NATURAL OCCURANCE OF FUNGI AND AFLATOXINS OF PALM KERNEL CAKE IN MALAYSIA: A PRELIMINARY STUDY

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ABSTRACT

Palm kernel cake (PKC), a by-product of palm oil is produced throughout the year in Malaysia and is used as animal feed especially for ruminants and poultry. The tropical climate in Malaysia is a conducive factor for aflatoxin-producing fungi growth. Aflatoxins contamination of PKC represents a serious food safety hazard. The aim of this study was to evaluate the fungal contamination and the presence of aflatoxins in PKC sampled in Malaysia. PKC samples were collected at selected PKC mills in Malaysia. These samples were cultured for fungal identification and analyzed for aflatoxins. The common fungi isolated from the PKC were Penicillium sp., Aspergillus sp., Fusarium sp., Mucor sp. and Rhizopus sp. All PKCs were analyzed for aflatoxins, with a detection below 1.0 µg/kg, hence complying with the requirements of maximum permissible limit (20 µg/kg) by FDA and European Union. Since the isolated aflatoxigenic fungi such as Aspergillus sp. can be detected in PKC it is recommended that a good storage management practice should implemented to reduce potential toxin risk.

Keyword: *Palm kernel cake, fungi, aflatoxins*

1. INTRODUCTION

Contamination of agricultural commodities with toxin-produced fungi is a worldwide concern. Discoloration, quality deterioration, reduction in commercial value and mycotoxin production have been linked to moldy contaminated foods and feeds [1]. Mold contamination will not only lead to great economic losses, can also cause fatal acute illness, and are associated with increased in cancer risk [2]. Every year, a significant percentage of the world's grain crops are contaminated with hazardous mycotoxins such as aflatoxins. Most countries have established maximum tolerated level for total aflatoxins ranging from 4-20 ng/g [3].

Aflatoxins are abundant and the most toxic mycotoxins. It can be found in food and feed commodities such as grains, peanuts, cotton seeds and protein sources such as rapeseed meal, cottonseed meal, soy bean meal, sunflower meal, corn gluten meal, copra meal and palm kernel meal [4]. Aflatoxins are harmful to human and animals such as fish, rodents, cattle, pigs, waterfowl and birds [5]. Aflatoxins are acute toxin, immunosuppressive, carcinogenic, teratogenic and mutagenic compounds. Aflatoxin is listed as carcinogenic in group I by IACR and WHO [6]. Although 20 types of aflatoxins have been identified, only 4 main types (B1, B2, G1 and G2) can contaminate foods [7]. According to FDA, the maximum limit of aflatoxin is 20 ppb and the consumption of foodstuff with level higher than 20 ppb is harmful for animals and may cause reduced milk production, loss of weight, rectal prolapsed, premature delivery and liver damage [8] [9].

The issues of aflatoxin negative effects have been reported to be more serious in tropical and subtropical regions of the world where climatic conditions of temperature and relative humidity favor the growth of Aspergillus flavus and Aspergillus parasiticus. Malaysia's climate is categorized as equatorial, being hot and humid throughout the year.

This condition is conducive for the growth of fungus and favorable for natural aflatoxin contamination of foods to occur.

Malaysia is the second largest manufacturer and exporter of palm kernel cake (PKC). PKC is obtained from palm kernel after the oil has been extracted by screw pressing method. It is an excellent source of protein and energy for livestock especially ruminants. In Malaysia, PKC is included in the feed for cattle up to 80% of the total ration and 50% for dairy cattle [10]. However, non-ruminants lack the ability to digest fibers in PKC as analysis showed that more than 60% of PKC is cell wall components that consists of 58% mannan, 12% cellulose and 4% xylan [11]. Thus, recommended level of PKC inclusion in non-ruminants diet such as swine, poultry and fish is only about 20% of the total ration [10].

Aflatoxin contamination of palm kernel cake has gained global significance due to its deleterious effects on human and animal health, and subsequently affecting the international trade. This contamination of carcinogenic agents can occur in the oil palm crop during the entire crop growth period and during storage. Both biotic factors such as soil insects and soil pathogens, and abiotic factors such as end-of-season drought and soil temperature during crop growth can lead to aflatoxin contamination.

The risk for the development of aflatoxin is greatest during major droughts. When soil moisture is below normal and the temperature is high, the number of Aspergillus spores in the air increases. These spores infect crops through areas of damage caused by insects, and inclement weather [12]. Improper post-harvest handling and storage of the produce such as high moisture, temperature and insect or mechanical damage can also influence the incidence and cause contamination [13] [14].

