



Development of sorbent-based microextraction technique for the determination of trace level aflatoxin in traditionally processed cassava products

Yanduru Mercy ^a, Hakim Nsubuga PhD ^{a*} and Francis Onyilo PhD

^aDepartment of chemistry Muni university, ^b Department of vocational studies Lira university

Commonwealth Chemistry Posters



Introduction

Aflatoxins are a class of naturally occurring mycotoxins that pose a serious threat to food safety and public health due to their potent carcinogenic, mutagenic, and hepatotoxic effects. The detection of aflatoxins in complex food matrices remains challenging due to matrix interferences, expensive and the labor-intensive nature of conventional sample preparation techniques such as IAC, LLE, SPE

In this study we developed a green, sensitive, and efficient analytical method for isolating and enriching aflatoxins prior to the quantification, by employing porous membrane-protected micro-solid phase extraction (μ -SPE) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS).

A series of eco-friendly sorbent materials were synthesized from water hyacinth biomass through chemical activation using eggshell-derived calcium carbonate and phosphoric acid. The resulting materials were characterized using X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), energy-dispersive X-ray spectroscopy (EDX), and scanning electron microscopy (SEM), followed by sorbents optimisation to determine the sorbent with best extraction efficiency.

Then Taguchi orthogonal array design was employed to optimize key extraction parameters including pH, extraction time, sorbent dosage, ionic strength, and desorption time that influence the extraction efficiency of aflatoxins, the method was validated by determining its accuracy, precision and sensitivity and the developed method was successfully applied to the trace-level determination of aflatoxins in 30 cassava flour samples collected from Arua City, Uganda.

Methods and Materials

Reagents and Equipment

❖ Reagents of Analytical grade and standard equipment were used.

❖ Afs standard, Acetonitrile, methanol, ultra pure water, HCl

❖ Equipment like LC-MS/MS, muffle furnace, magnetic stirrer, centrifuge, ultrasonicator, PH meter, sealing machine and characterization equipments were used

