Recovery of Aflatoxin B1 in a Range of Food Commodities Utilizing a Matrix Resistant ELISA

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利用抗基质效应的 ELISA 方法检测一系列食品商品中黄曲霉毒素 B1 的回收率

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Introduction

Aflatoxin B1 is a well-known potent human carcinogen produced by toxigenic fungi. Given its ubiquitous presence in a wide variety of foods and beverages, aflatoxin B1 levels must be measured and monitored to prevent contaminated products from reaching the consumer. Enzyme linked immunosorbent assays(ELISAs)are frequently employed as a rapid and inexpensive method to screen samples that may contain aflatoxin B1 concentrations above the legal permissible limit. To date, many available ELISAs are limited in the range of commodities that can be tested because they are subject to matrix interferences and require an additional clean-up step.

介绍

黄曲霉毒素 B1 是由产毒真菌产生的已知的强烈的人类致癌物。由于黄曲霉毒素 B1 普遍存在于各类食品和饮料中，所以其残留水平必须得到测量和监控，以防止受污染的产品到达消费者手中。酶联免疫吸附测定（ELISA）法经常作为快速、廉价的方法用来筛查那些可能含有超过法定允许限量的黄曲霉毒素 B1 的样品。迄今为止，许多可用的 ELISA 试剂盒检测范围往往受限于特定的商品种类，主要是由于受基质干扰并需要额外的净化步骤。

purpose

The aim of this study was to evaluate if a single ELISA kit can accurately detect aflatoxin B1 in commodities that typically pose matrix interferences, including nuts, spices, and other common cooking ingredients.

目的

本研究的目的是评估单个 ELISA 试剂盒能否准确地检测具有基质干扰的多种商品，包括坚果、香料、和其他常见的烹饪材料商品中黄曲霉毒素 B1 。

Materials and Methods

材料和方法

Materials:

The aflatoxin B1 Low Matrix ELISA kit was prepared in-house (Helica Biosystems,Inc;Santa Ana ,CA). The sodium chloride (NaCl), acetonitrle (ACN), hexane, methanol(MeOH),and aflatoxin B1(AFB1) were purchased from Sigma St. louis, MO). The various comestibles (almond, macadamia, peanuts, black pepper, chili, cinnamon, coriander, ginger, and coconut) were purchased from the local markets(Santa Ana,CA).

材料：

黄曲霉毒素 B1 低基质 ELISA 试剂盒是在实验室条件下准备的（HELICA 生物系统公司，圣安娜，CA）。 氯化钠（NaCl），乙腈（ACN），己烷，甲醇（MeOH）和黄曲霉毒素 B1（AFB1）等采购自 Sigma 公司（圣路易斯，密苏里州）。各种食物样品（杏仁，澳洲坚果，花生，黑胡椒，辣椒，肉桂，香菜，生姜和椰子）从本地市场获得（圣安娜，CA）。

Methods:

Sample preparation

for the spike and recovery studies, all commodities were finely ground in a blender. Ingredients were weighed and spiked with a pure solution of AFB1 at low, medium , and high concentrations. The AFB1 spiked commodity was dried overnight at room temperature prior to extraction.

Extraction and Dilution

The samples were extracted according to the general scheme (Figure1). A mass of each sample was transferred to a vessel and combined with 80% ACN. After a brief period of mixing, the samples were centrifuged and the supernatant was collected for analysis. Sample were diluted 1:10 into PBS-T prior to running the assay.

方法：

样品制备：

在加标回收研究中，对所有的商品进行研磨。称量研磨好的商品，并掺入分别为低、中和高浓度水平的 AFB1 纯溶液。添加有 AFB1 的商品在提取之前室温下干燥过夜。

提取和稀释：

样品按照常规方案萃取（图 1）。将一定量的样品转移到容器中，并与 80％ACN 混合。经过短时间混合之后，离心样品，收集上清液用于分析。 进行测试之前，样品按 1:10 稀释入 PBS-T 中。

图 1- 常规萃取方案

Due to high oil content, the peanut samples were extracted according to the following method: 5g of peanut paste, 0.5g of Nacl,30ml of 80%MeOH, and 10ml of hexane were combined and blended continuously for 3 minutes. A portion of the sample was filtered and the filtrate was diluted 1:10 into PBS-T prior to running the assay.