However, regular monitoring is crucial to ensure the safety of animals' or poultries' feeds. The purpose of this study is to determine mycoflora and total aflatoxin in collected PKCs from selected mills in Malaysia.

2. MATERIAL AND METHODS

2.1 Collection of Samples

A total of 12 PKC samples were collected from selected PKC Mills in Malaysia. The samples were labeled and packed in sterile polyethylene bags. Samples were used to assess any possible fungal contaminations, aflatoxin determination, and fungal identification. The samples were stored at 4°C until the beginning of laboratory analysis.

2.2 Isolation and Identification of Fungi

Introduction Fungal isolation was carried out using the method described by Jonathan and Olowolafe [15]. One gram of each sample was grinded and separately diluted serially in sterile distilled water. About 0.1 mL of each dilution was seeded into Petri dishes containing sterile potato dextrose agar (PDA) in which of chloramphenicol (30 mg/L) has been added to suppress the growth of bacteria. The plates were incubated at ambient room temperature (25 – 30 ^{0}C) for 7 days and the fungi developed were purified by repeated streaking on PDA. After the incubation period, the growing fungal cultures were examined microscopically using Lactophenol Cotton Blue (LPCB) stain and the identification of fungi. Each pure culture was characterized and identified based on their morphological and microscopic characteristics using the keys of Pitt and Hockings [16] and Raper and Fennel [17]. The isolation frequency (Fr) were calculated according to Ghiasian et al. [18], Pacin et al. [19] and Saleemi et al. [20] as follows:

$$\text{Fr (\%)}: \frac{\text{Number of samples with genus or species}}{\text{Total number of samples}} \times 100$$

2.3 Determination of Aflatoxins

The aflatoxins were extracted, purified by immunoaffinity column chromatography (Aflatest-VICAM) and analyzed by high-performance liquid chromatography (HPLC) consisting of a fluorescence detector (Agilent, USA). Aflatoxins were separated on HPLC column (C-18) with a mobile phase of water: methanol: acetonitrile (4:1:1, v/v/v), excitation and emission wavelengths of 360 and 440 nm respectively, flow rate of 1.0 mL/min. The aflatoxin

concentrations in the sample extract were determined and quantified by the retention time and peak areas, respectively.

3. RESULTS AND DISCUSSION

3.1 Prevalence of Fungi in Palm Kernel Cake Samples

The purpose of the present study is to assess the fungal contamination of selected PKC samples, and to subsequently determine the possible contaminations of these samples by aflatoxins. The contamination of PKC with microscopic filamentous fungi does not necessarily result in the presence of mycotoxins. The emergence of mycotoxins depends on several factors such as relative humidity, temperature, the properties of the substrate composition, and the degree of contamination [21].

A total of 6 different fungal isolates were obtained from samples of PKC, belonged to 5 different genera (Table 1). Of all the fungi encountered, Aspergillus flavus, a potential aflatoxin producer had the highest frequency of occurrence of 66.67% while Fusarium sp. was the lowest 11.11%. These fungi are easily found in PKC because PKC can provide an excellent substrate for mold growth and mycotoxin contamination. Aspergillus sp. and penicillium sp. are frequently isolated. They are the commonest and most widespread in nature, and have been shown as fungal contaminant of African foods and feeds [22] [23] [24] [25].

High level of Aspergillus species has previously been reported by Khosravi and his colleagues [26] in Iran. The most dominant species that were isolated from animal feed samples, belonged to Aspergillus (56%), Mucor (17%), Penicillium (15%), Fusarium (6%), Cladosporium (2%) and yeast (4%). In Malaysia, Reddy and Salleh [27] showed that in 80 samples of animal feeds; Aspergillus flavus (87%), Aspergillus niger (83%), Fusarium verticillioides (47%), Fusarium graminearum (43%), Fusarium proliferatum (42%), Fusarium equisieti (30%) and Penicillium sp. (5%) were the prevalent fungi in all samples and aflatoxin B1 was detected in 18 (22.5%) samples ranging 20.6 to 135 µg/kg.