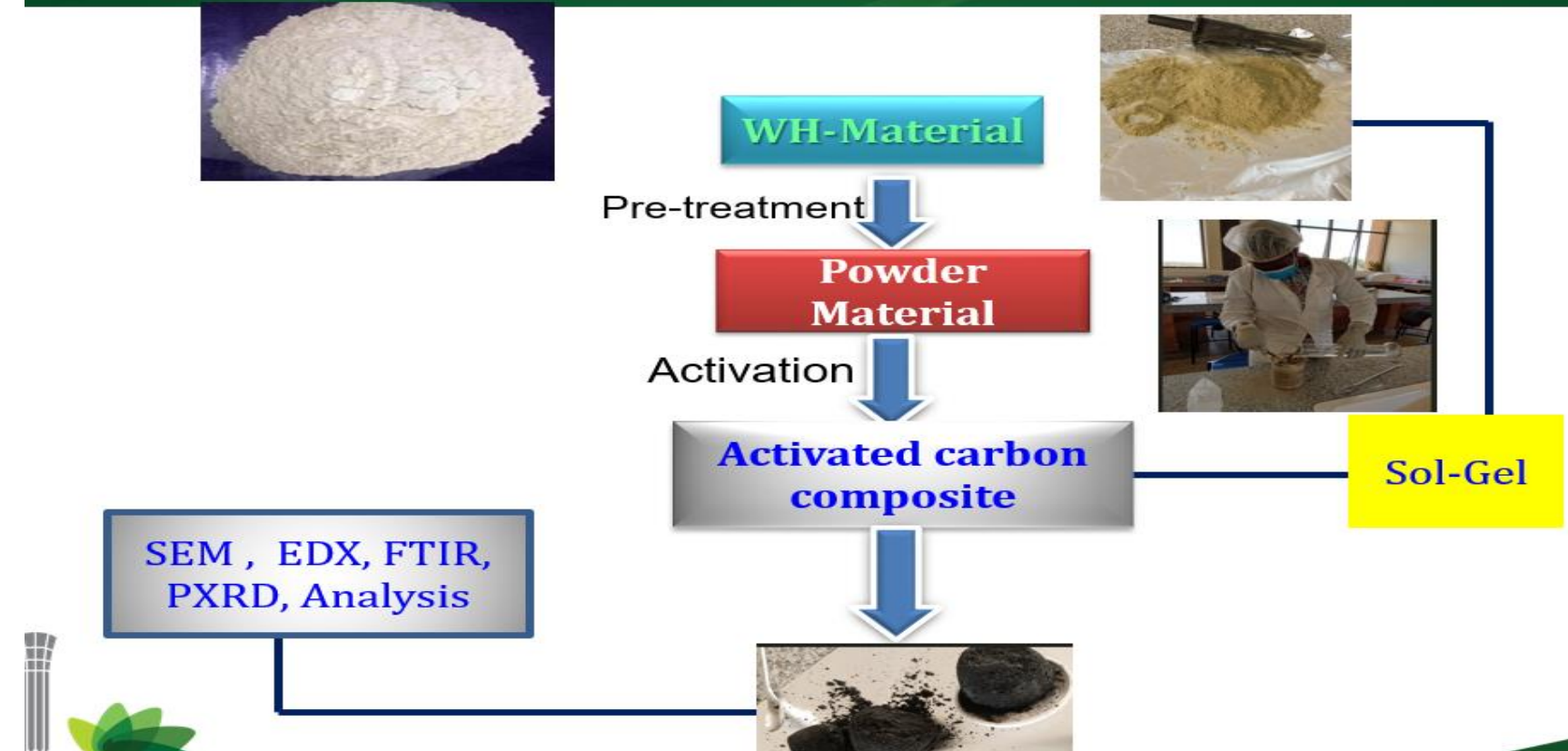
Sample collection

❖ Water hyacinth was collected from the shores of the River Nile in Rhino camp.