由于含油量高，花生样品根据以下方法提取：将 5g 花生糊，0.5 克氯化钠，30 毫升 80％甲醇，和 10ml 己烷混合，连续摇匀 3 分钟。取适量样品进行过滤，进行测试之前将滤液按 1:10 稀释入 PBS-T 中。

ELISA Method

The ELISA was performed according to the manufacturer`s instructions (Helica Biosystems, Inc., Santa Ana, CA). Briefly, all reagents were equilibrated to room temperature. 200ul of the assay diluent was transferred into each mixing well. 100ul of the standards or samples were pipetted into the appropriate mixing wells and mixed. 100ul of mixture was transferred to the appropriate antibody-coated wells in duplicate and incubated at ambient temperature for 30 minutes. The wells were washed three times with PBS-T and tapped dry. 100ul of Aflatoxin HRP-conjugate was added to each antibody-coated well and incubated at room temperature for 30minutes . The wells were washed three times with PBS-T and tapped dry. 100ul of the TMB substrate was added to each microwell and the plate was incubated at ambient temperature for 10 minutes. 100ul of stop solution was added to each well. The optical density (OD) of each microwell was read at 450nm on an EL×800UV absorbance reader (BioTek, Winooski, VT) using a differential filter of 630nm.

酶联免疫吸附法

ELISA 实验要依据制造商的说明书（Helica Biosystems 公司，圣安娜，CA）进行操作。简单地说，使用之前所有试剂恢复至室温。将 200μL 稀释液加入到相应的混合微孔中。100μL 标准品或样品分别转移至相应的混合微孔中并进行混合。将 100μL 混合液转移到相应的抗体包被的微孔中，每份两个平行样，并在室温下孵育 30 分钟。用 PBS-T 将微孔洗涤三次并拍干。100uL 黄曲霉毒素-HRP 藕合物加入到各个抗体包被的微孔中，并在室温下孵育 30 分钟。用 PBS-T 将微孔洗涤三次，并拍干。100μL TMB 底物加入每个微孔中并室温下孵育 10 分钟。接着 100μL 终止溶液加入至每个微孔中终止反应。用 ELx800 酶标仪（BioTek, Winooski, VT ）在 450nm(630nm)波长下读取微孔板吸光度值.

Calculation and Analysis

The %B/Bo was calculated by dividing the OD for the sample by the OD for the 0ppb standard times 100 to obtain a percentage. The standard concentrations were plotted along the x-axis on a log scale. The corresponding %B/Bo values were plotted along the y-axis. Gens5 software (BioTek) was used to fit the standard curve (4-paramater logistics) and the concentration of the samples were interpolated from the standard curve. The original concentration was calculated after taking the final dilution factor into account.

计算与分析

%B/B0 的计算是通过样品的 OD 值除以 0ppb 标准品的 OD 值再乘以 100 所得的百分比。标准品浓度的对数值沿着 x 轴绘制。相应的 $\%B/B_0$ 值沿着 y 轴绘制。Gens5 软件（BioTek）

用于绘制拟合标准曲线（**四参数拟合方程**），再通过标准曲线计算出样品浓度值。计算最终结果时必须乘以稀释倍数。

The limit of detection(LOD) was calculated by taking the mean of twenty replicates of negative samples and subtracting three times the standard deviation of the negative samples to obtain a %B/Bo. The %B/Bo was used to interpolate the concentration from the standard curve to obtain the LOD concentration.

