

The 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. Aflatoxins contamination of PKC represents a serious health threat to humans and livestock. Therefore, regular monitoring of level of aflatoxins in PKC is crucial to ensure the safety of animals' feeds. Aflatoxins belong to a group of toxigenic secondary metabolites mainly secreted by some species of *Aspergillus*. These constituents have been clearly identified as highly toxic, mutagenic, teratogenic and carcinogenic compounds. They can enter the food chain mainly by ingestion through the dietary channel of human and livestock. Malaysia has relatively high rainfall throughout the year, tropical climate at temperatures ranging from 28°C-36°C and high relative humidity up to 80%. Stored PKC under such conditions can easily be contaminated by *Aspergillus*.

THE TECHNOLOGY

Principle

Aflatoxins from PKC are isolated using immunoaffinity chromatography (IC) and quantified using high performance liquid

chromatography (HPLC) method and fluorescence detector.

METHODOLOGY

Sample Preparation

The aflatoxins are extracted from 25 g PKC sample with 100 ml of 70% methanol and 5 g sodium chloride in a blender jar for 2 min. The homogenised samples are centrifuged at 3000 g for 5 min and the supernatant is filtered through Whatman No. 1 filter and Vicam microfibre filter. The filtered extracts (eluate) are stored at 4°C until analysis.

Analysis of aflatoxins. The four aflatoxins are separated in a C18 HPLC column. The optimum chromatographic conditions are as follows: 1 ml min⁻¹ for the mobile phase flow rate, mobile phase with a mixture of methanol:acetonitrile:milli-Q water (30:15:60 v/v/v) in an isocratic elution, injection volume of 50 µl and column oven temperature of 50°C. The fluorescence detection is set at excitation wavelength of 360 nm and an emission wavelength of 440 nm. The four aflatoxins are separated in 20 min under the above mentioned conditions.



Sample
Palm kernel cake



Aflatoxins Cleanup
Immunoaffinity column



Determination of Aflatoxins
High performance liquid chromatography

Figure 1. Procedure of aflatoxins analysis.

RESULTS

Quality Control and Quality Assurance Data of HPLC Analysis

Method Performance (HPLC)

- Calibration Curve (5 points): 0.25, 0.5, 1, 5 and 10 $\mu\text{g kg}^{-1}$ of AFB1, AFB2, AFG1 and AFG2.
- Good linearity (r^2) > 0.9998, Good recovery: > 78%.

REFERENCE

Stroka, J; Anklam, E; Jörissen, U and Gilbert, J (2000). Immunoaffinity column cleanup with liquid chromatography using post-column bromination for determination of aflatoxins in peanut butter, pistachio paste, fig paste, and paprika powder: Collaborative study. *J. AOAC International*, 83(2): 320-340.

TABLE 1. ACCURACY OF THE METHOD

Compound	Linear range ($\mu\text{g kg}^{-1}$)	r^2	LOD ($\mu\text{g kg}^{-1}$)	LOQ ($\mu\text{g kg}^{-1}$)
Aflatoxin G2	0.25 to 10	0.9998	0.18	0.60
Aflatoxin G1	0.25 to 10	0.9996	0.18	0.60
Aflatoxin B2	0.25 to 10	0.9998	0.18	0.60
Aflatoxin B1	0.25 to 10	0.9999	0.16	0.50

Notes: r^2 : coefficients of correlation; LOD: limit of detection; LOQ: limit of quantification.

Recoveries of aflatoxins in spiked samples

TABLE 2. RECOVERY METHOD

Compound	Recovery (%) (n=3)		
	0.25 $\mu\text{g kg}^{-1}$	0.5 $\mu\text{g kg}^{-1}$	10 $\mu\text{g kg}^{-1}$
Aflatoxin G2	79.4	78.1	80.4
Aflatoxin G1	82.2	84.4	81.1
Aflatoxin B2	85.1	94.4	92.4
Aflatoxin B1	91.3	94.3	87.0

ADVANTAGES

- A sensitive, simple and reliable method for the detection and quantification of aflatoxins in PKC sample; and
- The service will facilitate the palm oil industry to monitor and control the presence of aflatoxins in PKC in order to assure the hygienic and nutritional quality of PKC as animal feeds ingredient, as well as to ensure the health and productivity of animal/poultry.

CONCLUSION

The developed method is specific and reliable, and is also suitable for the routine analysis of aflatoxins in PKC. The minimum requirement amount of PKC sample is 100 g. The cost for this analysis is RM250 per sample, including sample preparation and analysis. The cost is subjected to change without prior notice.

For more information, kindly contact:

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