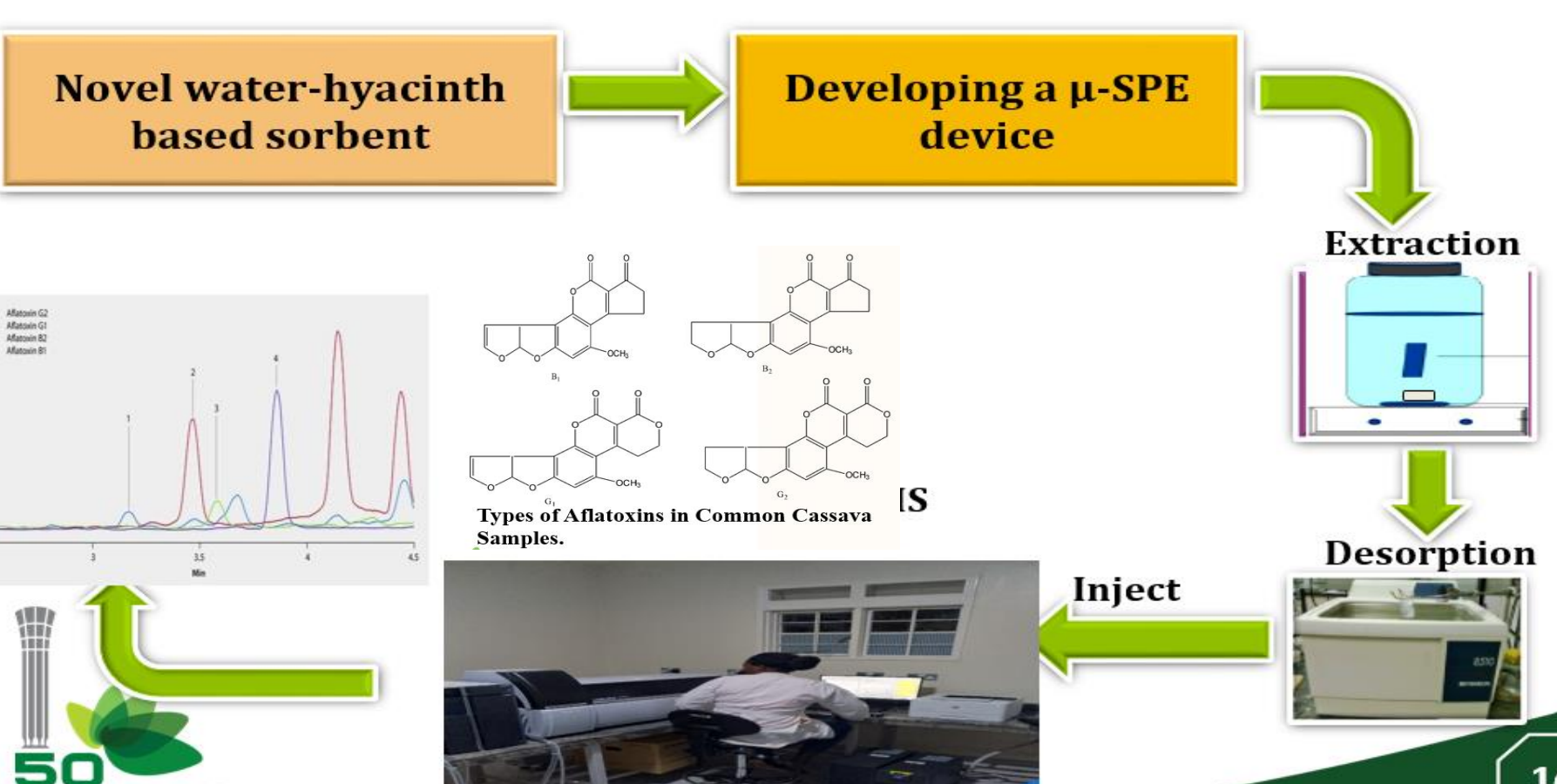
❖ Eggshell were collected from local Rolex vendors at Muni site.