计算检测限(LOD)时首先计算出 20 次重复的阴性样品的平均值，减去 3 倍的阴性样品的标准偏差值，以获得%B/B0。用%B/B0 从标准曲线上获得 LOD 值。

% recovery was tabulated by taking the difference of the amount of AFB1 spiked into the sample and the amount of AFB1 recovered from the assay divided by the amount of AFB1 spiked into the sample multiplied by 100 to obtain a percentage. In addition, the %CV was determined for each commodity at each spike level where each food sample was tested in triplicate at each spike level.

％回收率计算是将不同浓度的 AFB1 添加到样品中，通过检测得出 AFB1 浓度并除以 AFB1 加标量再乘以 100 得出的百分比值。此外，每个商品的每个加标浓度都计算了％CV 值，其中每个食品样本每个加标浓度都进行了三次平行。

Result and Discussion

AFB1 has long been recognized as a harmful contaminant present in food supplies throughout the economically developing world(1). Several commercial ELISA kits have been developed to detect AFB1 in foods. However, a major challenge of using ELISAs for food analysis is the matrix effect, in which interfering substances from the commodity hinder enzyme activity or reduce the interaction between antibody and antigen(2).

Previously, we have developed an ELISA kit for the quantitative detection of AFB1 in corn , wheat, hay, snaplage, paprika, and pistachio with minimal interference from the various matrices. Due to the ubiquitous nature of AFB1 in a wide array of dietary staples and agricultural products, we assessed the ability of our Aflatoxin B1 Low Matrix ELISA kit to accurately quantitate the level of AFB1 in additional food products(3).

Since food matrices can exert varying effects on an assay, we first determined the limit of detection (LOD) or sensitivity of the commodities under investigation. According to Table 1, the result demonstrate minimal interference by these particular matrices. All LOD values were lower than 2.5ppb indicating that it would be possible to test these food matrices at levels above 2.5ppb.

AFB1 一直被公认为是整个经济发展中世界食物供应中存在的有害污染物（1）。已有几种商业 ELISA 试剂盒被开发以检测食物中的 AFB1。但是，ELISA 用于食品分析的一个主要挑战是基质效应，某些商品中的干扰物质阻碍酶的活性或降低抗体和抗原（2）之间的相互作用。以前，我们开发了对于各种不同的基质干扰最小的一种 ELISA 试剂盒，用于定量检测玉米，

小麦，干草，snaplage，红辣椒，开心果和花生中的的 AFB1。由于 AFB1 在膳食主食和农产品中的广泛无处不在的性质，我们评估了我们的黄曲霉毒素 B1 低基质 ELISA 试剂盒准确定量检测更多食品商品中 AFB1 的残留量。

由于食品基质对测定有不同程度的影响，我们首先确定了所研究的商品的检测限（LOD）或灵敏度。根据表 1，结果表明这些特定基质的干扰很小。所有 LOD 值均低于 2.5ppb，这表明这些样品基质可以在高于 2.5ppb 水平进行检测。

表 1- 各种商品的检测限

基质 Matrix	LOD(μg/kg)
杏仁	0.7
澳洲坚果	2.4
花生	2.1
黑胡椒	2.0
辣椒	0.9
肉桂	0.3
香菜	0.8
生姜	1.2
椰子	0.8

Since the commodities did not exhibit significant matrix interferences, we performed spike and recovery studies. The commodities under evaluation were spiked at three different levels of AFb1 and the % recovery was determined. The overall recoveries were excellent for the nuts(Table 2). The recoveries were 96.2%,120.4%, and 99.8% for almond, macadamia, and peanut, respectively. At the low 2.5ppb spike level for peanut, recovery of AFB1 was not possible suggesting that the assay will not be useful for detecting low level of AFB1 in peanut.

