***Las** aflatoxinas*

Las micotoxina

Micotoxina (del griego μύκης (mykes, mukos) "hongo") es una toxina producida por un organismo del Reino Fungi que incluye setas, mohos y levaduras. La mayoría de los hongos son aeróbicos, se encuentran casi por todas partes en cantidades pequeñas debido a sus esporas, y son comunmente microscópicos. Dado que son organismos heterótrofos, deben consumir materia orgánica. Dondequiera que la humedad y la temperatura sean las adecuadas. Las micotoxinas poseen estructuras bioquímicas diversas: así pueden ser proteínas o no (como por ejemplo la penicilina, aflatoxina

Donde las condiciones son propicias, los hongos proliferan, formando colonias, y los niveles de micotoxina pueden llegar a ser altos. Las micotoxinas varían grandemente en su peligrosidad. Algunos hongos producen solamente toxinas severas en niveles específicos de humedad, de temperatura o de oxígeno en el aire. Otras son mortales, causan enfermedades o problemas de salud identificables, algunas debilitan el sistema inmune sin producir síntomas específicos, actúan como los alergénicos o irritantes, y otras no tienen ningún efecto conocido en el organismo humano. Algunas micotoxinas causan la muerte de animales del campo. La función de las micotoxinas proteger al organismo que las produce ante otros que pueden, si no matarlo, impedir su máximo desarrollo y crecimiento generando competencias. En este sentido, Fleming descubrió la penicilina como consecuencia de que los hongos que estaba cultivando en las placas de Petri inhibieron el crecimiento de bacterias a su alrededor.

Los hongos que crecen sobre los vegetales no sólo son responsables del deterioro de los mismos, sino que también producen una serie de metabolitos que actúan como antibióticos frente a otros organismos que vayan a ocupar ese nicho ecológico y que en determinadas ocasiones pueden llegar a ser ingeridos por los seres humanos, ya sea directamenteo bien porque las ingerimos con otros alimentos como carnes, leche o derivados. Las micotoxinas, son compuestos ubicuos que difieren mucho en sus propiedades químicas, biológicas y toxicológicas Lo que implica que el problema de las micotoxicosis comienza en el campo y continúa durante toda la cadena de transformación-comercialización que acaba en la mesa del consumidor. Por lo tanto, la contaminación de la fruta por hongos causa no sólo altas pérdidas después de la recolección sino que también constituye una fuente de sustancias tóxicas y peligrosas para el ser humano. Unas pocas micotoxinas se han comprobado en brotes de intoxicación animal y humana, y otras muchas se han ensayado en animales de experimentación. Se denomina micotoxicosis primaria a la que se produce al consumir vegetales contaminados, y secundaria a aquella que se produce al ingerir carne o leche de animales que comieron pastos o piensos con micotoxinas Las micotoxinas son sustancias muy específicas. Cuanto más compleja es la ruta biosintética de estos metabolitos secundarios, más restringido es el número de especies de hongos productores. Por ejemplo: las esporidesminas son formadas solamente por Pithomyces chartarum. La aflatoxina B1 es generada por tres especies estrechamente relacionadas Aspergillus flavus, Aspergillus nomius y Aspergillus parasiticus..

Las micotoxinas aparecen en la cadena de alimentos como resultado de la infección fúngica de la cosecha. Si una cosecha infectada no es comida por los seres humanos, la micotoxina sigue siendo peligrosa para la salud humana, porque la cosecha puede ser dada como alimento a los animales de granja. Las micotoxinas resisten la descomposición o no son inutilizadas durante la digestión, así que permanecen en la cadena de alimentos en carnes y productos lácteos. Incluso los tratamientos de temperatura, tales como cocinar y congelar, no destruyen todas las micotoxinas. Por ejemplo enfermedades del oidium (también se llama oidio) de Fusarium ssp en cereales, o la infección de productos almacenados.

http://es.wikipedia.org/wiki/Micotoxina

Las *aflatoxinas*

Las aflatoxinas son un grupo de sustancias producidas por algunos hongros en pequeña cantidad, como metanolito secundarios. Pertenecen al grupo de las microtoxinas..