❖ Cassava Samples were collected from the local markets in Arua city including the main central market.

Synthesis of a novel WH based Sorbents

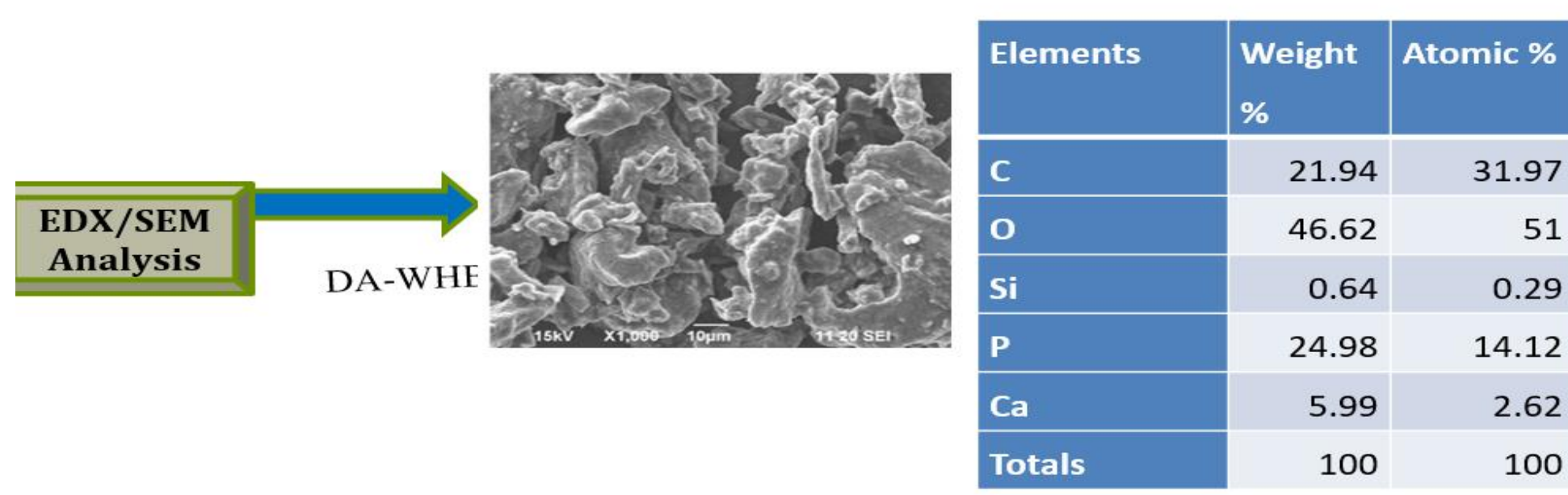


Method development (Developed Extraction Method)

