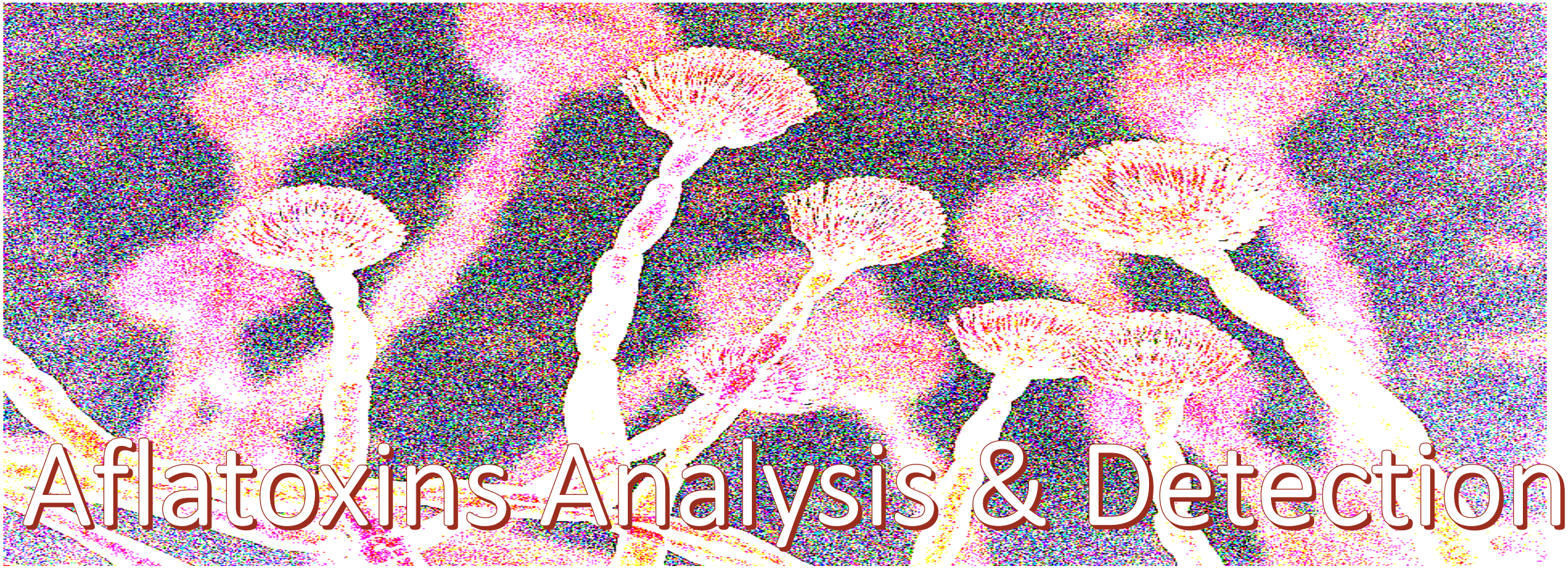

Aflatoxins Analysis & Detection

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Introduction:

In the past, fungal growth was generally treated as a regrettable nuisance which caused visible spoilage of food & feeds. (ergotism,, ATA, yellow rice syndrom).

The turning point was with the discovery of Aflatoxins in early 60s starting with the Tukey X-desease in England and isolation of the causative agent from ducklings in Uganda.

Aflatoxins, the secondary metabolites produced by *Aspergulus vlavus* & *Aspergulus parasiticus* are considered as the most potent toxin in food and agro-products.

The phrase used by scientists since early 70th. is still used to describe the health hazard posed by aflatoxin

(Aflatoxins are considered as the most potent carcinogenic, mutagenic, and teratogenic substance EVERKNOWN,,,,, and can exert it effect in ppb)

Introduction: (continued)

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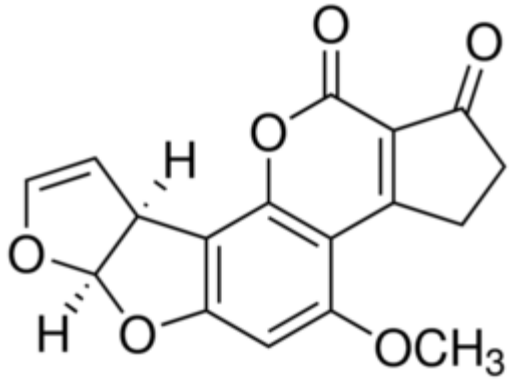
- hepatocellular carcinoma
- Nucleic acids and proteins interact covalently with aflatoxins and this results in alteration in base sequences in nucleic acids (both DNA and RNA) and in protein structures, leading to impairment of their activity.
- To inhibit protein synthesis.
- Aflatoxin B1 has also been reported to negatively impact carbohydrate metabolism, which results in both the reduction in hepatic glycogen and also the increased blood glucose levels.
- Consequently, the International Agency for Research on Cancer (IARC) of the

World Health Organization in 1987 classified aflatoxins and, in 1993, it classified aflatoxin B1 as Group 1 carcinogen.



