BIPM Capacity Building & Knowledge Transfer Programme

2021 BIPM - TÜBİTAK UME Project Placement

REPORT

Project Name	Production of Food Matrix Reference Materials for Assuring Quality of Sudanese Products
Description	The aim of this project is to take a part in production steps of candidate aflatoxin reference material (RM) for hazelnut; method validation and perform the validated method to (RM) certification studies (homogeneity and short term stability tests).
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Date	13.09.2021 – 10.12.2021

Motivation & Introduction

Aflatoxins (AFs) are a family of toxins produced by certain fungi that are found on crops such as corn, peanuts, hazelnut, etc. The main fungi that produce aflatoxins are Aspergillus flavus and Aspergillus parasiticus, which are abundant in warm and humid regions. Aflatoxin-producing fungi can contaminate crops in the field, at harvest, and during storage. Human can be exposed to aflatoxins by eating contaminated plant products, by consuming meat or dairy products of animals feed. Agricultural workers may be exposed by inhaling dust generated during the handling and processing of contaminated crops and feeds. Among the types of Aflatoxin G2, G1, B1 & B2, Aflatoxin B1 is the most toxic and has been classified as a Group I carcinogen by the International Agency for Research on Cancer (IARC). Exposure to aflatoxins is associated with an increased risk of liver cancer, and is estimated to cause as many as 26,000 deaths annually in Sub-Saharan Africa. Due to the seriousness of these toxins, various countries have been working on regulations to ensure the safety and quality of their products. According to the CODEX, the maximum residue of AFs level in nuts was determined as 5 µg/Kg for AFB1, and 10 µg/Kg for Total AFs (AFB1, AFB2, AFG1 and AFG2).
The concerns about the harmful effects of certain Aflatoxins have prompted the National Metrology Institute of Turkey to produce a series of reference materials according to ISO 17034 standard aimed at improving the quality and consistency of Aflatoxins analysis and monitoring laboratories overall performance on a reliable, systematic and comparable basis.
The importance of aflatoxins in peanut in Sudan stems from the huge production (> million tons/annum), its high consumption (as food and feed) and being an important export product. Peanut is very susceptible to aflatoxins contamination, and this fact is manifested by a lot of literature of research as similar problems observed with the hazelnuts in Turkey.

Research

The production of reference materials (RMs) is a key activity for the improvement and maintenance of worldwide coherent measurement system. RMs with different characteristic are used in measurements, such as calibration, quality control, proficiency testing and method validation, as well

as for the assignment of values to other materials. This project is a part of candidate CRM; UME CRM 1321 production project that is designed to comprise the following;

1. **Material Processing**
   a. Homogenization of hazelnut puree and taking samples from homogenized content for analysis, using UME randomized stratified sampling software program TRaNS.
   b. Filling, labeling the bottles and separation of samples for tests and sales.
2. **Method Validation.**
3. **Homogeneity study.**
4. **Short-term stability study.**

Material Processing

Homogenization

100 Kg of contaminated hazelnut sample is homogenized by mixing with Ultra Turrax homogenizer for 8 hours.

Figure 1. Ultra-Turrax homogenizer (UTC 115kt)

Figure 2. Filling machine

Procedure

1. Samples

Contaminated hazelnut samples were procured from local company. The raw materials have been tested of AFs level by the local company before bought by TUBITAK UME. Homogenized hazelnut samples of at least 60 g have been filled in aluminum sachets, vacuumed and transported to storage room and preserved at – 20 °C until the experimental process could be conducted. Some of the units selected randomly stratified for analysis using software program TRaNS. The selected units were analyzed for the content of AFs. All experiments were performed with at least three replicates.

Figure 3. Samples in the aluminum sachets

2. Validation of the analytical method

Reproducibility, intermediate precision and recovery studies were performed for method validation study. The reproducibility study for the method, 6 sub-samples were analyzed on the same day. For the intermediate precision (between days) of the method, 6 sub-samples were measured from 2 different bottles on 2 different days. NIST CRM-2387 (peanut butter) was used for the recovery study. The results were evaluated according to the ERM Note 1. İt was seen that there is no significant difference between measurement result and certified value at a confidence level of approximately %95 according to ERM application Note 1.