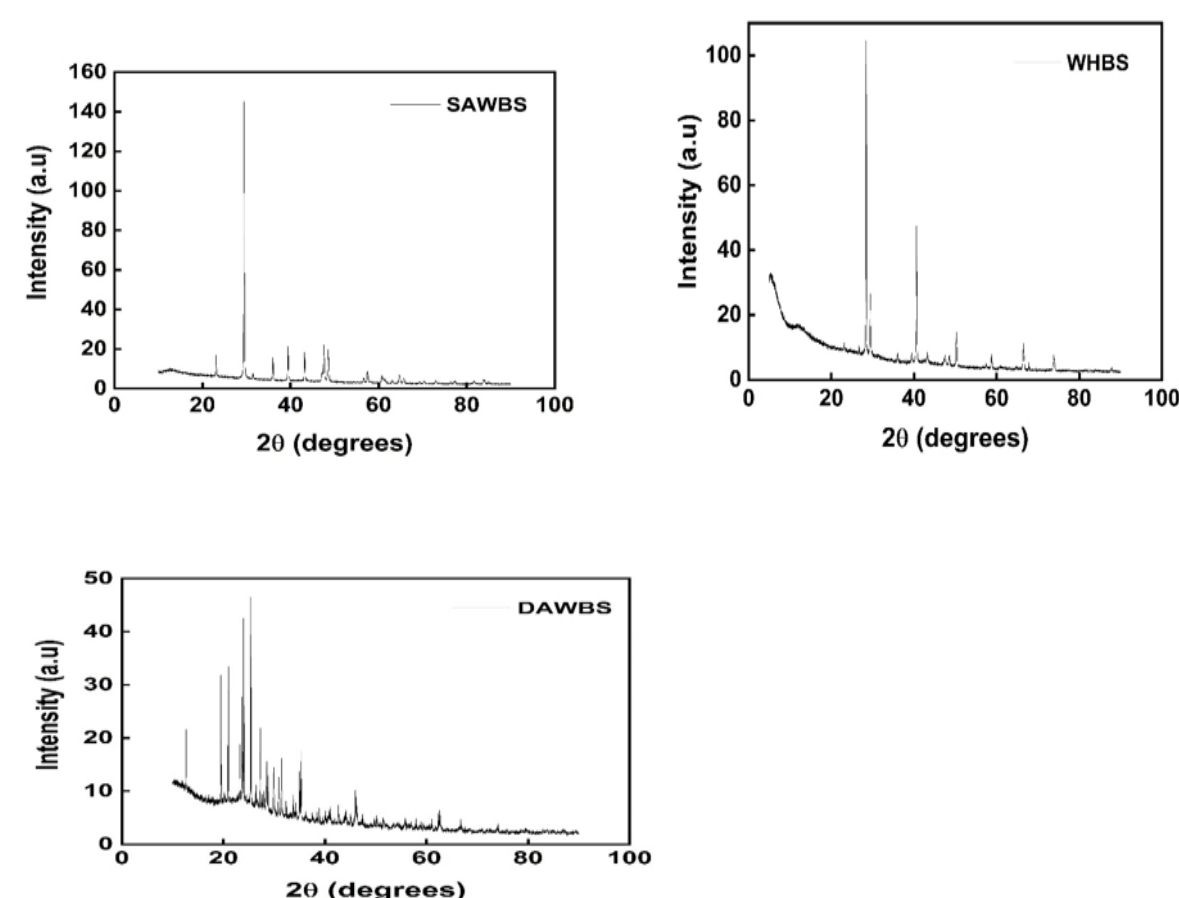


CHARACTERIZATION RESULTS OF THE SORBENTS

EDX/SEM ANALYSIS CONT.



PXRD ANALYSIS.



Introduction cont.

MOTIVATION: To develop a green, sensitive, and efficient method for detecting trace-level aflatoxins in Cassava food products to enhance food safety.

Aflatoxins in Cassava Food stuffs

- Produced by Aspergillus species
- Present at trace levels in food matrices
- Difficult to detect

Carcinogenic and Hepatotoxic at trace level concentrations

Analytical Challenges

- ✓ Complex food matrices
- ✓ Laborious Sample preparation
- ✓ Risk of contamination during analysis
- ✓ Use of toxic solvents in extraction
- ✓ Expensive