Introduction: (continued)

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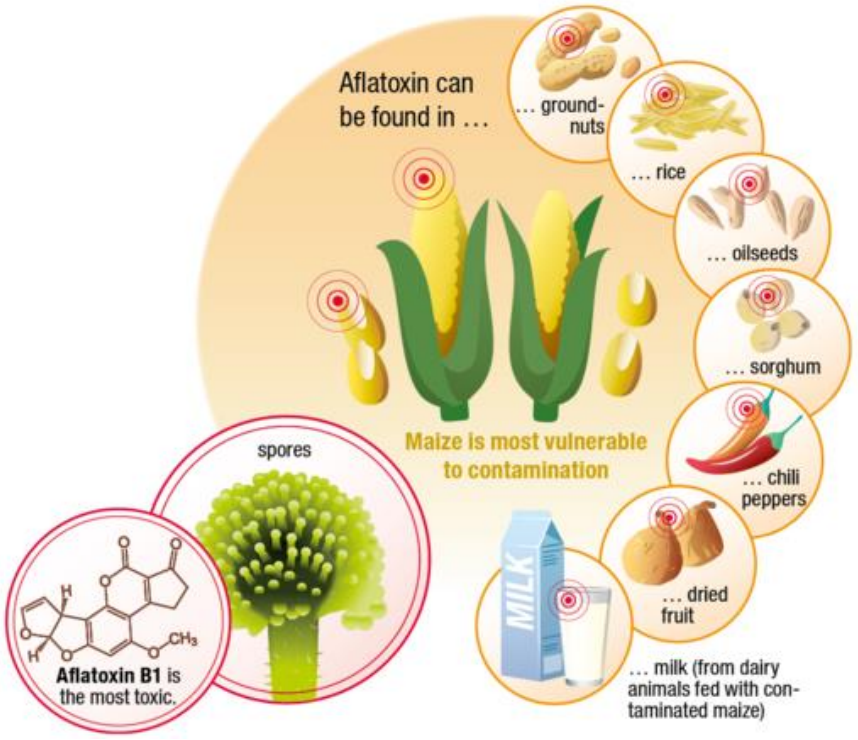
The potent toxicity and carcinogenicity of aflatoxins to laboratory animals stimulate a vast amount of research through out the world to assess the risk to Man.

Most of early research concentrated on peanuts as the crop concern, but later studies confirmed that Aflatoxins were present in a wide range of food products as a post and pre havest problem:-

- **Nuts:** ground nut, peanut butter, pistachio, walnuts, almonds, pecans, cashew nuts and hazel nuts
- **Grains:** corn, wheat, barley, sorghum, oats, rye, millet and rice.
- **Oilseeds:** cotton seeds, sunflower seeds, safflower seeds, sesame seeds, linseed, copra and coconut, palm kernel and soybean.
- **Root crops:** cassava, sweet potatoes, yam and radish
- **Miscellaneous**: figs, date fruits, spices, pulses, alfalfa hay and green coffee peans.
- **Animal products:** diary products, liver, meat, kidney, poults and egg.



Factor affecting aflatoxins production:

