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# *ASPERGILLUS FLAVUS* POPULATION AND AFLATOXIN $B_1$ CONTENT IN PROCESSED PEANUT PRODUCTS IN MUNICIPALITY OF BOGOR, WEST JAVA, INDONESIA

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## ABSTRACT

The objective of this study was to document the population of *A. flavus* and aflatoxin $B_1$ content of five processed peanut products collected from different retailers in *Kecamatan Bogor Tengah* (Subdistrict of Central Bogor), Municipality of Bogor. A total of 129 samples of processed peanut products were collected. The products consisted of roasted nut-in shell peanuts (33 samples), flour-coated peanut kernels (33), *siomay* sauce (18), *pecel/gado-gado* sauce (33) and *satai* sauce (12). Sample size varied from 2000 g for roasted nut-in shell peanuts and flour-coated peanut kernels, to 1500 g for *siomay* sauce, *pecel/gado-gado* sauce as well as *satai* sauce samples. The samples were mixed homogeneously. It was then divided into two parts manually, and then each part was also divided into two parts to obtain working samples to determine *A. flavus* population, AFB1 content and a reserve sample. Kernels of roasted nut-in shell peanuts and flour-coated peanut kernels were obtained by shelling their skin pods and removing the seed coat and the batter coat of tapioca flour manually, respectively. *Aspergillus flavus* in peanut processed products was isolated using a serial dilution method, followed by pour plate method on Aspergillus Flavus and Parasiticus Agar (AFPA). $AFB_1$ content was determined using Thin Layer Chromatography method. Two replicates were used for each sample.The results showed that the population of *A. flavus* in roasted nut- in shell peanuts, flour-coated peanut kernels, *siomay* sauce, *pecel/gado-gado* sauce and *satai* sauce were 0.3, 0.1, 0.3, 13.2 and 0.4 cfu/g (wet basis), respectively. The highest $AFB_1$ content of 43.2 ppb was found in roasted peanut nut-in shell, followed by flour-coated peanut kernels (34.3 ppb), *satai* sauce (23.2 ppb), *pecel/gado-gado* sauce (17.1 ppb) and *siomay* sauce (4.4 ppb).

**Key words:** *Aspergillus flavus,* aflatoxin $B_1$, processed peanut products, Municipality of Bogor

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## INTRODUCTION

In Indonesia peanuts (*Arachis hypogaea*) is the third most important secondary crop after maize and soybean (BPS 2013). They are consumed as human dietary supplements and processed into various snack foods such as, roasted nut-in shell peanuts, flour-coated kernels, siomay, *gado-gado* and *satai* sauces. According to BPS (2010) in Indonesia in 2009 the production of peanuts was 763 507 tonnes.

In tropical and humid countries such as Indonesia, fungal infection can occur before as well as after harvest. According to Sauer *et al.* (1992) fungal infection after harvest could decrease the physical quality and nutritional content, discolouration of seeds (grains) and mycotoxin production, among others aflatoxin. Aflatoxin can cause liver cancer in human and domestic animals, produced by certain strains of *Aspergillus flavus*. There are four kinds of aflatoxins which are generally found in foodstuff and their processed products, i.e. aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$. The most dangerous of the toxins is AFB1.

Lilieanny*et al.* (2005) reported on the percentage of *A. flavus* infection and total aflatoxin content in roasted nut-in shell peanuts (47 samples), flour-coated peanut kernels (22), *pecel* sauce (12), and *enting-enting gepuk* (4) obtained from several factories, supermarkets, and traditional markets in Bogor, Malang, Pati and Yogyakarta. The percentages of samples infected by *A. flavus* in roasted nut-in shell peanuts, flour-coated peanut kernels, *pecel* sauce and *enting-enting gepuk* were 38.3, 27.3, 50.0 and 100%, respectively. Total aflatoxin content in the products was 1.8, 5.2, 41.6 and 20.8 ppb, respectively. In Indonesia the maximum tolerable limit of AFB1 content in peanuts and their processed products is 15 ppb (SNI 2009).

The objective of this study was to investigate the population of *A. flavus* and $AFB_1$ content of five processed peanut products collected from retailers in *Kecamatan Bogor Tengah* (Subdistrict of Central Bogor), Municipality of Bogor.