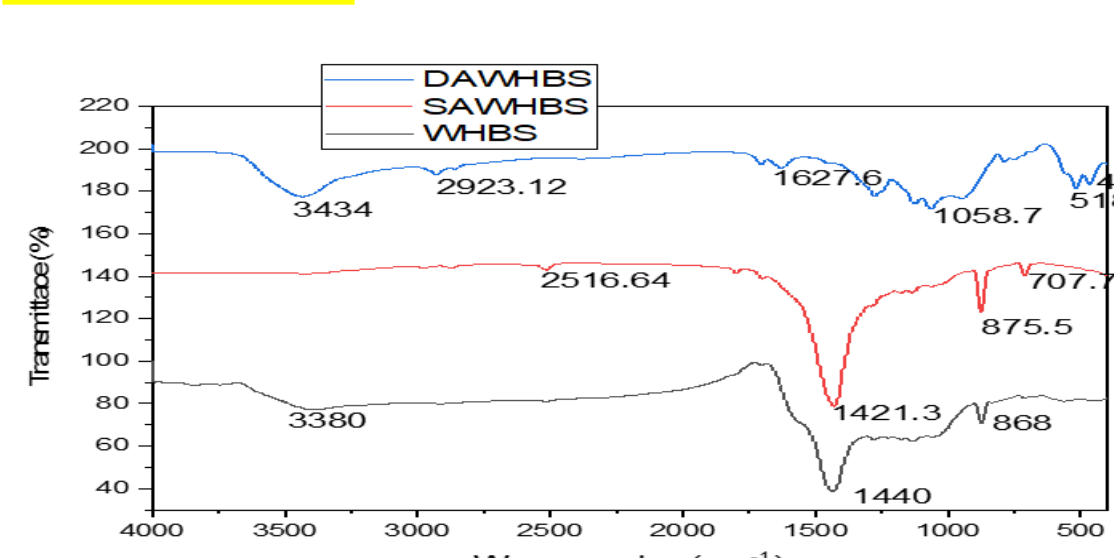
Proposed Solution

Need for Greener, sensitive and more efficient Methods

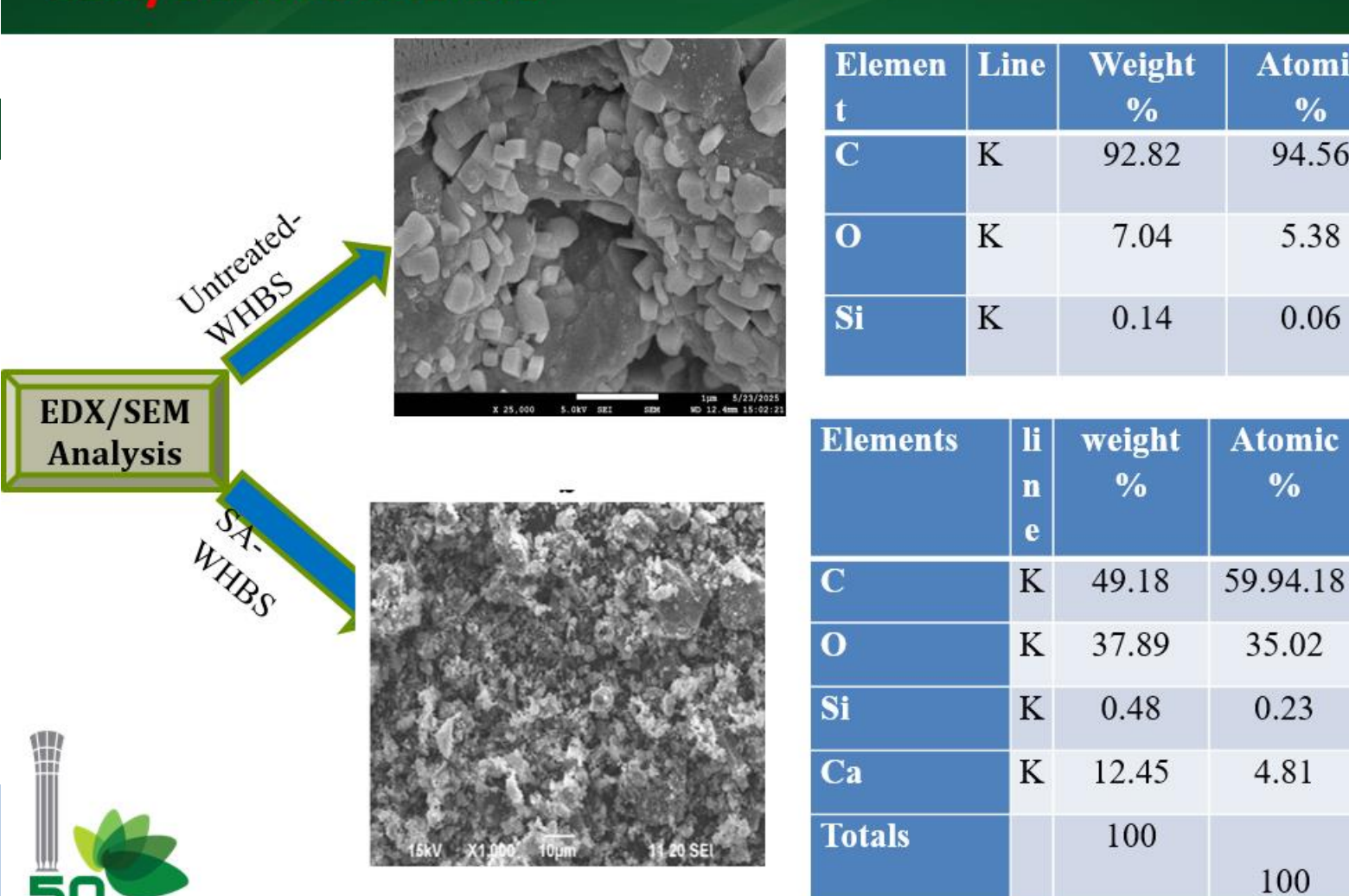
- 1) (Lavkor & Var, 2017)
- 2) (Awuchi et al., 2020)