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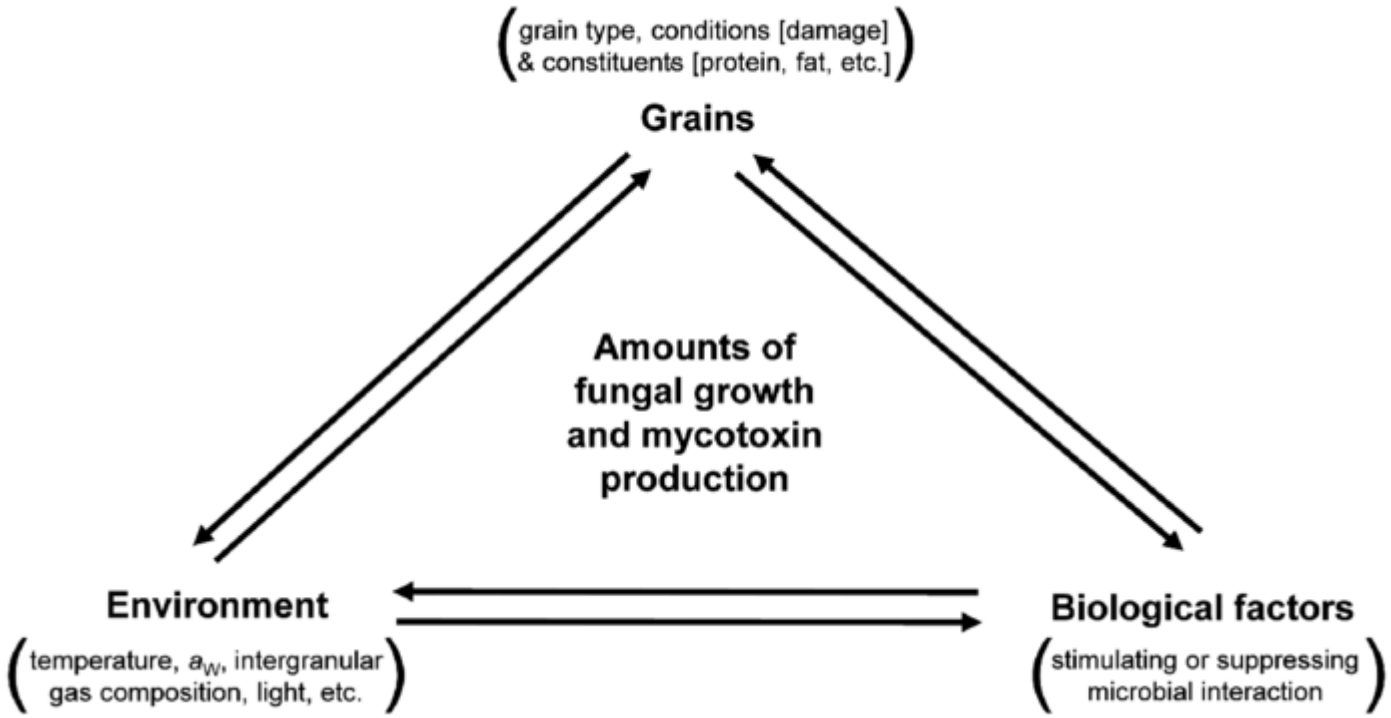
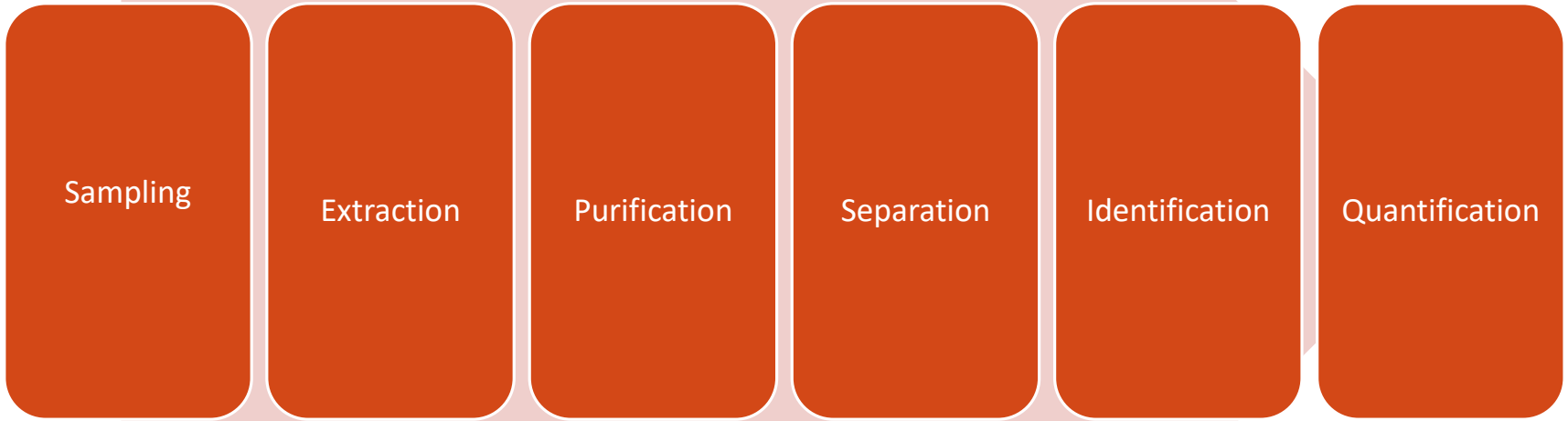
- The organism
- The Substrate
- Environmental Factors.



Steps of Alfatoxins Analysis:

- Sampling
- Extraction
- Purification & Clean-up
- Separation
- Identifications
- Quantifications



Problems associated with Aflatoxin analysis:

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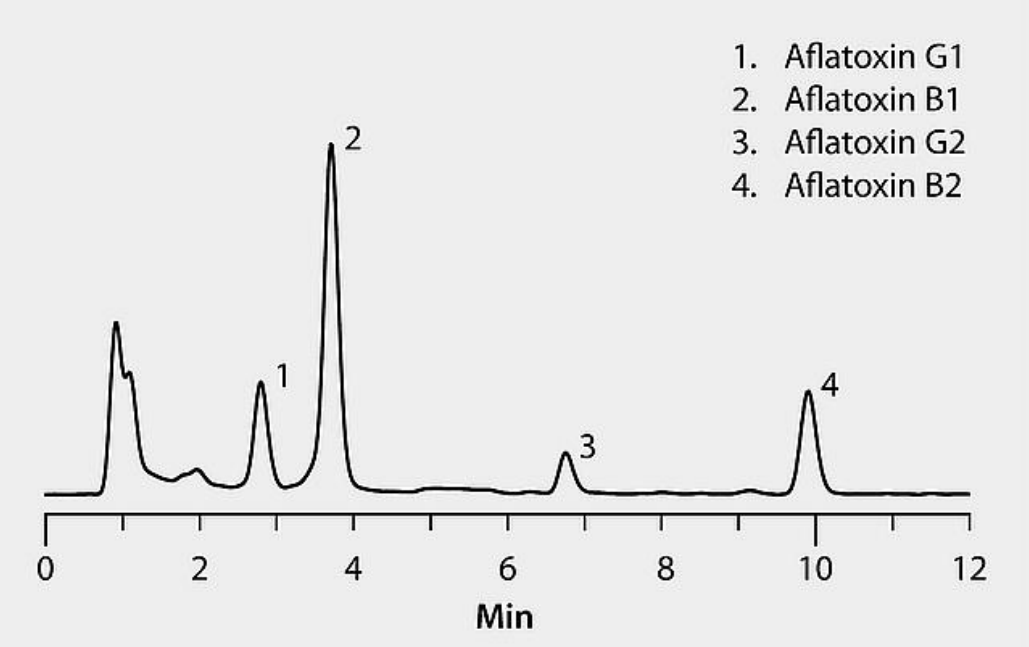
- Sampling.
- Toxicity & health hazard.
- Handling of Std. & high Concentrated samples.
- Very low concentrations.
- Interference substances.
- Percentage recovery.
- Sensitivity to light and some chemicals.
- Detoxification.



Sampling:

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Sampling is considered as the major misleading factor in aflatoxin determination in unprocessed agric. Products.

Due to the ubiquitous nature of aspergilli, aflatoxin contaminations can occur at numerous site on invasion giving rise to small local bathes of highly contaminated products, and this situation often contributes great variability to the results of aflatoxin analysis.

- ***Sampling contributes the greatest single source of error.***

Effort to minimize the effect of sampling of aflatoxin analysis by: particle size reduction and mixing,,,, lot of sample to be randomly divided and subdivided to obtain representative sample.

Extraction:

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At the beginning they use to extract aflatoxin by Soxhlet extractors.

Better extraction was then achieved by aqueous solvents with the help of high shear blenders or shakers.

Aflatoxins are generally soluble in polar solvents such as methanol, acetone, chloroform, and acetonitrile,,, mixed in different proportion with small amounts of water.

Water is added in small quantities to aid the penetration of the solvents in the substrate.

Two adopted methods by AOAC are widely used for aflatoxins analysis namely BC (Contamination Branch) and BF (Best Food).

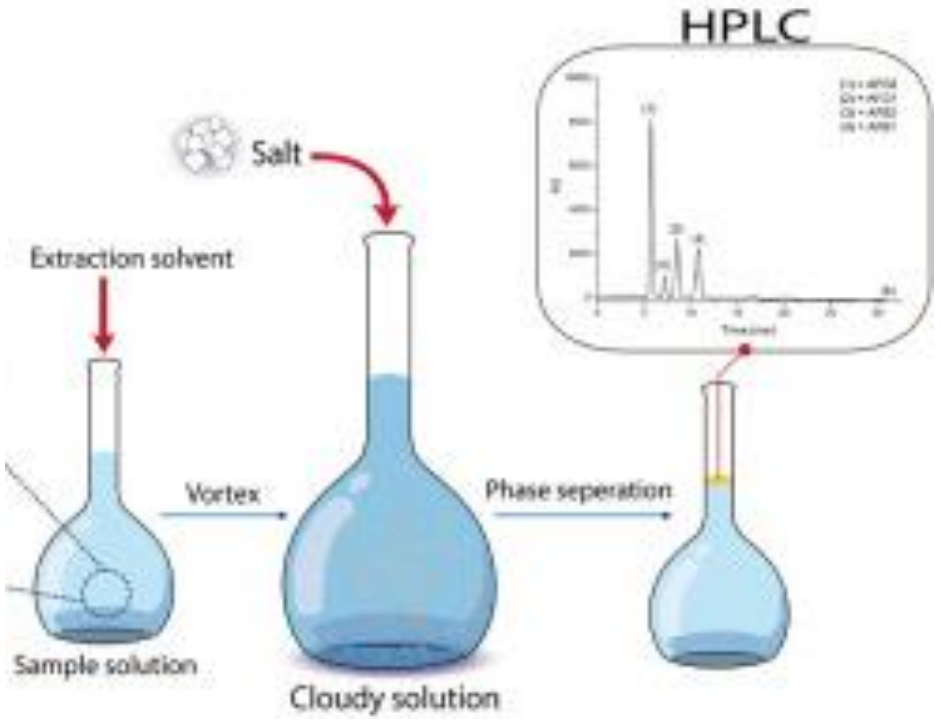

Table 1. Comparison of the CB and BF Methods of Extracting Aflatoxins from Date Fruit Pieces of Lulu Variety at Khalal Stage Inoculated with *A. parasiticus* (IMI 91091b)[a]