## MATERIALS AND METHOD

### Processed Peanut Products

Based on rankings given by 342 respondents in 11 *kelurahan* subdistricts of Central Bogor who consumed 11 processed peanut products, roasted nut-in shell peanuts was ranked as first choice, followed by flour-coated peanut kernels; *siomay*, *pecel*/*gado-gado* and *satai* sauces (Dharmaputra *et al.* 2010).

### Location of Sampling

The five processed peanut products were obtained from 11 shops in *kelurahan* (subdistrict) of Central Bogor from 23 July until 21 August 2009. Subdistrict of Central Bogor was selected for conducting survey and sampling, because it is the most populated subdistrict in the Municipality of Bogor. Roasted nut-in shell peanuts, flour-coated peanut kernels and *pecel*/*gado-gado* sauce were obtained from *warung* (small shops). *Siomay* was obtained from traveling salesman, *satai* was obtained from both

*warung* and traveling salesman. *Pecel* and *gado-gado* are salad made from cooked vegetables. *Satai* is small pieces of meat roasted on skewer. *Siomay* is steamed ravioli filled with meat and open on the top. *Pecel*, *gado-gado*, *satai* and *siomay* are served with peanut sauces.

**Sampling and to Obtain Working Samples**

A total of 129 processed peanut products were collected. They consisted of roasted nut-in shell peanuts (33 samples), flour-coated peanut kernels (33), *siomay* sauce (18), *pecel*/*gado-gado* sauce (33), and *satai* sauce (12). Roasted nut-in shell peanuts and flour-coated peanut kernel samples were a labeled products, while pasta sauces samplings were collected randomly without knowing their processing and their sanitations during processing. At the time of purchasing, the sauces of *siomay*, *pecel*/*gado-gado* and *satai* were packed separately from the main materials. Locations of sampling, the kinds and numbers of processed peanut products are presented in Table 1.

Each sample consisted of five portions of processed peanut products in the form of pasta (*pecel*/*gado-gado*, *siomay* and *satai* sauces) and 2 kg (100 @ 20 g packets) of roasted nut-in shell peanuts and flour-coated peanut kernels. One portion of gado-gado, siomay and satai sauces contained 75, 50 and 60 g of peanut sauces, respectively. Each sample was mixed manually and homogeneously, and it was then divided into two parts manually. Each part was also divided into two parts to obtain working samples for the determination of *A. flavus* population and AFB1 content, and a reserve sample. Peanut kernels of roasted nut-in-shell peanuts and flour-coated peanut kernels were obtained by shelling their skin pods and peeling the batter coat of tapioca flour manually, respectively.

Table 1. Location of sampling, kinds and number of processed peanut product samples

| Location of sampling | Number of samples | | | | | Total |
|---|---|---|---|---|---|---|
| | Roasted nut-in shell peanuts | Flour-coated peanut kernels | *Siomay* sauce | *Pecel*/ *gado-gado* sauce | *Satai* sauce | |
| *Kelurahan* Tegallega | 3 | 3 | 2 | 3 | 1 | 12 |
| *Kelurahan* Babakan | 3 | 3 | 2 | 3 | - | 11 |
| *Kelurahan* Sempur | 3 | 3 | 3 | 3 | 2 | 14 |
| *Kelurahan* Panaragan | 3 | 3 | 1 | 3 | 3 | 13 |
| *Kelurahan* Gudang | 3 | 3 | 1 | 3 | 3 | 13 |
| *Kelurahan* Kebon Kelapa | 3 | 3 | 2 | 3 | 1 | 12 |
| *Kelurahan* Ciwaringin | 3 | 3 | 1 | 3 | - | 10 |
| *Kelurahan* Cibogor | 3 | 3 | - | 3 | - | 9 |
| *Kelurahan* Babakan Pasar | 3 | 3 | 1 | 3 | - | 10 |
| *Kelurahan* Pabaton | 3 | 3 | 2 | 3 | 2 | 13 |
| *Kelurahan* Paledang | 3 | 3 | 3 | 3 | - | 12 |
| Total | 33 | 33 | 18 | 33 | 12 | 129 |