Results and discussion

FTIR analysis



EDX/SEM ANALYSIS



Contact

<YANDURU MERCY>

<MUNI UNIVERSITY UGANDA>

Email:mercyyanduru7@gmail.com

Website:

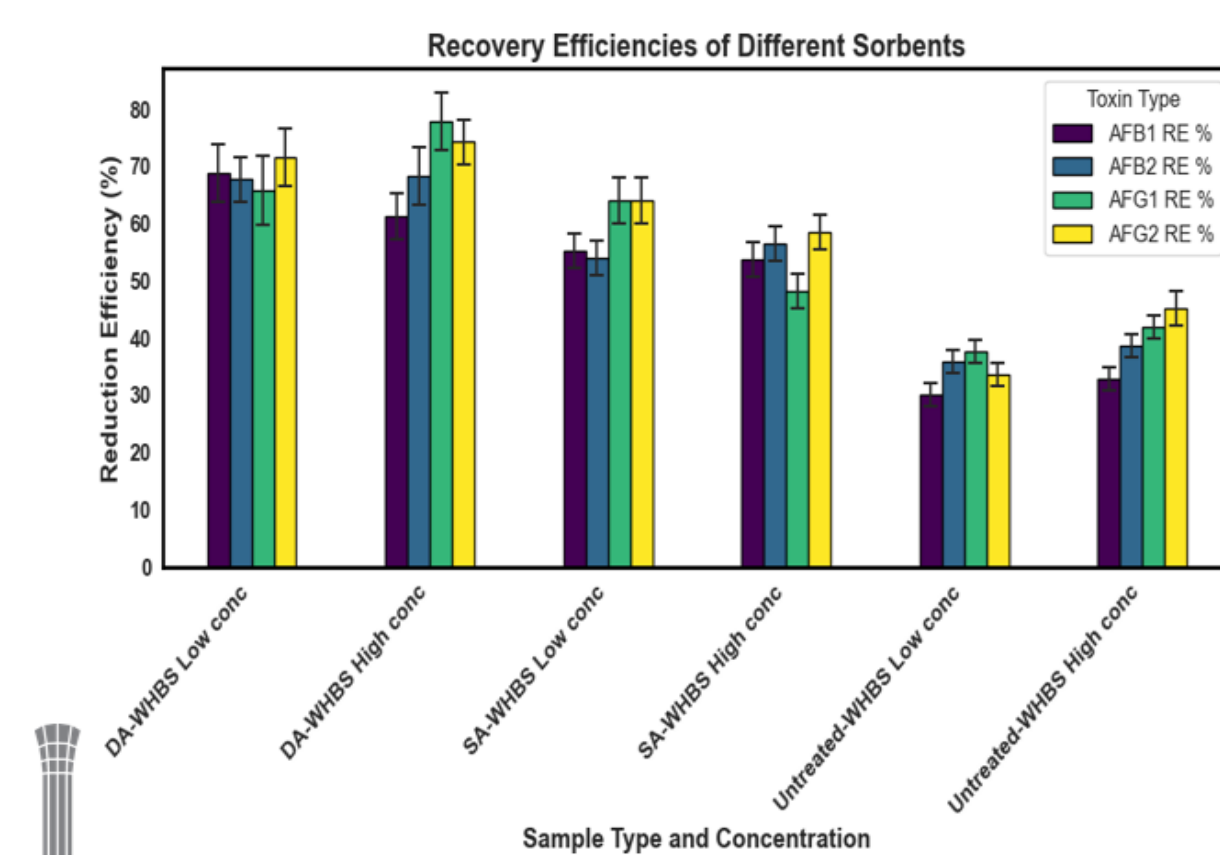
Phone:0779077796

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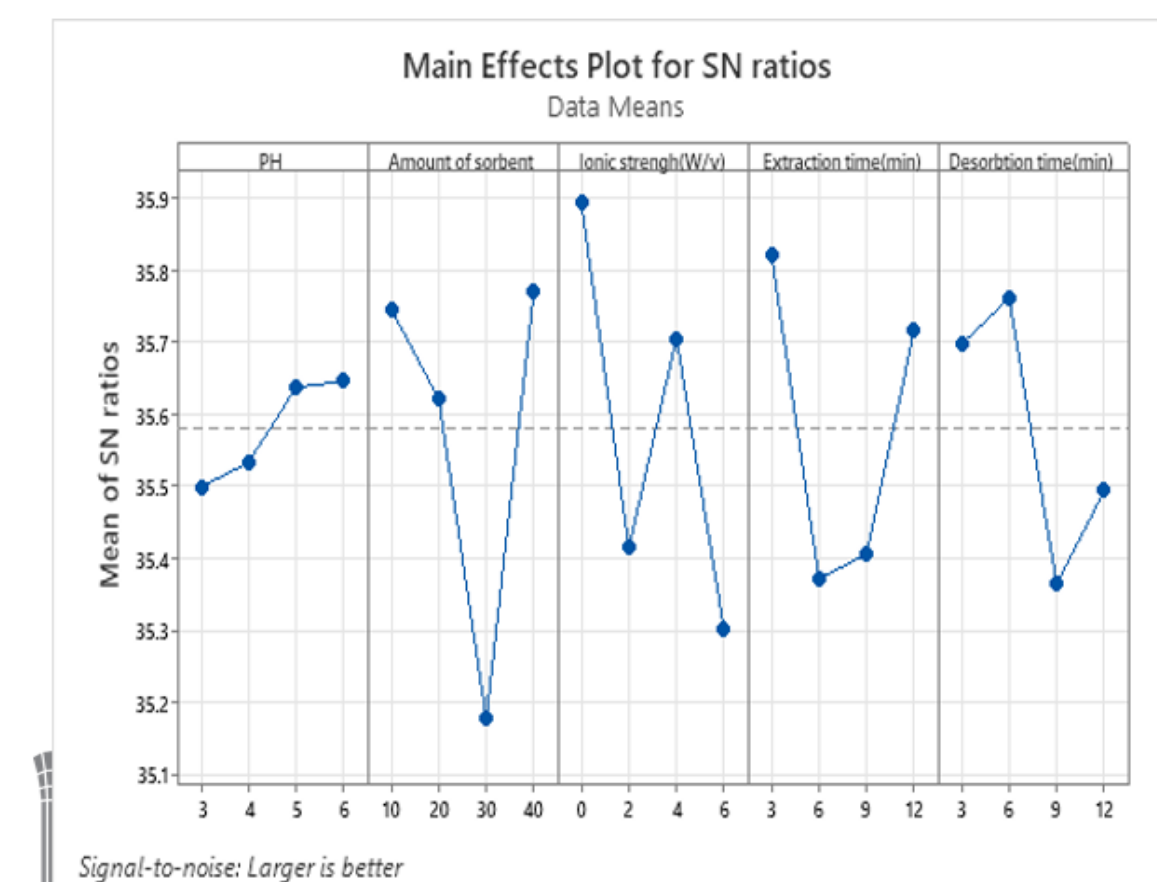
Results and discussion

Results and discussion



Sorbent optimization
DA-WHBS>SA-WHBS>untreated-WHBS due to multiple pores created during activation

Taguchi optimization



Optimal values are
PH= 6, sorbent amount= 40 mg, 3 mins extraction time without ionic strength and 6 mins of desorption

