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MONITORING OF AFLATOXIN CONTAMINATION AT MARKET FOOD CHAIN IN EAST JAVA

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Seed moisture content
Damage seed

ABSTRACT

Peanut is a cheapest source of protein especially for developing countries communities and mostly it obtained from traditional markets. Earlier studies showed that aflatoxin incidence was relatively less at the farmer/trader levels while it is significantly higher at retail levels especially in traditional markets. Present study was conducted to understand the factors leading to the post-harvest building up of aflatoxin in peanuts sold in traditional market and in supermarket. This study was carried out at Pasuruan regency, East Java Province, Indonesia from March 2005 to June 2006. During study period peanut grains were collected from wholesalers, retailers and supermarkets at three months interval. In each sampling point, 2kg of grains was obtained and then was divided into eight parts for the analysis of parameters namely seed moisture content, physical quality, *Aspergillus flavus* infection and aflatoxin B₁ contamination. The results showed that seed water contents at wholesalers, collectors, and retailers in traditional wet markets were almost lower than 10%. They were thus 'safe' from aflatoxin B₁ contamination as seed moisture contents were below the aflatoxin risk zone. Time of sampling did not affect the level of aflatoxin B₁ contamination. Under controlled condition generated from air-tight container, the influence of seed moisture content and *A. flavus* infection on aflatoxin production was significant.

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1 Introduction

Community awareness related to the risk of aflatoxin contamination is still low especially for people who have limited access to education and information. Aflatoxin is a carcinogen, immune suppressing and anti-nutritional natural contaminant of peanuts and hence is a major food and feed quality problem worldwide (Wotton & Strange, 1985; Paramawati et al., 2006). This toxin produced by soil-borne fungi *Aspergillus flavus*, *A. paraciticus* and *A. nomius* (Pitt & Hocking, 1997) in peanut kernels. These fungi can colonized and infect peanut even when the crops are on farm, which represent that aflatoxin production in peanut seeds occur at both pre and post harvesting stages. Pre-harvested aflatoxin contamination occurs when peanut suffered from drought stress especially during late generative growth phase (seed maturation). Meanwhile post-harvest contamination usually proceeds when the seeds are in warm and humid conditions either during storage or transportation (Yameogo & Kassamba, 1999).

Extensive surveys on aflatoxin contamination at various levels of the Indonesian peanut supply chain have found that aflatoxin incidence was very minor at the farmer and collector level where peanut is produced and sun-dried, respectively (Dharmaputra et al 2003; Dharmaputra et al 2007; Rahmianna et al 2007a). The toxin contamination increased as peanut seeds went through the next level of supply chain i.e. the marketing sectors especially at retail level in traditional market; here the contamination was generally found to be the highest. This indicates that aflatoxin contaminated peanuts entered in the marketing chain, post-harvest contamination of aflatoxin build up due to poor storage conditions (Dharmaputra et al., 2003; Dharmaputra et al., 2004; Rahmianna et al., 2007; Ndung'u 2013).

Early results of various studies confirmed that *A. flavus* is highly prevalent in kernel samples stored at wholesaler and wet markets with temperature (25 to 35°C) and relative humidity (up to 60%) conditions being highly conducive for aflatoxin production (Dharmaputra et al., 2003, Dharmaputra et al., 2004; Rahmianna et al., 2007). Though Peanut is a cheapest and important source of protein for population of developing countries, and the current study is proposed to enhance our understanding of factors leading to the post-harvest build up of aflatoxin in peanuts during storage in three different selling points (i.e. wholesaler, traditional 'wet' market and supermarket) in Indonesia. The objective of this research was to understand the incidence of aflatoxin contamination at three market types during wet and dry seasons of study period.

2 Materials & Methods

A monitoring study was conducted by collecting peanut kernels from the available points of market food chain in one

of the central production areas at East Java Province, i.e. Pasuruan regency. In this regency, peanut is an important secondary food crop and the grains are popularly consumed either as food (peanut sauce, peanut cracker, etc) or in various snacks. The monitoring study was conducted for 16 months (from March 2005 to June 2006) with sampling scheduled in every three months during the storage period (a total of 6 samplings: March, June, September, and December in year 2005, while in year 2006 was March and June). These six samplings covered both dry and wet seasons, where from April to October was dry season and November to March was wet season. Peanut samples were collected from one wholesaler (who distributes peanuts to traditional/wet market), three shops/retailers in traditional market, and one supermarket.

