A survey of aflatoxin B_1 and total aflatoxin contamination in baby food, peanut and corn products sold at retail in Indonesia analysed by ELISA and HPLC

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Abstract

Aflatoxin contamination has been well known as a world-wide health-threatening problem in tropical countries including Indonesia. This research was undertaken to determine the degree of aflatoxin contamination in different Indonesian foodstuffs. A preliminary survey was carried out to evaluate the level of total aflatoxin (AfT) and aflatoxin B_1 (AfB_1) contamination of baby foods, peanut products, and corn products, which were purchased from traditional markets and supermarkets in Indonesia during the year 2001-2002. Eighty two peanut products, 12 baby foods products, and 11 corn products from different brands were analysed for AfT and AfB_1 using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The results indicate that, of the brands analysed, 35% of the peanut products were contaminated with aflatoxins at various levels (range 5 to 870 µg/kg). Peanut-chilli sauces had the highest percentage of AfT contamination 9/12 (75%), which was followed by traditional snacks 5/11 (45%), peanut butter 4/11 (40%), flour egg coated peanut 6/16 (37%), and peanut cake 3/10 (30%). Fried peanuts and roasted peanut were found to contain aflatoxin at relatively lower percentages of 9% and 8%, respectively. From the 12 analysed baby food samples, on the other hand, no sample was found to be contaminated with aflatoxins. Two of 11 samples (18%) of corn based products were contaminated with AfT, ranging between 5.8 and 12.4 µg/kg. Additionally, 30 selected samples in different concentration ranges were further analysed to verify the correlation between ELISA and HPLC techniques and results were compared.

Keywords: aflatoxin, peanut, baby foods, corn, retail

Introduction

Aflatoxins – toxic metabolites produced by *Aspergillus* spp., mainly *Aspergillus flavus* and *A. parasiticus*, are frequently found in many raw and processed feeds and foodstuffs. High ambient temperatures and high relative humidity in tropical regions such as in Indonesia are highly favourable for the development of such fungi. Aflatoxin B_1 (AfB_1) was recognised as one of the most toxic mycotoxins and world-wide regulatory control limits for AfB_1 in food and feed are well established [1, 2]. In the European Union, the threshold level for total aflatoxin (AfT) contamination in foodstuffs is set at 4 μg/kg, and 2 μg/kg for AfB_1, whereas the US FDA has established the action level for human food at 20 μg/kg for AfT.

Among agricultural products, peanuts are very susceptible to aflatoxin contamination; yet, peanut based foods are a diet staple of the population. Indonesia is the third largest peanut producing country in the world, after India and China. In Indonesia, peanuts and its processed products i.e. roasted peanuts, egg-coated peanuts or flour coated peanuts and peanut-chilli sauces are staple foods. In the Javanese area, egg-coated peanuts and flour coated peanuts are generally consumed as snacks by children whereas peanut-chilli sauces are commonly consumed as condiments. Such products are consumed either fresh or processed such as edible oil, flour, peanut butter, crackers, candies, snack, and sauce. Hence, consumers could be put at high risk of exposure to aflatoxin.

The extent of the fungal and mycotoxin problems in Indonesia is largely unknown [3]. A previous report indicated that aflatoxin-contaminated feed increases dramatically during the wet season (December to May) [4]. A comparison of aflatoxin contamination of groundnut samples has indicated that higher levels of aflatoxins in retail groundnuts are attributed to climatic conditions. It has been reported that higher levels of aflatoxin were found in groundnut used for making oil than those destined for the retail market. Contamination of commercial foods with high levels of aflatoxin is a very important issue for Indonesia since those foodstuffs are very popular among children.

Widiastuti *et al.* [3] reported that cyclopiazonic acid was accompanied by other mycotoxins especially aflatoxins, in poultry feed samples collected at feed mills. Sardjono *et al.* [5] found that 28% of the peanut and 64% of the corn sample collected at farms, from middlemen, and retailers from several areas in Indonesia, were contaminated with *A. flavus/A. parasiticus*. In another survey [6] 11 of 16 corn samples (69%), collected in Indonesia, were contaminated with aflatoxin (mean level of 119 ng/g; maximum level 487 ng/g). Goto *et al.* [7] investigated 26 agricultural samples from markets in Central and East Java and Bali, Indonesia. They found that AfB_1 was presented in five of eight peanut (62.5%) and four of five maize (80%) samples. They also reported that the highest level of AfB_1 found approached 6 ppm in a peanut sample and 300 μg/kg in a maize sample. In another study [8] 91% out of the 34 corn samples tested were contaminated with aflatoxins, with the total concentration ranging from 22 to 6171μg/kg. The dominant species found was *A. flavus* [8]. In a preliminary study of our group, 118 peanut samples were analysed and 63.6% of samples were found to be contaminated with AfB_1 in different concentration ranges [9].