由于这些商品并没有表现出明显的基质干扰，我们接着进行了加标和回收率研究。这些受评估的样品中添加了三个不同浓度水平的 AFB1，并计算了回收率。坚果的平均回收率是非常好的（表 2）。杏仁，澳洲坚果和花生回收率分别为 96.2%，120.4%和 99.8%。 对于花生的 2.5ppb 低水平的加标，AFB1 回收率并不代表该测定将不能用于检测的花生的 AFB1 低浓度水平。

表 2-坚果中黄曲霉毒素 B1 回收率

基质 Matrix		**加标水平(μg/kg) Spike level[a]**	**重复性（%CV） Repeatability**	**回收率(%) Recovery**
Almond	杏仁	2.5	2.5	82.6
		5	17.6	100.5
		10	17.1	105.5
		平均	**12.4**	**96.2**
Macadamia	澳洲坚果	2.5	8.3	137.3
		5	14.7	132.2
		10	6.9	91.8

		平均	**10.0**	**120.4**
Peanut	花生	10	4.7	85.9
		20	9.5	113.8
		平均	**7.1**	**99.8**

a 每个商品每个加标浓度测试了三个平行。

b 平均回收率为所有加标水平的平均值。

Excellent recoveries of AFB1 in various spices were also obtained with the exception of cinnamon(Table 3). The overall recoveries for black pepper, chili, cinnamon, coriander, and ginger were 113.%, 92.0%,57.0%,104.2%,and 93.8%, respectively. The cause for low recovery in cinnamon is unclear, though it is possible that co- extractants in this spice may bind to and mask AFB1 detection. In addition, dried coconut was also tested and the overall recovery was excellent at 94.5%(Table 4).

各种香料都获得到了非常好的 AFB 1 回收率，除了肉桂（表 3）。黑胡椒，辣椒，肉桂，香菜，和生姜平均回收率分别为 113%，92.0%，57.0%，104.2%和 93.8%。肉桂回收率低的原因不清楚，有可能是这种香料在协同萃取过程中结合并掩蔽了 AFB 1 检测。此外，还对干椰子进行了测试，获得了非常好的平均回收率 94.5%（表 4）。

表 3- 各种调料的黄曲霉毒素 B1 回收率

基质 Matrix		**加标水平(μg/kg) Spike level[a]**	**重复性（%CV） Repeatability**	**回收率(%) Recovery**
Black Pepper	黑胡椒	2.5	11.9	117.3
		5	22.0	101.7
		10	13.7	124.7
		平均	**17.9**	**113.2**
Chili	辣椒	2.5	7.2	100.0
		5	3.6	96.0
		10	3.0	88.0
		平均	**3.3**	**92.0**
Cinnamon	肉桂	2.5	31.8	56.9
		5	4.2	63.6
		10	15.1	50.6
		平均	**9.6**	**57.0**
Coriander	香菜	2.5	4.0	99.0
		5	5.5	100.3
		10	7.9	108.0
		平均	**6.7**	**104.2**
Ginger	生姜	2.5	8.7	92.7
		5	3.3	90.0
		10	4.2	97.5
		平均	**3.8**	**93.8**

a 每个商品每个加标浓度测试了三个平行。

b 平均回收率为所有加标水平的平均值。

表 4-椰子中黄曲霉毒素 B1 回收率

基质 Matrix		加标水平(μg/kg) Spike level[a]	重复性（%CV） Repeatability	回收率(%) Recovery
Coconut	椰子	2.5	14.2	94.3
		5	6.7	92.7
		10	7.1	96.3
		平均	**6.9**	**94.5**

a 每个商品每个加标浓度测试了三个平行。

b 平均回收率为所有加标水平的平均值。

In summary, all food items exhibited minimal matrix interferences. All commodities showed excellent overall recoveries of 92.0-120.4% with %CVs of less than 18%. The only exception was cinnamon, which had a recovery of 57%.

总之，实验结果表明，所有的食物样本显示出了极小的基质干扰。所有商品均表现出非常好的平均回收率 92.0-120.4％，同时 CV％值小于 18％ 。唯一例外的是肉桂，其回收率为 57％ 。

Significance
重要意义

The data demonstrate that a single ELISA kit can be used to successfully quantify AFB1 in most commodities without the need for special extraction methods or clean-up procedures.

这些数据表明，单一的 ELISA 试剂盒可以成功地定量检测大多数商品的 AFB1，并且不需要特殊的提取方法或净化程序。

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