At retailer, wholesaler and supermarket levels, three samples were obtained in every sampling time as replication. An analysis of variance was performed using a randomized block design with two factors and three replications. Time of sampling (six sampling times) was factor 1 and market type (three market types) as factor 2.

The size of each sample was two kg of seeds. The air dried seed were divided in eight parts by using a seed divider to obtain working samples for analysing moisture content, physical quality, percent of kernel infected by *A. flavus* and aflatoxin B₁ content (Dharmaputra et al., 2004). The procedure for obtaining the working samples from the main peanut samples described in the figure 1.

The moisture content of peanut kernels was measured by gravimetric method. Physical quality of seed kernels was quantified by manually segregating seed sample into three seed qualities i.e. sound mature kernel (SMK), shriveled and damaged kernels (only data on damaged kernels provided in this paper). The damaged kernels included those cracked, broken, discolored, and damaged by insects or fungi. The percentage of seeds infected by *A. flavus* was determined by plating 100 seeds per sample onto the *Aspergillus flavus* and *parasiticus* agar (AFPA) media in 10 petri dishes (as replications) or 10 seeds per each petri dish. The seeds were incubated for 3 days.

The *A. flavus* infection was easily determined by the presence of fungal colonies as shown by dark yellow or orange colour at the bottom of the media. Aflatoxin B₁ content in the seeds was determined using Elisa method (Lee & Kennedy 2002). Briefly, samples for aflatoxin B₁ were extracted with methanol. Supernatant was separated from the pellet. A competitive ELISA was performed to measure aflatoxin B₁ in the supertanant. The absorbance values were determined using spectrophotometer and plotted against a standard curve of aflatoxin B₁. The aflatoxin B₁ contents of the samples were expressed in part per billion (ppb).

Figure 1 The procedure for obtaining the working samples from the main peanut samples. [Source: Dharmaputra et al. (2004)]

3 Results

3.1 Seed Moisture Contents

Entire samples obtained from three market types during the various study periods, the seed water moisture contents were found to be <10%, except from one sample that revealed kernels with moisture content >10%. This high seed moisture content was obtained from retailer at traditional market in March 2006. Possible reason for this could be the usage of

opened-wooden box for the storage of seeds in the shops for at least one week at ambient temperatures. The seeds obtained from supermarket, however, had lowest moisture content throughout the sampling times because peanut seeds were properly packed in air-tight packaging and stored under air-conditioned environment. Samples obtained from wholesalers consistently contained 8-9 % moisture, as the seeds stayed for very short period with maximum 7 days in this point, and during storage, the seeds were packed in the gunny bag with minimum contact to the surrounding air (Table 1).

Table 1 Seed moisture contents of peanuts collected from three different market types in Pasuruan Regency from March 2005 to June 2006.

Sampling times	Market types		
	Traditional market	Wholesaler	Supermarket
March 2005	8.9 b	8.6 b	6.3 cd
June 2005	6.4 cd	8.2 bc	7.5 bcd
September 2005	7.9 bc	7.9 bc	6.3 cd
Januari 2006	8.0 bc	7.7 bcd	6.3 cd
March 2006	12.1 a	8.3 bc	7.2 bcd
June 2006	5.6 d	8.8 b	6.2 cd

Numbers followed by the same letter(s) indicated not significantly different based on DMRT 5%

Table 2 Percent of damaged seeds of peanuts collected from three different market types in Pasuruan Regency from March 2005 to June 2006.

Sampling times	Market types		
	Traditional market	Wholesaler	Supermarket
March 2005	29.6 ij	55.6 b	39.9 efgh
June 2005	50.0 bcde	24.6 j	52.1 bc
September 2005	35.0 hi	45.4 bcdefg	49.4 bcdef
December 2005	50.5 bcd	46.3 bcdef	65.9 a
March 2006	46.3 bcdef	35.6 ghi	40.2 defgh
June 2006	42.5 cdefgh	34.2 hi	39.5

Numbers followed by the same letter(s) indicated not significantly different based on DMRT 5%

Table 3 Percent of seeds infected by *Aspergillus flavus* on peanuts collected from three different market types in Pasuruan Regency from March 2005 to June 2006.