method		AFB1	AFB2	AFG1	AFG2	total
BF	1	186	111	933	143	1373
	2	176	87	777	112	1151
	3	216	107	964	137	1424
	4	213	102	933	137	1386
	5	194	98	925	130	1347
	6	206	101	925	132	1364
	mean	198	101	910	132	1341
	SD	15	7	66	9	95
	CV, %	7.6	6.9	7.2	6.8	7.1
CB	1	451	141	1243	173	2008
	2	517	152	1464	183	2315
	3	454	133	1278	147	2011
	4	406	123	1150	149	1828
	5	432	127	1364	158	2080
	6	440	130	1324	163	2056
	mean	450	134	1304	162	2050
	SD	37	10	103	13	156
	CV, %	8.2	7.5	8	8	7.6

[a] All figures are in μg/kg.

Purification & Clean-up

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Almost all solvents used are good solvents for lipids, plant pigments and other food components which, unless otherwise removed, will interfere with aflatoxin detection.

Lipids can be removed by using non-polar solvents (hexane, iso octane, petroleum ether).

Transfer of aflatoxin to chloroform helps in the clean up stage.

Passing the extract through silica or Celite column was proved to be more effective in purification step.

Disposable, monoclonal antibody affinity columns gain acceptance these days for complete and rapid removal of interfering materials from biologically diverse samples.

Figure 1. Photocopy of a TLC plate covered with transparent self-adhesive plastic film showing aflatoxins extracted from contaminated dates using the CB, BE, and RMC extraction and purification methods. A standard solution (STD) of aflatoxins was spotted centrally, and the four major aflatoxins were revealed, namely (from top to bottom), B_1, B_2, G_1, and G_2. The developing solvent was chloroform−acetone (9 v/v).

d) Isolation and Quantification:

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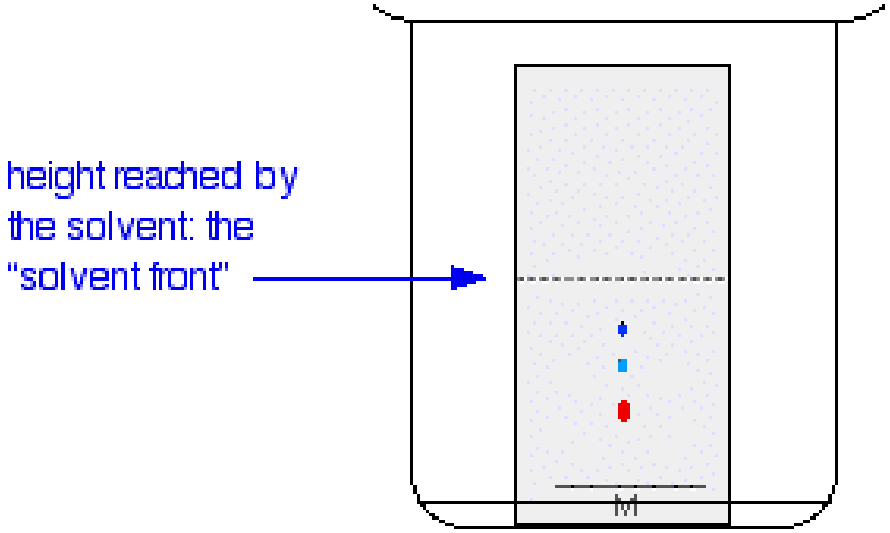
The chromatographic separation of aflatoxins, coupled with their inherent fluorescence properties, has provided the basis for their detection and quantification.

The use of TLC plates, coated with different silica gels has become the most widely used technique for detection and confirmation of identity of aflatoxins (very sensitive 0.5 ng per spot).

The most significant advantage of the TLC technique is the low cost of each analysis, and the major disadvantage is the quantification step.



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d) Isolation and Quantification: (continued)

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Aiming to improve the quantitative accuracy and precision of aflatoxin analysis, the recent trend has been towards the use of **HPLC.**

NP columns were discontinued as the chlorinated solvents tend to quench the fluorescence of B1 and B2.

Now day RP columns are used with methanol / acetonitrile along with water to achieve excellent separation and improved reproducibility.