The purpose of this study was to conduct a survey to determine the degree of aflatoxin contamination of foods sold in traditional markets and supermarkets, in Indonesia. For the first time, foodstuffs that are consumed directly such as snacks, baby foods, and other products, were analysed for aflatoxin contamination, and these data are presented. Additionally, a comparison of the ELISA method verses HPLC has been carried out to verify the results of ELISA.

Materials and Methods

Commercial peanut, baby food, and corn samples: From 82 peanuts products, 12 baby foods products and 11 corn products with different trade marks were purchased from traditional markets and supermarkets in Indonesia. The peanut samples consisted of 12 roasted peanuts, 16 flour and egg-coated peanuts, 11 traditional snacks, (fried floured peanut, peanuts with brown sugar, and sweet-grounded peanuts), 10 peanut cakes, 11 fried peanuts, 10 peanut butters, and 12 peanut-chilli sauces. The baby foods samples consisted of one baby cake and 11 baby porridges mainly of rice. The corn products consisted of 3 industrial-produced snacks and 8 home-made snacks.

Chemicals and reagents, HPLC: Acetonitrile and methanol were purchased from LOBA Feinchemie AG (Fischamend, Austria). Buffer salts of analytical grade were obtained from Merck (Darmstadt, Germany). Water was purified in a UPW2 system (F&L, Vienna, Austria). Ridascreen® kits were purchased from R-Biopharm GmbH (Darmstadt, Germany). Aflaplate® kits and aflatoxin standard solution as well as immunoaffinity columns (Aflaprep®) were purchased from R-Biopharm Rhône LTD (Glasgow, Scotland). The HPLC system consisted of a Waters 626-LC pump, a Waters 717plus autosampler (Milford, MA, USA) and Waters 474 fluorescence detector combined with a Kobra cell® from R-Biopharm Rhône LTD (Glasgow, Scotland) as electrochemical reaction cell. As analytical column a Lichrospher 100 RP 18 E 5µm 250 mm x 4.6 mm (Bischoff Chromatography, Leobenberg, Germany) was used and column temperature was kept at 40°C. The isocratic mobile phase consisted of water-acetonitrile-methanol (62:22:16, v/v/v) with 350 µl of 4M nitric acid and 119 mg of potassium bromide per litre and a flow rate of 1 ml/min. The aflatoxins were detected after derivatisation in Kobra cell (current 100 µA) by fluorescence detection at λ_{ex} = 360nm, λ_{em} = 435 nm. The aflatoxins B_1, B_2, G_1 and G_2 were determined by HPLC and the four peaks quantified.

Sample extraction and preparation: Samples (50 g) were grounded and extracted using methanol/water (60/40, v/v) and homogenised for 1 minute using a high speed Ultra-Turrax homogeniser (Janke & Kunkel IKA-Labortechnik, Staufen, Germany). For AfB_1 analysis, 4 g NaCl (Merck, Darmstadt, Germany) was added to the extract. The extract was filtered through a Schleicher & Schuell 595½ filter paper and the filtrate was collected. The filtrate was then analysed for AfT and AfB_1 by ELISA or applied to the immunoaffinity columns prior to HPLC analysis. For HPLC analysis, 300 µl of the eluent from the immunaffinity column was injected into the system. Quantitative analysis was carried out on all samples using the ELISA technique and employing commercial kits (Ridascreen® for total aflatoxins and Aflaplate® for B_1). The absorbance of the samples was measured using a Bio-Rad Microplate Reader Model 450 (with a 450-nm filter) and Microplate Manager®/PC Version 4.0 software (Bio-Rad Laboratories).

Statistical analysis: Student's t-test was calculated for the analysis of correlation coefficient on the comparison of ELISA and HPLC techniques. p-value ≤ 0.05 was then considered significantly different.

Results and Discussions

The results, illustrated in Table 1 and Table 2, indicated that, of the brands analysed, 35 % of the peanut products were contaminated with total aflatoxins at various levels (range 5 to 870 µg/kg). Peanut-chilli sauces had the highest percentage of AfT contamination (75 %), which was followed by traditional snacks (45 %), peanut butter (40 %), flour/egg coated peanut (37 %), peanut cake (30 %), and corn product (18 %), whereas the percentages of fried peanut and roasted peanut samples found to contain AfT were relatively lower than others at 8 % and 9 %, respectively.

