB1 (23.47 µg/kg), AFG1 (2.90 µg/kg) and AFB2 (2.05 µg/kg); AFG2 was not detected. AFB1, the most toxic analogue, accounted for approximately 82% of the total. These values exceed the EU limits for total aflatoxins and AFB1 approximately 7-fold and 12-fold, respectively [14], but are just below the Sri Lankan national limit of 30 µg/kg [15, 16]. Aflatoxins were not detected in fresh areca nut, karunka or mada-puwak. As one composite sample was analysed per product, these values are point estimates rather than means of replicate measurements.

Table 1
Aflatoxin concentrations ($\mu\text{g/kg}$) in composite samples of areca nut products

Product	AFB1	AFB2	AFG1	AFG2	Total
Fresh areca nut	ND	ND	ND	ND	ND
Sun-dried (karunka)	ND	ND	ND	ND	ND
Fermented (mada-puwak)	ND	ND	ND	ND	ND
Sweetened (pani-puwak)	23.47	2.05	2.90	ND	28.50
EU maximum level [14]	2.00	–	–	–	4.00

ND: not detected (below the limit of detection). One composite sample was analysed per product; values are point estimates.

Discussion

To our knowledge, this is the first report identifying pani-puwak as an aflatoxin-contaminated areca nut product. Pani-puwak is prepared by boiling or soaking areca nut in sugar syrup or jaggery, which increases moisture and supplies a sugar substrate that favours *Aspergillus* growth, particularly during prolonged ambient storage or in poorly sealed packaging [17]. *A. flavus* grows above a water activity (a_w) of about 0.80–0.83 but produces aflatoxin only above an a_w of about 0.85, maximally near 0.95–0.99 at 25–30°C [18]. Syrup-soaked pani-puwak stored under humid tropical conditions plausibly falls within this range, whereas the low moisture of karunka and the acidification of mada-puwak would keep a_w below this threshold, consistent with the non-detects observed. The predominance of AFB1 is typical of *A. flavus* contamination, whereas the presence of AFG1 suggests that G-producing species such as *A. parasiticus* may also be involved, as *A. flavus* generally produces only B-type aflatoxins [17].

The AFB1 level in pani-puwak lies at the upper end of that reported for raw areca nut in India and Taiwan [7] and is consistent with contaminated Sri Lankan-origin consignments in Pakistan [10], suggesting that this is not an isolated occurrence and that aflatoxin exposure through Sri Lankan areca nut may extend beyond the single product and market surveyed here. Notably, this level was found in a processed, packaged retail product rather than in raw nuts, indicating that contamination may arise or increase after harvest, during processing, packaging or storage.

The public health implications are twofold. First, areca nut is already an established oral carcinogen [7]; the added presence of AFB1, which acts through DNA adduct formation [2], alongside arecoline-induced oxidative stress [9], raises the possibility of combined carcinogenic exposure, although whether these distinct mechanisms act additively or synergistically has not been investigated. Second, pani-puwak is often perceived as a milder form of areca nut, including among younger consumers, so awareness of its risks may be low. Because pani-puwak is held in the mouth for prolonged periods and partly swallowed,

users may be exposed repeatedly, and regular users may therefore accumulate a meaningful dietary AFB1 burden. A margin of exposure assessment using the European Food Safety Authority (EFSA) benchmark dose lower confidence limit (BMDL₁₀ 0.4 µg/kg body weight per day) [19] will require consumption data and is a priority for future work.

Sri Lanka has no aflatoxin limit specific to areca nut products. The general national limit of 30 µg/kg total aflatoxins for all foods, set under the Food (Labelling and Miscellaneous) Regulations 1993 [15, 16], would classify the pani-puwak analysed here (28.50 µg/kg) as compliant, indicating that it is outdated. Sri Lanka Standards Institution provisions for other commodities adopt EU maximum levels as their reference [14, 20], and recent regulatory action, such as the 0.5 µg/kg limit for processed liquid milk effective from January 2027, does not extend to areca nut [20]. Closing this gap, particularly for pani-puwak, merits consideration. In the interim, good manufacturing practices for pani-puwak, including adequate drying before sweetening, moisture-proof packaging, limited shelf life and storage away from heat and humidity, could reduce the risk of fungal growth, and routine surveillance of sweetened areca nut products by food control authorities would help to identify contaminated batches.

Limitations

This exploratory study has the following limitations:

- Samples were collected from three markets in a single district, so the findings do not represent island-wide contamination.
- Only one composite sample per product was analysed, without replicates or seasonal sampling, so results are point estimates and cannot be compared statistically.
- Only one of several sweetened areca nut products on the market was tested; the findings show that pani-puwak can be contaminated rather than estimating prevalence across brands.
- Sampling was conducted at a single time point, so seasonal variation in contamination related to humidity and storage duration could not be assessed.
- Aflatoxins were identified by retention time with fluorescence detection without confirmation by mass spectrometry.
- Moisture content, water activity and fungal load were not measured, and fungal species were not isolated or identified, so the proposed mechanism for the selective contamination of pani-puwak remains to be confirmed.
- Consumption data were not collected, so dietary exposure could not be estimated.

Future island-wide surveys covering multiple brands, with replicate and seasonal sampling, are needed to establish prevalence and identify where contamination occurs, and extracts from contaminated samples will be tested on healthy cell lines to assess carcinogenic potential.

Conclusion

Pani-puwak contained aflatoxins substantially exceeding EU limits, whereas fresh, sun-dried and fermented areca nut did not. This previously unrecognised carcinogenic exposure supports regulatory monitoring of aflatoxins in areca nut products, improved processing and storage standards, and public awareness.

Abbreviations

AFB1: aflatoxin B1; AFB2: aflatoxin B2; AFG1: aflatoxin G1; AFG2: aflatoxin G2; AOAC: Association of Official Analytical Collaboration (AOAC International); a_w : water activity; BMDL₁₀: benchmark dose lower confidence limit for a 10% response; EFSA: European Food Safety Authority; EU: European Union; UHPLC-FLD: ultrahigh-performance liquid chromatography with fluorescence detection; IARC: International Agency for Research on Cancer; ND: not detected.

Declarations

Ethics approval and consent to participate

Not applicable. This study did not involve human participants, human data or animals.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article. The HPLC chromatograms are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

RDJ and RF conceived and designed the study. RR, KS, PMPAB collected the samples. RMSKK, RF performed the laboratory analysis. RR, KS and RMSKK analyzed and interpreted the data. KS and RR drafted the manuscript. All authors reviewed and approved the final manuscript.

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Figures



Figure 1

Areca nut products analysed in this study. **a** Fresh areca nut; **b** sun-dried areca nut (karunka); **c** fermented areca nut (mada-puwak); **d** commercially packaged sweetened areca nut (pani-puwak), with brand details redacted