However, the major problem associated with the RP system is the weak fluorescence of B1 and G1, but this lack of intensity can be solved either by pre-column derivatization using trifluoracetic acid (TFA), or post-column derivatization (PCD) reaction using iodine at elevated temperature (100^{o}C).

The fluorescence detection system has proved to be more sensitive, more selective and less liable to background interference than UV and can achieve measurement of picograms level of aflatoxins

Table 2. Results of Injecting Two Randomly Selected Samples of Contaminated Date Extract for 6 Consecutive Days To Test the Repeatability of the HPLC Determination Procedure[a]

		AFB1	AFB2	AFG1	AFG2	total
sample 1	1	19.1	0.71	10.8	ND	30.6
	2	24.6	0.99	13.7	ND	39.4
	3	23.9	0.95	13.5	ND	38.4
	4	24	0.91	13.5	ND	38.4
	5	25.8	1.04	12.6	ND	39.4
	6	24.7	1.0	11.8	ND	37.5
	mean	23.7	0.9	12.7	0.0	37.3
	SD	0.7	0.0	0.7	0.0	0.7
	CV, %	2.9	0.0	5.5	0.0	1.9
sample 2	1	29.9	1.39	19.5	0.41	50.9
	2	27.2	1.15	16.2	0.34	44.9
	3	26.9	1.09	15.8	0.32	44.1
	4	29.6	1.32	18.0	0.37	49.3
	5	29.1	1.38	18.1	0.37	49.0
	6	28.37	1.14	15.8	ND	45.3
	mean	28.5	1.3	17.2	0.4	47.3
	SD	1.0	0.1	1.0	0.0	2.2
	CV, %	3.5	7.6	5.8	0.0	4.7

[a] All figures are in μg/L.

HPLC repeatability

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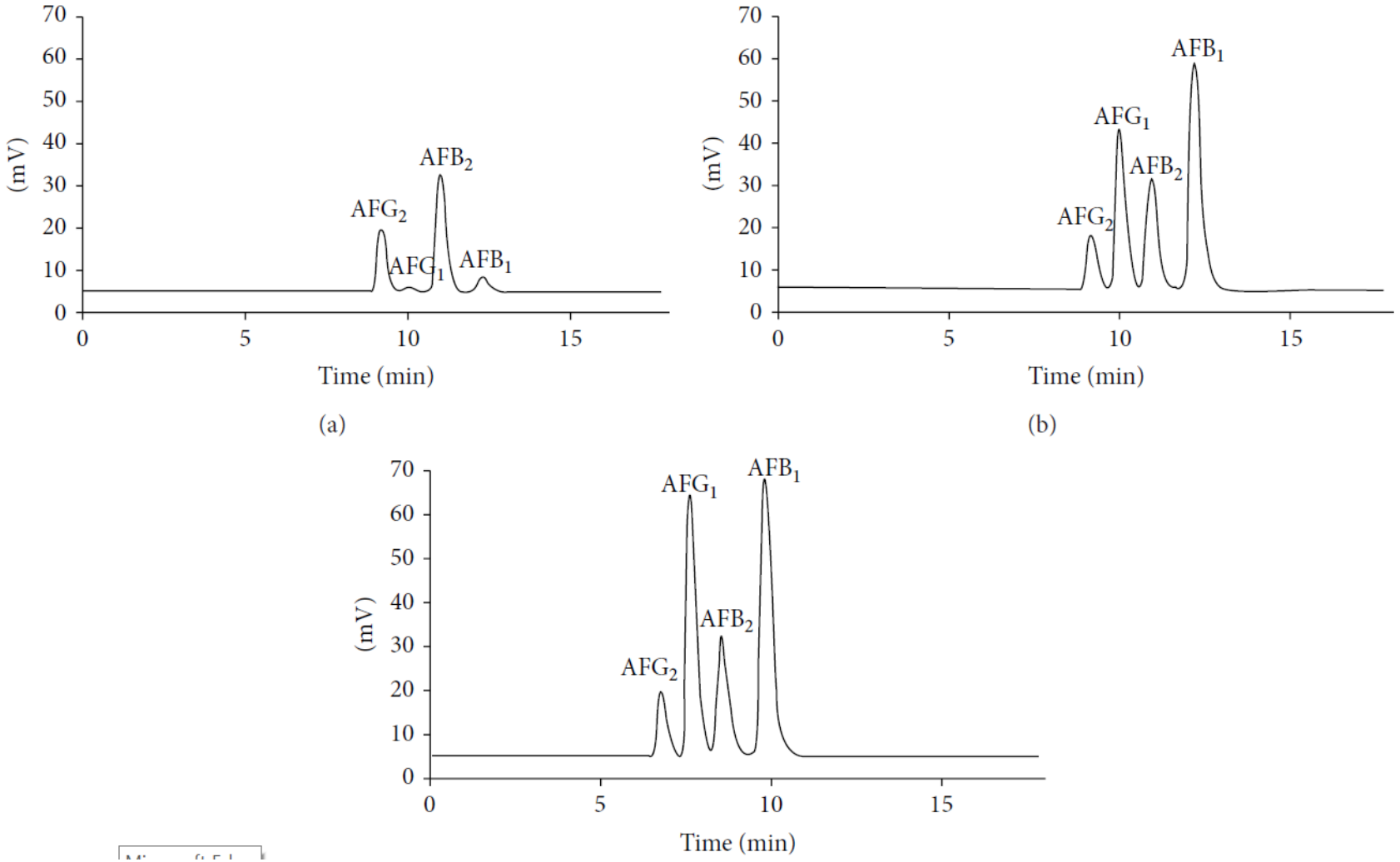