Note : - = No sample

**Determination of *Aspergillus flavus* Population**

*Aspergillus flavus* was isolated using serial dilution method followed by plating method on Aspergillus Flavus and Parasiticus Agar (AFPA) (Pitt *et al.* 1983, 1992). The kernels derived from roasted nut-in-shell peanuts and flour-coated peanuts were ground. A 25 g of ground sample was placed in an Erlenmeyer flask (volume 500 mL), and then sterile distilled water was added to make up the volume to 250 mL. This process resulted in dilution of 1 : 10. The Erlenmeyer flask with the suspension was shaked vigorously using a shaker KOTTERMANN 4020 for two minutes as much as 250 times to obtain a homogeneous suspension. A 10 mL of the suspension was taken using a volumetric pipet, then it was placed in a separate 250 mL Erlenmeyer flask containing 90 mL distilled water, to make a 1 : 100 dilution. The same step was repeated to obtain a serial dilution of 1 : 1 000.Two replicates were used for each sample.

A 1 mL of each dilution of each sample was transferred in a Petri dish (9 cm in diameter) using a volumetric pipet, then 15 mL AFPA media (45°C) was poured into the dish. Three Petri dishes were used for each dilution . The Petri dishes were shaked manually to obtain a homogeneous dispersion of the suspension in the media, they were then incubated at room temperature (28°C) for 4 days. Population of *A. flavus* per gram kernels of roasted nut in-shell peanuts or that of flour-coated peanut kernels; *siomay*, *pecel/gado-gado* or *satai* sauces (based on wet basis, w.b.) from each replicate was determined using the following formula:

$$AFP = \frac{1}{X \times Y} \text{ cfu/g (w.b.)}$$

Where:

AFP = *Aspergillus flavus* population per gram each processed peanut product per replicate
X = Volume of each processed peanut product suspension placed in each Petri dish
Y = Dilution which gives *A. flavus* colony separately
Z = Mean of colony number of *A. flavus* from three Petri dishes

**Determination of AFB1 Content**

AFB1 content was determined using Thin Layer Chromatography (AOAC 2005). This method is applicable to determine 5-25 ng/g AFB1 in processed peanut products. Dilution is needed for higher concentration of AFB1. Limit of detection for this method is 0.5 ng/g, while that of recovery and precision are 87-101% and 12.6%, respectively (as RSD intra laboratory study). AFB1 stock standard solution 1000 ppm was made from 1 mg crystalline AFB1 using 1 mL methanol. Lower concentrations of AFB1 (100 and 20 ppm) was prepared from 1000 ppm stock solution and were verified using a spectrophotometer (AOAC Official Method 970.44). Extraction: as much as a mixture of 1100 g of kernels of roasted nut-in shell peanuts or flour-coated peanut kernels of each sample, 1500 mL distilled water and 22 g NaCl were ground using a blender for three minutes. A 200 g of this mixture was then packed in a polyethylene bag and stored in a freezer as retain samples. About 130 g of each kernel sample which has been in the form of pasta and each sample of processed peanut

products in the form of sauces was placed in a 250 mL Erlenmeyer flask. Fifty mL NaCl 2.2%, 150 mL methanol p.a. and 100 mL n- hexane were added and the mixture was stirred using a magnetic stirrer for 30 minutes. It was then left for 30 minutes to obtain a good separation.

As much as 25 mL methanol phase was taken using a volumetric pipette, it was then filtered and placed in a separated funnel (volume 250 mL). This part was extracted using 25 mL chloroform p.a. After separation, chloroform fraction in bottom layer was placed in a 100 mL vial. The liquid as the result from extraction was evaporated until it was almost dried. The obtained residue was dissolved again using chloroform p.a., it was then transferred into a vial and re-evaporated. Before processing for TLC, the residue left after evaporation was dissolved again using 500 uL chlorofom solution p.a.

Identification was carried out using a chromatography tank containing eluent, i.e. chloroform p.a. : acetone p.a. (9 : 1). A 5 and 10 uL samples of aliquots were spotted on chromatography plate using a 10 uL microsyringe. On the same plate AFB1 standard solutions 1-10 uL were also spotted. The known concentrations of aflatoxin $B_1$ standards used were also spotted between 1-4 uL. Chromatography plate was placed in chromatography tank containing eluent, it was then eluted from the bottom up to the top until the eluent attained the limit of the top. The result of elution was then dried using a hair dryer and was observed under long wave (365 nm) ultra violet length.