Table 1 - Occurrence of total aflatoxins (AfT) in Indonesian foodstuffs

Samples	Number of samples		Total aflatoxins (µg/kg)			Median of positives
	Total	Positive				
Roasted peanut	12	1	n.d.	-	204	204
Flour/egg coated peanuts	16	6	5	-	870	20
Traditional snack	11	5	7	-	112	77
Peanut cake	10	3	5	-	302	11
Fried peanut	11	1	n.d.	-	27	27
Peanut butter	10	4	7	-	228	45
Peanut-chilli sauces	12	9	7	-	613	46
Baby foods	12	0	-	-	-	-
Corn products	11	2	6	-	12	9

LOD ≤ 1.7 µg/kg

Table 2 - Occurrence of aflatoxin B1 (AfB1) in Indonesian foodstuffs

Samples	Number of samples		Aflatoxin B_1 (µg/kg)			Median of positives
	Total	Positive				
Roasted peanut	12	1	n.d.	-	162	162
Flour/egg coated peanuts	16	6	4	-	357	14
Traditional snack	11	5	8	-	75	48
Peanut cake	10	3	4	-	117	7
Fried peanut	11	1	n.d.	-	13	13
Peanut butter	10	4	5	-	61	27
Peanut-chilli sauces	12	7	8	-	207	40
Baby foods	12	0		-		-
Corn products	11	2	5	-	8	6

LOD ≤ 2 µg/kg

The low aflatoxin contamination in the fried peanut samples might be the result of the high intense heat applied during frying [12]. The highest amount of AfT found was that for a flour/egg coated peanut samples which contained 870 µg/kg. This particular sample also contained 357 µg/kg AfB_1. Given the known adverse effects of aflatoxins on human health, such highly contaminated foods must be avoided. For traditional snacks the maximum levels of AfT and AfB1 found in these snacks were 112 µg/kg and 75 µg/kg, respectively. The peanut cakes were contaminated in a range of 5 to 302 µg/kg of AfT and 4 to 117 µg/kg of AfB_1. The total concentration of aflatoxins in the one fried peanut sample was 27 µg/kg and AfB_1 was 13 µg/kg. Similarly, the peanut butters were contaminated with levels up to 228 µg/kg and 61 µg/kg of AfT and AfB_1, respectively. The total concentration of aflatoxins in peanut-chilli sauce samples ranged from 7 to 613 µg/kg; AfB_1 was between 8 to 207 µg/kg.

Out of the 12 baby food samples analysed, no traces of aflatoxin were found in baby foods. These results are encouraging, since infants are especially susceptible to aflatoxicoses. Of the corn snacks tested, 18 % of the samples were contaminated at levels up to 12 for AfT and 8 µg/kg for AfB1, respectively. In 11 corn products analysed, the highest levels of AfT and AfB_1 were found in two snack samples produced from small traditional manufacturers, which, however, were below the 20 µg/kg level. Interestingly, all samples of roasted peanut, flour/egg coated peanut, traditional snacks, peanut cake, peanut butter, and peanut-chilli sauces contained AfT at the levels greater than the tolerance levels allowed by FAO (20 µg/kg for AfT). In contrast, baby food samples exhibited no contamination and in corn snacks as well as fried peanuts, low concentrations of aflatoxins were recorded.

Correlation between ELISA and HPLC results

In this study ELISA technique was conducted because of simplicity of installation and performance in laboratories with limited possibilities regarding either the equipments or the know-how. The verification of ELISA technique for aflatoxin was determined in order to compare its reliability with that of HPLC, a well-established technique for aflatoxin determination. Thirty samples were selected in different concentration ranges and analysed by HPLC in the combination with immunoaffinity cleanup. According to the HPLC analysis, the main fungal contaminant would appear to be *A. parasiticus which* produces all types of aflatoxins (AfB_1, AfB_2, AfG_1, AfG_2). In 3 samples based on peanut, only AfB_1, AfB_2 could be detected which indicated the contamination of *A. flavus*. Overall correlations of HPLC versus ELISA for AfT and AfB_1 are illustrated in Fig. 1 and Fig. 2.

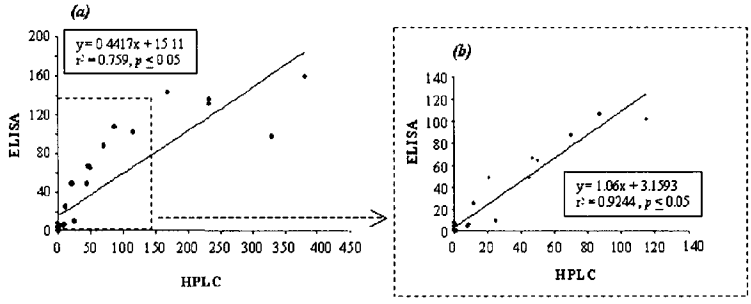

Figure 1 - Comparison of the analysis of total aflatoxin (AfT) from selected peanut products between ELISA and HPLC *(a)* for the range from 0-400 µg/kg (n = 30) and *(b)* for the range from 0-120 µg/kg (n = 25)