Validation results (recovery study)

Table 4. 4 Recovery Results of spiked samples at Optimum Conditions

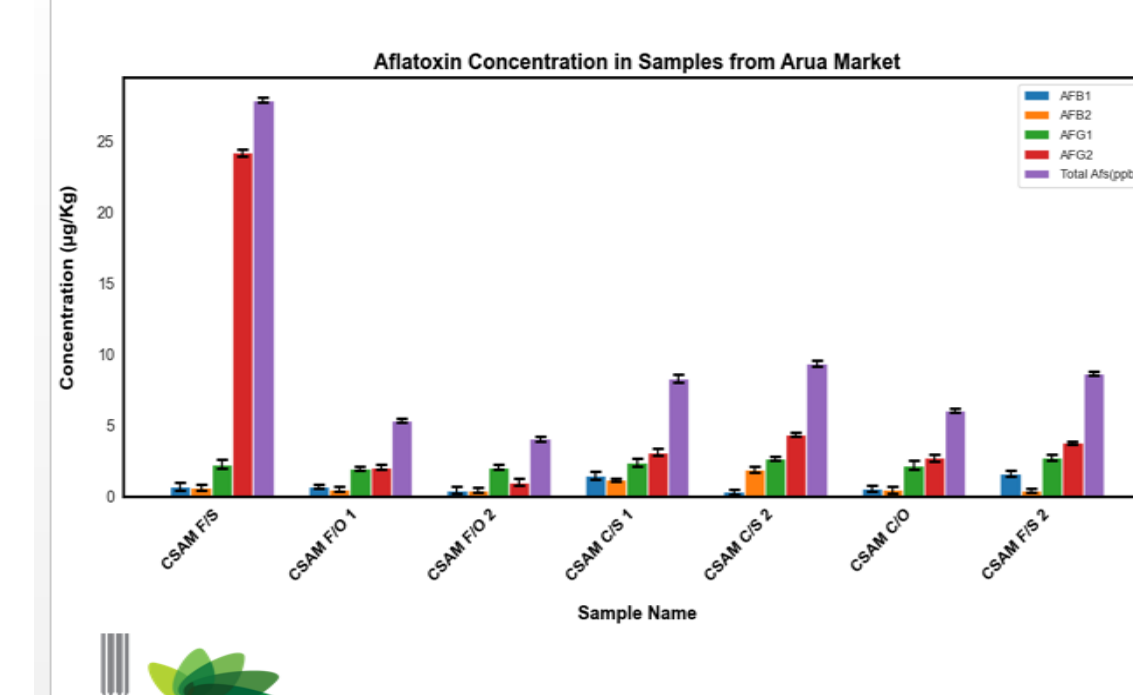
Spiked-cassava sample Concentration (ng/ml)	Aflatoxin Type	Mean recovery (%)	Percentage Relative Standard Deviation (%RSD)	LOQ (µg/kg)	LOD (µg/kg)	Recoveries are within the range 80–115 % set by international organisation for standards (ISO, 2013).
2.5	B1	91.5	4.41	0.25	0.08	
	B2	89.7	4.55	0.20	0.06	
	G1	90.8	4.05	0.30	0.09	
	G2	90.6	4.63	0.22	0.07	
5	B1	91.9	4.24			
	B2	92.2	4.30			
	G1	90.0	4.15			
	G2	82.6	4.16			

Aflatoxin levels in real cassava samples.

The lower levels of aflatoxin in un-preconcentrated samples could be due to less exposure of the aflatoxins for detection, which resulted from the presence of interfering ions (Shabeer et al., 2022), therefore this result confirmed the necessity of preconcentration of samples prior to quantification.

- All the preconcentrated samples (Table A.4) have aflatoxin levels higher than the non-preconcentrated samples

Aflatoxin levels in cassava samples from arua market



- Afs are highest in fermented cassava samples from stores, followed by chips from stores, and then fermented and cassava chip samples from open market, have relatively low aflatoxin concentration.

Conclusions

In conclusion, the study involved development of green sorbent based micro solid phase extraction technique.

The results indicated that fermented cassava samples from stores have highest aflatoxin levels, followed by chips from stores, then fermented cassava samples and cassava chips from open market have lower and comparable AF levels and cassava soaked in water have the least aflatoxin concentration due to poor storage practices during the preparation process