Sampling times	Market types		
	Traditional market	Wholesaler	Supermarket
March 2005	11.6 g	34.6 defg	42.3 bcde
June 2005	59.5 bc	33.8 defg	94.6 a
September 2005	34.3 defg	30.0 defg	16.0 fg
Januari 2006	25.6 efg	40.2 cde	21.0 efg
March 2006	52.5 bcd	63.2 b	36.5 def
June 2006	23.3 efg	41.6 bcde	30.5 defg

Numbers followed by the same letter(s) indicated not significantly different based on DMRT 5%

3.2 Physical quality

Despite three categories of physical quality observed, only data of damaged seeds are presented in this paper since the damage seeds had more risk in or were more prone to *A. flavus* infection and aflatoxin B₁ contamination based on our previous studies (Rahmianna et al 2007a, Rahmianna et al 2007b). In general, the data indicated that percent of damage seeds were high ranging from 24.8% to 65.9% in three market points. Percent of damaged seeds at wholesaler was generally lower than those at retailer level from six sampling times, except for samples taken in March and September 2005 (Table 2).

Peanuts sold in the supermarket did not always have good quality seeds in terms of seed coat color, intactness and healthiness as pointed out by the sample obtained in June, September, and ultimately December (Table 2).

3.3 Infection of *A. flavus*

Peanut seeds from all market chains were infected by *A. flavus* at various levels, ranging from 11% to almost totally infected (Table 3). The percentage of infection range was very wide, especially for peanut from retailers in traditional market. Among sampling time, samples taken in September 2005 had low infection rate in all three market points.

3.4 Aflatoxin B₁ contamination

The aflatoxin B₁ (AFB₁) contamination in most samples from all three market types in Pasuruan regency was >10 ppb. In addition, some samples had unacceptable levels of aflatoxin since of its extremely high contamination (>3000 ppb) (Table 4). In general, the number of samples with aflatoxin content >10 ppb from retailers was higher compared to those obtained from wholesale and supermarket, except samples taken in March and June 2006 (Table 5).

The higher contamination incidence at retailer level was due to the longer period of storage under conditions that favorable for the fungus to produce this secondary metabolite before the seeds were processed for food.

4 Discussion and Conclusions

Any peanut kernel with moisture content $\geq 10\%$ is stayed in aflatoxin risk zone as the critical moisture for aflatoxin production is in the range of 10-30% (Crop Link, 2000). Having low aflatoxin contamination, the kernels should contain 7-8% moisture and therefore the dry seeds had to be kept under air-tight 0.05 mm poly propylene bag. This condition was experienced by peanuts in wholesaler, where they were closely packed. Dried condition of seeds together with air-tight packed resulted in safe kernels with low aflatoxin contamination even up to six months storage (Dharmaputra, 2002; Ginting, 2006).

Table 4 Aflatoxin B₁ content of peanuts collected from three different market types in Pasuruan regency from March 2005 to June 2006.

Market type	Aflatoxin B ₁ content (ppb) of peanuts samples					
	March 2005	June 2005	Sept 2005	Dec 2005	March 2006	June 2006
Whole-sale	6.4 – 10.7	8.3 – 418.8	6.7 – 137.5	3.5 – 8.7	5.6 – 453.4	4.8 – 3583
Super-market*	6.5	11.5	9.1	7.7	6.3	265.8
Retailer in traditional market	1.7 – 18.1	8.1 – 3074.9	5.6 – 478.3	3.8 – 90.9	4.8 – 290.8	4.4 – 187.7

* one sample only

Table 5 Percentage of seeds with aflatoxin B₁ content lower and higher than 10 ppb of peanuts collected from three different market types in Pasuruan regency from March 2005 to June 2006.