Qualitative test was conducted by comparing the retention factor (RF) of sample and standard spots, while quantitative test was carried out by comparing the fluorescence intensity of sample spot and standard spot. If AFB1 was not detected, the fluorescence intensity of sample spot was compared with that of standard spot. If the fluorescence intensity of sample spot was too intense to match the standards, the sample extracts should be diluted and re-chromatographied.AFB1content was determined using the following formula:

$$\text{Aflatoxin } B_1 \text{ content (ppb)} = \frac{S \times Y \times V \times fp}{W \times Z}$$

Where:

S = volume of AFB1 standard (μL) which gives fluorescence equivalent with Z uL of sample
Y = concentration of standard AFB1 (μg/mL)
Z = volume of sample extract (μL) required to give fluorescence equivalent with S μL AFB1 standard
W = weight (g) of extracted sample
V = volume of the solvent (μL) required to diluted final extract
fp = dilution factor 150/25.

## RESULTS AND DISCUSSION

### *Aspergillus flavus* Population and AFB1 Content of Processed Peanut Products.

The percentage of roasted nut-in-shell peanuts and flour-coated peanut kernels; *siomay*, *pecel*/*gado-gado* and *satai* sauce samples infected by *A. flavus* were 15.2, 6.1, 5.6,

Table 2. Percentage of processed peanut product samples infected by *A. flavus*, contaminated by aflatoxin $B_1$, and non-detected aflatoxin $B_1$ content

| Processed peanut products | Number of sample | Number (%) of samples infected by *A. flavus* | Number (%) of sample contaminated by AFB1 | Number (%) of non-detected AFB1 content samples (0.0 ppb)* |
|---|---|---|---|---|
| Roasted nut-in shell peanuts | 33 | 5 (15.2) | 14 (42.4) | 19 (57.6) |
| Flour-coated peanut kernels | 33 | 2 (6.1) | 10 (30.3) | 23 (69.7) |
| *Siomay* sauce | 18 | 1 (5.6) | 2 (11.1) | 16 (88.9) |
| *Pecel/gado-gado* sauce | 33 | 19 (57.6) | 9 (27.3) | 24 (72.7) |
| *Satai* sauce | 12 | 1 (8.3) | 2 (16.7) | 10 (83.3) |

* = AFB1 content < detection limit for AFB1 (0.5 ppb) using Thin Layer Chromatography method

Table 3. Range and mean of *A. flavus* population, aflatoxin $B_1$ content, and percentage of processed peanut product samples containing aflatoxin $B_1$ exceeded 15 ppb

| Processed peanut products | Range (mean) of *A. flavus* population (cfu/g (w.b)) | Range (mean) of AFB1 content (ppb) | Samples containing AFB1 exceeded 15 ppb (%) |
|---|---|---|---|
| Roasted peanuts with skin pods | 0.0 – 3.3 (0.3) | 0.0 – 316.8 (43.2) | 42.4 |
| Flour-coated peanuts | 0.0 – 1.7 (0.1) | 0.0 – 160.0 (34.3) | 30.3 |
| *Siomay* sauce | 0.0 – 5.0 (0.3) | 0.0 – 39.9 (4.4) | 11.1 |
| *Pecel/gado- gado* sauce | 0.0 – 255.0 (13.2) | 0.0 – 197.8 (17.1) | 21.2 |
| *Satai* sauce | 0.0 – 5.0 (0.4) | 0.0 – 198.6 (23.2) | 16.7 |

57.6 and 8.3%, respectively, while those contaminated by $AFB_1$ were 42.4, 30.3,11.1, 27.3 and 16.7%, respectively. The percentage of processed peanut product samples infected by *A. flavus* and those of non-detected $AFB_1$ contents are presented in Table 2.