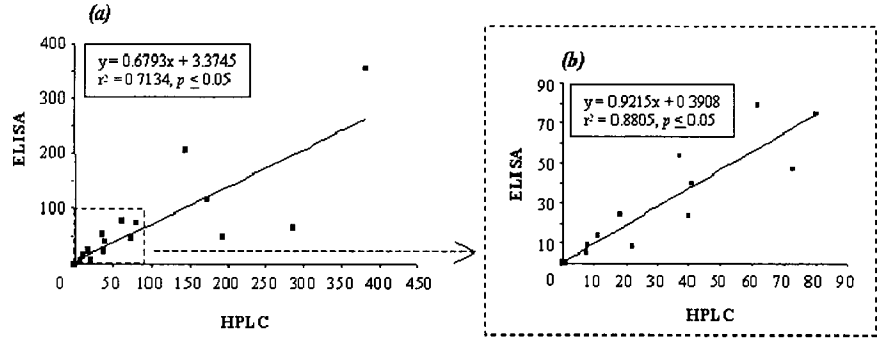

Figure 2 - Comparison of the analysis of aflatoxinB1 (AB1) from selected peanut products between ELISA and HPLC *(a)* for the range from 0-400 µg/kg (n = 30) and *(b)* for the range from 0-80 µg/kg (n = 25)

It is noteworthy that the better correlation coefficient between two techniques was observed at the lower concentrations of both AfT and AfB1 as compared to the whole range of the aflatoxin levels (Fig. 1 and Fig. 2). At lower levels of AfT (0-120 µg/kg), the correlation between ELISA and HPLC techniques were more comparable than at higher AfT levels (0-400 µg/kg) as determined by the higher correlation coefficient (0.9244 *vs.* 0.7590). Likewise, the correlation coefficient between ELISA and HPLC technique appeared to be greater at the lower concentrations of AfB1 (0-80 µg/kg) than that at higher concentraion (0-400 µg/kg) (0.8805 *vs.* 0.7234).

Park *et al.* [10] have compared ELISA and HPLC methods for the analysis of AfB_1, fumonisin B_1 and ochratoxin A in barley and corn foods and reported correlation factors ranging between 0.81-0.87. The results of the method comparison in our study revealed that, at low concentration ranges, better correlations between ELISA and HPLC could be obtained, indicating the reliability of either of these techniques for aflatoxin determination. However, HPLC appeared to be the method of choice for the measurement of high concentration ranges of aflatoxins. This might also indicate the limitation of ELISA technique for the determination of aflatoxins at high concentration which was possibly due to the quenching of absorbance.

High contamination of prepared foods for direct consumption may have resulted from high aflatoxin contamination in raw materials used. This supports the assumption of Goto [7] who reported that peanuts and maize samples collected from Bali, Central Java, and East Java, contained AfB_1 in the range of 8 to 299 µg/kg. The high concentrations of AfT and AfB_1 in peanut and corn products may be due to the time of harvesting, as peanuts and corn were harvested during the rainy season (February to May) and only sun drying was used to reduce the moisture content. The results of a study by Pitt *et al.* [11] supported this hypothesis. Pitt *et al.* [11] analysed 256 peanut samples which were obtained from fields at harvest season during the 1991 to 1994 period. Tests revealed that 98% of the samples were contaminated with *A. flavus* and 80% with *A. niger*.

However, it should be noted that the high concentrations of aflatoxins were registered in mainly home-made products or in products which were produced by small

traditional manufacturers and directly sold to the market. This may also have resulted from the way agricultural products such as peanuts and corn as raw material were handled after harvesting. Improper handling of these commodities are often the result of limited technology of the farmer and food manufacturer. Slow air drying, under atmospheric conditions, is probably the main reason for such high concentrations of aflatoxin in these commodities. Sardjono *et al.* [5] found that freshly harvested peanuts and corn were contaminated with *A. flavus/A. parasiticus* and the content of mycotoxins increased during storage.

Conclusions

The results presented here indicate that aflatoxin contamination in some Indonesian foodstuffs is a very serious problem. The extent of aflatoxin contamination in mainly home-made foods and their products, for the most part, had not been previously determined. The contamination of industrially produced samples was in general lower than those of home-made or small traditional manufacturers. This is the result of better quality control of coming raw agricultural products in the food industry in Indonesia.

Although this study was limited in both the number of samples and number of locations where samples were collected, aflatoxin contaminated peanut products were consistently found. Also, the improper handling of these commodities, such as slow drying processes and/or improper storage conditions have been observed at both the primary producer and retail level. These data show the need for regulations to limit the level of aflatoxin contamination in food products in Indonesia and support the conclusions of the others [6,8] who have suggested that strict regulation be put in place and enforced to limit the degree of aflatoxin contamination in Indonesian foodstuffs. Given the results presented and discussed here, a concerted effort should be undertaken by all levels of government to reduce and regulate the degree of aflatoxin contamination in Indonesian foodstuffs.

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