Son de gran importancia en la industria de cereales almacenados, ya que su potencial de toxicidad es muy elevado, pueden provocar la muerte de cualquier ser vivo que consuma algún cereal infectado con alguna de las toxinas conocidas.

Actualmente se conocen unos 20 compuestos químicamente similares, de elevada toxícidad y carcinogenicidad. Las aflatoxinas fueron descubiertas en 1960 por un grupo de investigación británico. Su nombre procede de la toxina del Aspergillus flavus y fue propuesto en 1962 por sus descubridores.

http://es.wikipedia.org/wiki/Aflatoxinas

Determination of Aflatoxins in Botanical Roots by a Modification of AOAC Official MethodSM 991.31: Single-Laboratory Validation

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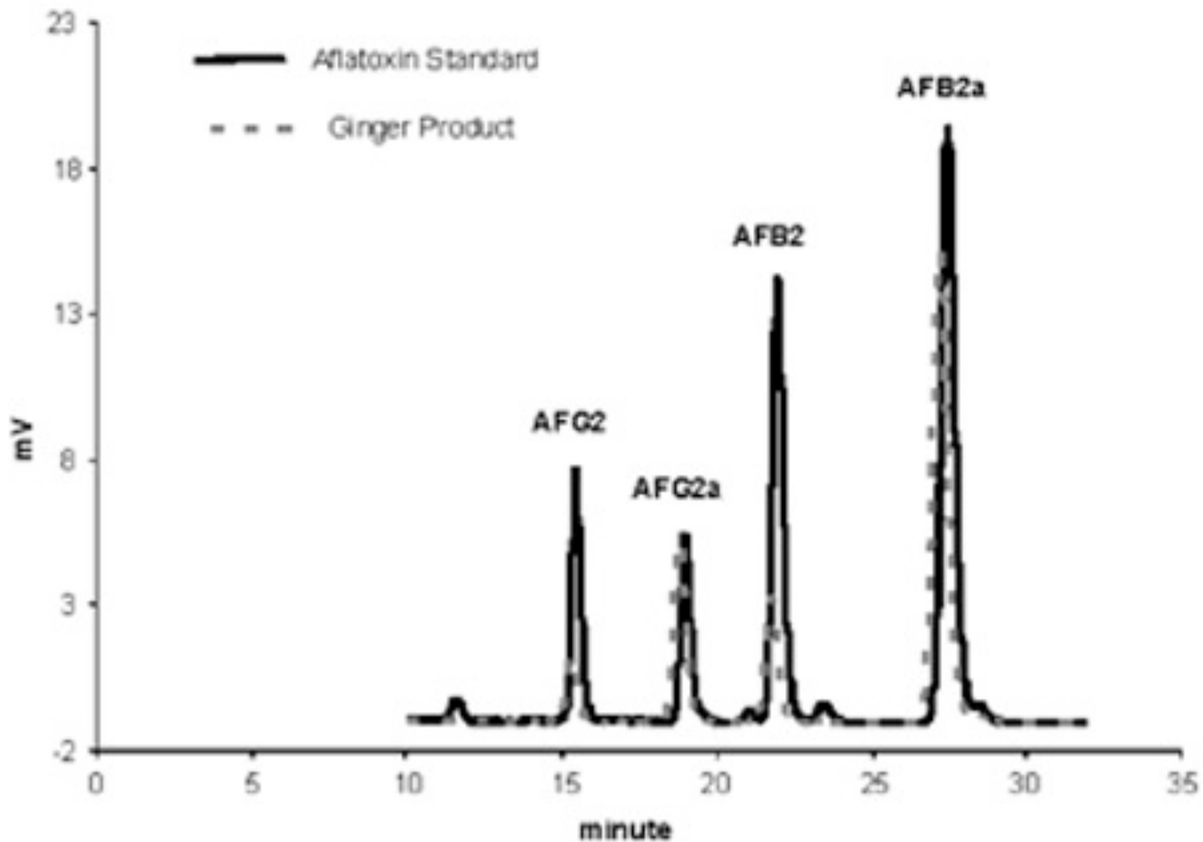
AOAC Official MethodSM 991.31 for the determination of aflatoxins (AFs; sum of aflatoxins B_1,B_2, G_1, and G_2) in corn, raw peanuts, and peanut butter by using immunoaffinity column cleanup with LC has been modified and applied to the determination of AFs in botanical roots. The modifications were necessary to improve the performance of the method for matrixes beyond corn and peanuts. The extraction solvent was changed from a mixture of methanol and water to acetonitrile and water. The accuracy, repeatability, and reproducibility characteristics of this method were determined. Replicates of 10 test portions of each powdered root (black cohosh, echinacea, ginger, ginseng, kava kava, and valerian) at each spiking level (AFs at 0, 2, 4, 8, and 16 ng/g) were analyzed on 3 separate days. Test portions were extracted with acetonitrile-water (84 + 16, v/v), and the extracts were centrifuged, diluted with phosphate-buffered saline, filtered, and applied to an immunoaffinity column containing antibodies specific for AFs. After the column was washed with water, the toxins were eluted from the column with methanol and quantified by HPLC with fluorescence detection. All test materials except kava kava were found to contain AF at <0.1 ng/g. Kava kava was naturally contaminated with AFs at 0.5 ng/g. Average within-day and between-days recoveries of AFs from botanical roots ranged from 88 to 112 and from 86 to 118%, respectively. Total RSD values for within-day and between-days repeatability ranged from 1.4 to 15.9%. HorRat values were <0.4 for all of the matrixes examined. The modified AOAC Official Method 991.31 was found to be applicable to an analysis of the six botanical roots.