HPLC Chromatogram comparing PCD

d) Isolation and Quantification: (continued)

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It is important usually to confirm the identity of aflatoxins as some interfering substances, which can fluorescence and show same retention time as aflatoxins, lead to false positive.

- Pistachio showed spot which appeared in the area of B1 & G1
- Fig showed purple fluorescence in the region of B2 – G
- Sunflower seed meal, extracted by CB method, showed compound resemble aflatoxins behavior.
- Some workers reported the formation of new spots with long storage of contaminated peanuts with same Rf value.

❖ confirmation could be done by:

✓ Internal standards under different chromatographic conditions.

✓ Spray of TLC plates with 25% methanolic sulfuric acid.

✓ Formation of derivatives.

Typical Aflatoxin Laboratory

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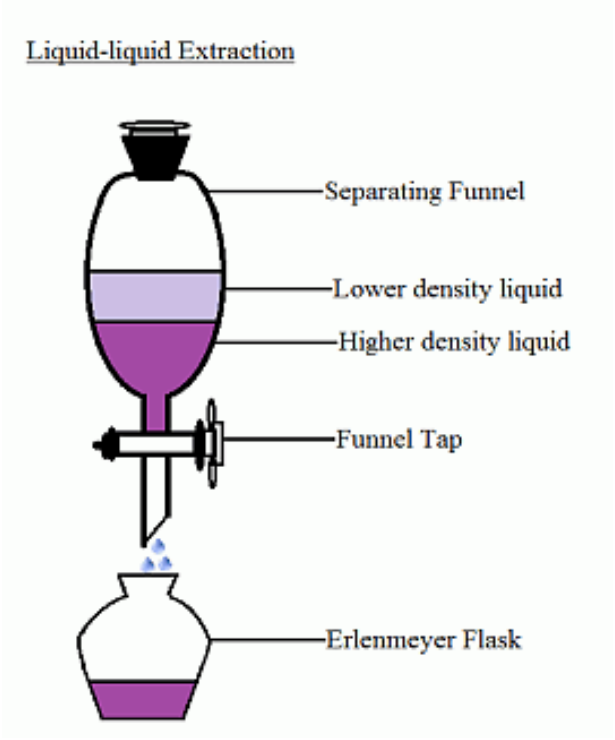
Liquid Extraction



Purification by immunoaffinity columns

High Performance Liquid Chromatography (HPLC)

Typical HPLC Setup for Aflatoxins

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C18 HPLC Columns 4.6 x 250 mm, 5 microns

Diode array detectors

Fluorescence detectors

Post-column derivatization module

Other Detection Methods

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1. Gas Chromatography – with ECD and Mass Spectrometer
   - Requires highly skilled personnel
   - Repeatability issues for aflatoxins analysis
   - More expensive than HPLC
2. Spectrophotometric methods – like fluorimetry and FTIR
   - Very quick methods
   - Cheaper than HPLC
   - Not suitable for very low concentrations of aflatoxins esp. for export to EU
3. Immunoassay methods – like ELISA
   - Quick tests
   - Not requiring skilled personnel
   - Washing after testing is time consuming
   - Requires ELISA kits for operation