Attitallam and his colleagues [28] reported that in 20 samples of animal feeds, 10 mold genuses including: Aspergillus, Penicillum, Fusarium, Rhizopus, Mucor, Alternariam, Rhizoctonia, Pythium, Phyllactinia and Sacharomyces cerevisiae have been isolated. Mucor sp. and Rhizopus sp. obtained were the result of these feed contain higher level of carbohydrate. The high level of Mucor sp. and Rhizopus sp. found is in concomitant with studied by Infeanyi et al. [29] which showed a high frequency of these fungi in poultry feed ingredients.

Table 1: Fungal contamination in PKC and their frequency of occurrence (%)

Fungal Isolates	Occurrence	Frequency of Occurrence (%)
	Toxigenic Fungi	
Aspergillus flavus	9/12	75.00
Aspergillus niger	10/12	83.33
Penicillium sp.	6/12	50.00
Fusarium sp	3/12	25.00
	Non Toxigenic Fungi	
Rhizopus sp.	9/12	75.00
Mucor sp.	4/12	33.33

3.2 Method Validation

All analytical works were performed in triplicate to verify the accuracy of the method performance. In addition, the performance of the HPLC method was also evaluated in terms of linearity, limit of detection (LOD) and recovery studies. All relevant parameters regarding method validation are presented in Table 2. The calibration curves were linear over the evaluated range of 0.25 - 10 ng/mL. The coefficient of determination (R2) values for aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) ranged between 0.9993 and 0.9998. LOD for AFB1, AFB2, AFG1 and AFG2 were 0.16 ng/mL, 0.18 ng/mL, 0.18 ng/mL, and 0.18 ng/mL respectively. Recovery study of aflatoxins in spike PKC was more than 85%.

Table 2: **Method validation for the quantification of aflatoxins (AFs; AFB1, AFB2, AFG1, and AFG2) in palm kernel cake**

Aflatoxin	LOD (ng/mL, ppb)	R^2	Recovery (%) 0.5, 2, 5 (ng/mL, ppb)
AFB1	0.16	0.997	>85%
AFB2	0.18	0.997	>85%
AFG1	0.18	0.998	>85%
AFG2	0.18	0.993	>85%

3.3 Aflatoxin Contamination Level in Palm Kernel Cake Samples

The level of aflatoxin in the PKC samples collected in Malaysia were less than 1 µg/kg (Table 3). This indicates that the samples were fit for animal consumption (Acceptable limit of total aflatoxin is 20 µg/kg according to FDA). Previous studied by Khayoon et al. [30] that showed no aflatoxin was detected in PKC produced in Malaysia. However studied by Pranowo et al. [31] showed that the level of aflatoxin in PKC produced in Indonesia is ranging from 5.9 to 93.10 µg/kg. Reither and his colleagues [32] also showed that the level of aflatoxin in PKC from Indonesia ranging from 0.19 to 0.43 µg/kg.

Despite the fact that the total aflatoxin estimated in the PKC samples was lower than the FDA limits, the fungal contamination rate should not be neglected. Isolation of mycotoxigenic fungi such as Aspergillus sp., Penicillium sp. and Fusarium sp. is of vital importance in food industry. The present study has provided information about the contamination of toxigenic mycoflora of PKC in Malaysia. Aspergillus fungi should be of concern because of their association with aflatoxin. The presence of other contaminating fungi like Penicillium and Fusarium suggested the possible contamination by several mycotoxin. The presence of toxigenic fungi in PKC demands the adoption of effective measures to have a good storage management practice to be implemented to reduce potential toxin risk.

Table 3: **Aflatoxin production (µg/kg) in PKC samples from different region in Malaysia**

Mill Area	Number of Mill	Average Total Aflatoxin (µg/kg) ± SD
Southern Malaysia	6	0.242 ± 0.192
Central Malaysia	3	0.267 ± 0.057
Eastern Malaysia	3	0.333 ± 0.076

** SD = Standard Deviation*

4. CONCLUSIONS

In the present study, a total of 12 palm kernel cake samples were collected from Malaysia and investigated for the presence of fungal pathogens and aflatoxin contamination levels. The Aspergillus species was the most common fungal isolate in PKC samples. The samples were found to contain total aflatoxin not more than 1 µg/kg significantly lower than the permissible limits of the FDA and also fit for animal consumption. Although low levels of aflatoxin was detected in PKC samples, it is suggested that frequent monitoring program on the prevention and control of aflatoxin in animal feed industry are needed in order to assure the hygienic and nutritional quality of animal feeds as well as to ensure health and productivity of poultry/animal as well as prevent human foodborne diseases.

5. ACKNOWLEDGEMENT

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6. REFERENCES

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