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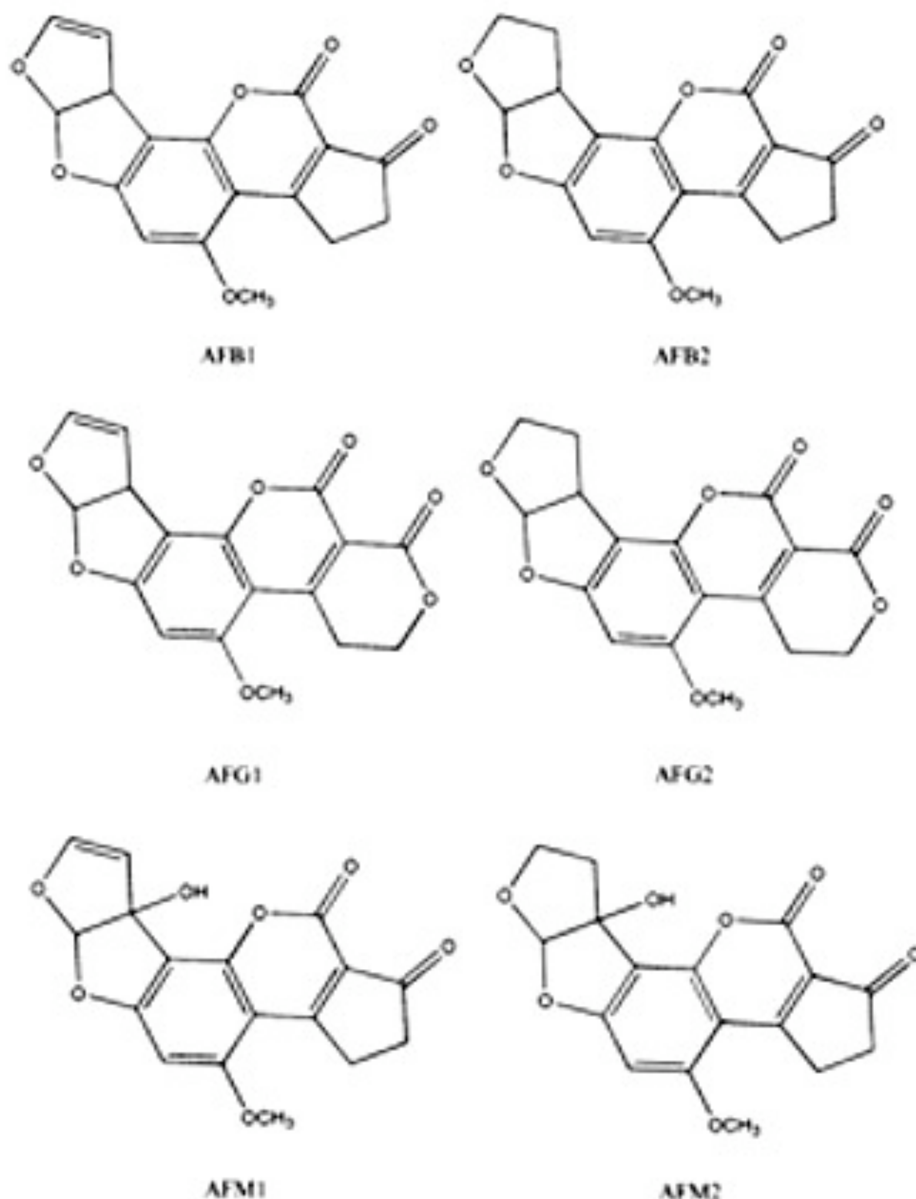
http://genemol.org/sheila/sheila-01.pdf