Summary and Final Takeaways

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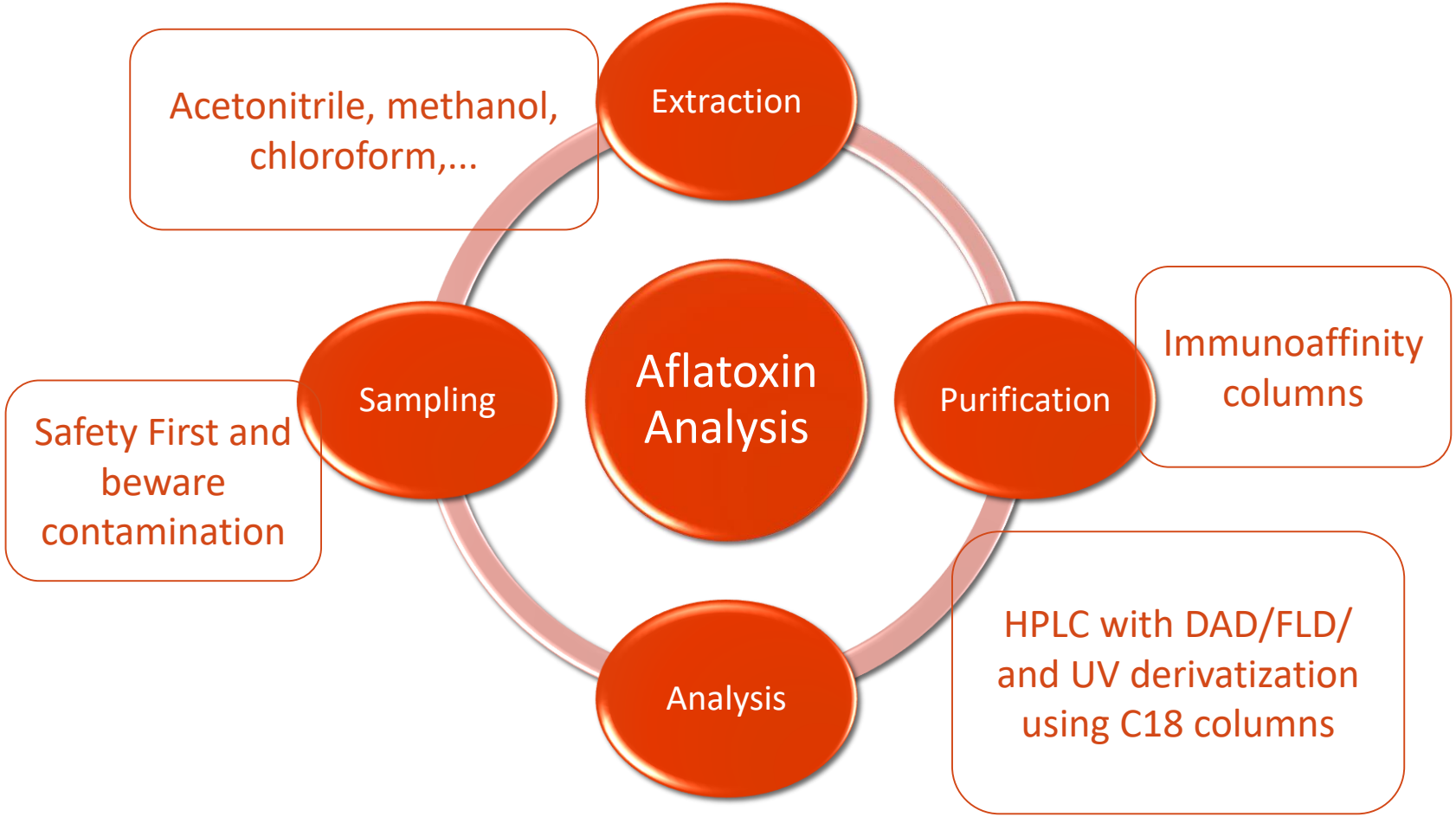

For Further information

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For more information on the aflatoxin testing methods and other methods for food and agricultural analysis please visit our website:

www. prime.sd

> Industries > Agriculture and Forestry

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رب الطيران الإسرائيلي لقافلة سيارات بالبحر الأحمر

أخبار سريعة

تحذير من فطريات «الافلاتوكسين» الموجودة بالفول السوداني والذرة

الخرطوم:اشراقة الحلو :حذر الخبير بمجال السموم الفطرية، الدكتور عماد علي أحمد، من ان فطريات «الافلاتوكسين «أحد أخطر المواد المسرطنة ،مشيرا الى تواجدها في الفول السوداني والذرة.

وقال أحمد ، في منتدى الجمعية السودانية لحماية المستهلك الاسبوعى أمس، « ان الحالات السرطانية الموجودة على الساحة الطبية الان يجب ان يتم ربطها مع الغذاء الملوث بالسموم الفطرية» ،مشيرا الى ان حبة الفول السوداني الواحدة الملوثة بالافلاتوكسين كفيلة بتسميم 30 كيلو من المحصول، مبينا ان سم الافلاتوكسين يقاوم درجات الحرارة العالية التي تفوق الـ250 درجة وهى درجة التحميص .

من ناحيته، كشف رئيس مجلس أمناء مركز السموم الفطرية، الشيخ محجوب جعفر، عن احتواء 70% من انتاج البلاد من الذرة على فطر الافلاتوكسين، وقال ان التلوث بدأ بالفول السودانى وتفاقم الامر عندما بدأ التلوث يطال محصول الذرة، غذاء اغلب اهل البلاد الرئيسى، مبينا ان هذه السموم وراء معظم امراض الكلى .

من جانبها، ارجعت عضو مجلس امناء مركز السموم الفطرية الدكتورة، نفيسة الماحي، تلوث الذرة والفول

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