All product types were infected by *A.flavus*, but the highest population of *A. flavus* was found in *pecel/gado-gado* sauce, followed by *satai* and *siomay* sauces, roasted nut-in shell peanuts and flour-coated peanut kernels. The range and the mean of *A. flavus* population, AFB1 content and the percentage of processed peanut product samples contaminated by AFB1 exceeded 15 ppb are presented in Table 3.

AFB1 contents in processed peanut products were varied. The mean of highest AFB1 content was found in roasted nut-in shell peanuts (43.2 ppb) (Table 3). The percentage of roasted nut-in shell peanuts samples containing AFB1> 15 ppb was also 42.4%. The percentage of processed peanut product samples contaminated by AFB1exceeded 15 ppb were as follows: roasted nut-in shell peanuts 42.4%, flour-coated peanut kernels 30.3%, *siomay* sauce 11.1%, *pecel/gado-gado* sauce 21.2%, and *satai* sauce 16.7%.

Fungi can be killed by heating, while aflatoxins could not be easily degraded by heating, because they have high melting point. The melting point of AFB1 is 267°C (Buchi & Rae 1969). Therefore, AFB1 was able to be detected on the processed peanut products used in this study, although they were heated during processing (Table 2). Although the percentage of samples infected by *A. flavus* was low, the processed peanut products could be contaminated by AFB1 due to the toxin produced during post-harvest handling (drying and shelling). AFB1 can only be produced by toxigenic strains of *A. flavus*. AFB1 contents were lower than 0.5 ppb in some samples.

The mean of highest *A. flavus* population was found in *pecel/gado-gado* sauce, followed by *satai* and *siomay* sauces, roasted nut-in shell peanuts and flour-coated peanut kernels (Table 3). Dharmaputra *et al.* (2010) reported that *A. flavus* population in raw peanut kernels collected from two traditional markets in Bogor was as high as 4865.8 cfu/g wet basis. In this study *A.flavus* population in the processed peanut products were lower than those in raw peanut kernels, because the kernels were heated during processing.

*Aspergillus flavus* population in *pecel/gado-gado*sauce was much higher compared to that in the other processed peanut products (Table 3). At the time of purchasing, *pecel/gado-gado* sauce was not ready to be consumed, but it was prepared by the seller when people come to buy *pecel/gado-gado*. It was assumed, that *A. flavus* was not only derived from the peanut kernels, but it also from the ingredients and tools that could have been contaminated, for example, a mortar to grind the peanut kernels or a glass jar to store the kernels could be the source of contamination.The level of *A. flavus* population in the products could also be due to the sanitation during processing, which was not tested in this study. The mean of *A. flavus* population in roasted nut-in shell peanuts and flour-coated peanut kernels, *siomay* and *satai* sauces were very low, i.e. < 1 cfu/g (w.b.), because raw peanut kernels were already heated.

*T*he roasted nut-in shell peanuts had high aflatoxin levels as a consequence aflatoxin production could have occurred in the post-harvest storage *Aspergillus flavus* was still found in processed peanut products, although they were heated during processing. Certain fungal species have dormant structures. Fungi can also contaminate processed peanut products during production, packaging and transportation.

The mean of highest AFB1 content was found in roasted nut-in shell peanuts, i.e. 43.2 ppb (Table 3). AFB1content was affected among others by the quality of the peanuts and the method of processing.

Bankole and Eseigbe (2004) reported that 43.4% of 106 fried peanuts without oil samples collected in Nigeria were infected by *A. flavus*. The range of AFB1 content of 64.2% of all samples positive contaminated by the toxin was 5 - 106 ppb. The peanuts were in the form of kernels and they were processed traditionally by roasting using hot sands on fire place made from clay.

## CONCLUSIONS

The population *of A. flavus* in roasted nut-in shell peanuts, flour-coated peanut kernels*, siomay, pecel/gado-gado* and *satai* sauces were relatively low (0.1-13.2 cfu/g wet basis). The highest AFB1 content was found in roasted nut-in shell peanuts (43.2 ppb), followed by flour-coated peanut kernels (34.3 ppb), *satai* sauce (23.2 ppb), *pecel/gado-gado* sauce (17.1 ppb) and *siomay* sauce (4.4ppb). To minimize aflatoxin contamination in processed peanut products, it is important to conduct a good handling practice from farmer up to table.

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