Detection and quantification of Aspergillus section Flavi spp. in stored peanuts by real-time PCR of nor-1 gene, and effects of storage conditions on aflatoxin production

María Alejandra Passone, Laura Cristina Rosso, Aurelio Ciancio b, Miriam Etcheverry

Aspergillus flavus and A. parasiticus are the main species from section Flavi responsible for aflatoxin accumulation in stored peanuts. A real-time PCR (RT-PCR) system directed against the nor-1 gene of the aflatoxin biosynthetic pathway as target sequence was applied to monitor and quantify Aspergillus section Flavi population in peanuts. Kernels were conditioned at four water activity (aW) levels and stored during a 4-month period. The quantification of fungal genomic DNA in naturally contaminated peanut samples was performed using TaqMan fluorescent probe technology. Sensitivity tests demonstrated that DNA amounts accounting for a single conidium of A. parasiticus RCP08300 can be detected. A standard curve relating nor-1 copy numbers to colony forming units (cfu) was constructed. Counts of species of Aspergillus section Flavi from unknown samples obtained by molecular and conventional count (CC) methodologies were compared. A correlation between cfu data obtained by RT-PCR and CC methods was observed (r=0.613; p<0.0001); and the former always showed values higher by 0.5–1 log units. A decrease of fungal density was observed throughout the storage period, regardless of the quantification methodology applied. Total aflatoxin levels ranging from 1.1 to 200.4 ng/g were registered in peanuts conditioned at the higher aW values (0.94–0.84 aW). The RT-PCR assay developed appears to be a promising tool in the prediction of potential aflatoxigenic risk in stored peanuts, even in case of low-level infections, and suitable for rapid, automated and high throughput analysis.



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http://genemol.org/sheila/sheila-02.pdf

Simultaneous determination of aflatoxins B1, B2, G1, G2, M1 and M2 in peanuts and their derivative products by ultra-high-performance liquid chromatography–tandem mass spectrometry.

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A reliable ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method for simultaneous determination of aflatoxins B1, B2, G1, G2, M1 and M2 in peanuts and their derivative products was developed. The sample was extracted by 84% of acetonitrile aqueous solution and the extract was purified by a reliable solid phase extraction-based clean-up method. Then, the analytes were separated on Acquity UPLC HSS T3 column (100mm˚ø2.1mm,1.8mparticle size), and eluted with a mobile phase consisting of (A) water containing 0.1% formic acid and (B) acetonitrile/methanol (50/50, v/v). The separated compounds were detected with a Waters Micromass Quattro Ultima Pt tandem quadrupole mass spectrometer operating in positive electro-spray ionization using multiple reaction monitoring mode. The established method was extensively validated by determining the linearity (R2 ≥0.9990), average recovery (74.7-86.8%) and precision (relative standard deviation≤10.9%).

It was shown to be a suitable method for simultaneous determination of the six aflatoxins in peanuts and their derivative products. Finally, a total of 73 samples randomly collected from different areas in Zhejiang province were screened for aflatoxins with the proposed method. The results showed that 31 samples of peanut butter, 14 samples of fresh peanut and 5 samples of musty peanut were contaminated with aflatoxins. Meanwhile, this was the first report on aflatoxins M1 and M2, which were found in unprocessed peanuts and their derivative products.