Market type	March 2005		June 2005		Sept 2005		Dec 2005		March 2006		June 2006	
	≤10 ppb	>10 ppb	≤10 ppb	>10 ppb	≤10 ppb	>10 ppb	≤10 ppb	>10 ppb	≤10 ppb	>10 ppb	≤10 ppb	>10 ppb
Whole-sale	72.7	27.3	70.0	30.0	76.9	23.1	100	0	20.0	80.0	60.0	40.0
Super-market*	100	0	100	0	100	0	100	0	100	0	0	100
Traditional market	81.3	18.7	38.1	61.9	64.7	35.3	89.0	11.0	89.0	11.0	85.7	14.3

* one sample only

High number of damaged seeds in traditional market was in accordance to the results obtained from six retailers at traditional markets in Banjarnegara region of Central Java, where there was an average of 45% damaged seeds with the range from 5 to 73.5% (Rahmianna et al., 2007a). The storage system practiced by retailers in the traditional market at Pasuruan (*i.e.* storing the grains in opened containers during the trading hours under ambient temperature) favored high incidence of damage seeds. Ginting (2006) concluded that when seeds were stored in opened containers under ambient temperature for 4 months generated huge damaged seeds by 80.9% compared to those kept in the 0.05 mm and 0.08 poly propylene bags with only 1.3 and 1.9% of damaged seeds, respectively. Seeds that were exposed to environment with free air rich in oxygen in the traditional market was stimulate the activity of microorganism both fungi and pest. Also, improper post harvest handling such as shelling (both manually and machinery) contributed to testa and/or cotyledons damage. This physical damage did not only found in peanut seeds obtained from in retailer level but that also found in wholesale level.

High *A. Flavus* infected seeds obtained from traditional market were also found in peanut seeds collected from traditional markets at Banjarnegara (Rahmianna et al., 2007), Pati (Dharmaputra et al., 2003), and Wonogiri regencies (Dharmaputra et al., 2004). In this monitoring study, high incidence of *A. flavus* infection in peanut obtained from supermarket was surprisingly. Since *A. flavus* is a soil borne fungus, the spores of *A. flavus* infected the pods during pre-harvest. The physical condition of pods and the intactness of testa plays an important role in entering the spores into the seeds and the development of hyphae onto the seeds, respectively (Mehan et al., 1983, Cole et al., 1985; Van Eden et al., 1994). Generally the fungal infection was getting serious when the seeds stayed in the marketing chains with poor storage condition as pointed out by high number of seeds

infected by *A. flavus*. In contrast, the number of seeds infected by *A. flavus* was very low (4.8-5.6% in average) at harvesting time in the farmer's field at Banjarnegara: (Rahmianna et al., 2007). According to Lubulwa & Davis (1994) aflatoxin content of 1000 ppb resulted in acute liver damage to human, livestock and poultry. High contamination at retailer level also happened in Cote-d'Ivoire of Africa (Pollet et al., 1992) as aflatoxin contamination built up during storage and transportation (Yameogo & Kassamba, 1999).

Based on the data obtained in all market points during six times of sampling undertaken both in dry and wet seasons, aflatoxin contamination was directly governed mostly by seed moisture content rather than by the number of seeds infected by *A. flavus* and physical quality (*i.e.* damaged seeds) as pointed out by correlation coefficient values of 0.517, 0.384 and 0.194 respectively.

Theoretically, aflatoxin production was governed by the level of *A. flavus* infection. Seed damage, among others, as a result of *A. flavus* infection, also played a significant role in aflatoxin production. The production of this secondary metabolite was also influenced by seed moisture content. All these theories were found significant in peanut seeds obtained from supermarket. These phenomena did not find in peanut seeds obtained from retailers and wholesalers. Air-tight container absolutely prevents the seeds from the interference of surrounding environment and air-conditioned storage room warrants the stable air temperature and relative humidity. Rahmianna et al. (2007) revealed the positive correlation between seed moisture content, which was from 4.2 to 17.6%, in line with the moisture content which risky to aflatoxin contamination by 10-30% (Crop Link, 2000) with aflatoxin contamination. The positive correlation was also significant between *A. flavus* infection and aflatoxin, both for peanut obtained from farmer's level.

Kernel moisture content most of peanut kernels sampled in wholesale and retail outlets was lower than 10% at most of the sampling times and therefore the incidence of aflatoxin contamination was also low or under the safe level of contamination. Despite a substantial proportion of damaged kernels both caused by mechanical and biological damage in seed lots obtained at each sampling time, these two elements did not govern the level of aflatoxin contamination. Time of sampling throughout the year did not affect the level of aflatoxin contamination. Under controlled condition generated from air-tight container and controlled condition, the influence of *A. flavus* infection and seed moisture content on aflatoxin production was significant.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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