Analytica Chimica Acta 662 (2010) 62–68

http://genemol.org/sheila/sheila-03.pdf

Lipid profile of rats fed high-fat diets based on flaxseed, peanut, trout, or chicken skin

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Objective: Dietary saturated fatty acids are associated with coronary disease. Conversely, dietary monounsaturated polyunsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) seem to exert a protective effect. This study evaluated the lipid profile of rats fed high-fat (HF) diets, with fat added as different sources of PUFA (flaxseed and trout), MUFA (peanut), and saturated fatty acid (chicken skin).
Methods: Adult male Wistar rats were placed into six dietary groups (n 10): control (normal); high fat, with 1% cholesterol, 10% soy oil, and 5% lard; and four groups fed similar HF diets, with 10% lipid as trout, flaxseed, peanut, or chicken skin. After 28 d the animals were killed. Blood, livers, and adipose tissue samples were collected.
Results: A higher level (P 0.05) of total serum cholesterol was observed in rats fed the normal diet (93.57 14.95 mg/dL) compared with those fed the HF diet (67.57 12.54 mg/dL). Total cholesterol levels in rats fed the flaxseed diet were lower (P 0.05) than in rats fed the other fats. No difference was observed in cholesterol levels between groups fed the peanut and chicken skin diets (P
0.05). Animals fed the peanut diet showed decreased body weight gain than did animals in the other treatment groups. There were large lipid and cholesterol depositions in livers of rats fed the HF diet. Lipid deposition in adipose tissue followed the same dietary fatty acid profile, i.e., high levels of -3 PUFA in the flaxseed group, high levels of MUFA in the peanut and chicken skin groups and high levels of -6 PUFA in the trout group.
Conclusions: These data indicate that flaxseed is promising for dietary manipulation of hyperlipidemia. *© 2006 Elsevier Inc. All rights reserved.*

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http://genemol.org/sheila/sheila-04.pdf

Development of a Novel Immunoaffinity Column for Aflatoxin Analysis Using an Organic Solvent-Tolerant Monoclonal Antibody

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AND MOTOHIRO NISHIJIMA

An organic solvent-tolerant monoclonal antibody specific to aflatoxins B1, B2, G1, G2, and M1 (AFB1, AFB2, AFG1, AFG2, and AFM1) was prepared. In an indirect competitive enzyme-linked immunosorbent assay, the half maximal inhibitory concentration (IC50) values were 1.9, 2.1, 2.1, 2.4, and 2.8 ng/mL for AFB1, AFB2, AFG1, AFG2, and AFM1, respectively. Antibody reactivity was retained at 40% methanol concentration or at acetonitrile concentrations up to 40%. An immunoaffinity column (IAC) was prepared using agarose gel beads with bound antibody. The IAC retained the tested AFs that were 89, 90, 95, 90, and 89% for AFB1, AFB2, AFG1, AFG2, and AFM1 at 20% acetonitrile concentrations or that were 81, 87, 79, and 83% for AFB1, AFB2, AFG1, and AFG2 at 60% methanol concentrations. Roasted peanuts and seven kinds of spices were spiked with 8.0, 1.0, 6.0, and 1.0 ng for AFB1, AFB2, AFG1, and AFG2 per 1 g sample and extracted with 90% acetonitrile. The roasted peanuts and cayenne pepper out of the spices were also extracted with 70% methanol. The

extracts were diluted 5-fold with phosphate-buffered saline and applied to the IAC. The spiked aflatoxins were recovered with satisfactory rates: 78 (RSD, 2.1%) to 127% (RSD, 1.7%). The developed IAC was used for the analysis of aflatoxins in naturally contaminated samples of roasted peanuts and cayenne pepper. The newly developed IAC showed substantially organic solvent tolerance at the concentration that could not be used for existing IACs, and the column showed good ability to clean up samples for food analysis.

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http://genemol.org/sheila/sheila-05.pdf