3. Aflatoxin analysis and extraction process

The analysis was performed using modified BS EN 14123:2007. 6 g of homogenized test portion was taken into falcon tube, 0.6 g of sodium chloride and 12 mL of water is added, and then the mixture is blended for 2 min at high speed vortex (to produce a slurry). 18 mL of methanol was added to the slurry and blended again for 2 min with high speed vortex. After that the extract was filtered through coarse filter paper, and the filtrate was collected into a 50 mL falcon tube. 5 mL of the clear filtrate (equivalent to 1g of sample) was taken into a falcon and diluted with 15 mL of phosphate buffer saline PBS solution (pH 7.4). The immunoaffinity column IAC is allowed to reach the room temperature, then connected to the vacuum manifold and attached the reservoir before using.

The diluted sample extract was added to the reservoir connected to the conditioned immunoaffinity column IAC and proceeded as follows; 20 mL of the reconstituted extract were passed through the IAC at flow rate of 3 mL/min (approximately one drop per second). After that 20 mL ultrapure water was passed through the column (for cleaning) and dried by applying little vacuum for 5 seconds, AFs bound to the specific antibody were eluted with 1.5 mL methanol and diluted with 1.5 mL ultrapure water, then filtered using 0.45 µm PTFE syringe filter and transferred into HPLC vials

4. Homogeneity study

The homogeneity study is designed to show that assigned value is valid for all units within the stated uncertainty. Homogeneity study between units was performed with number of samples representing the whole batch. In this project, 10 samples were selected by using random stratified sampling software (TRaNS) and were reserved for the study of homogeneity. Two subsamples from each unit were analyzed and each subsamples injected three times to HPLC-FLD under repeatability conditions. The graphs of the homogeneity study as follows;

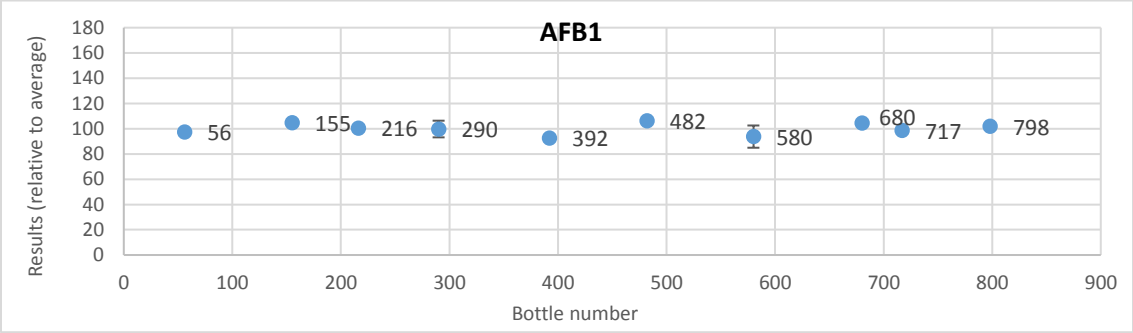

Figure 4.1. AFB1, Homogeneity plot

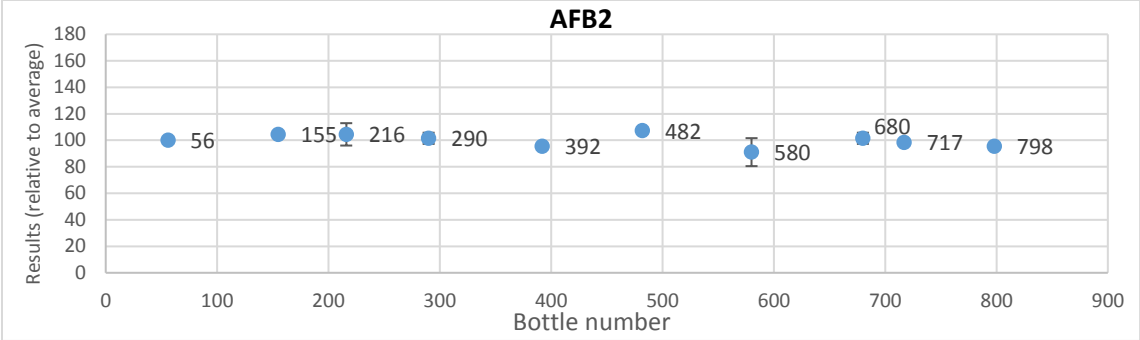

Figure 4.2. AFB2, Homogeneity plot

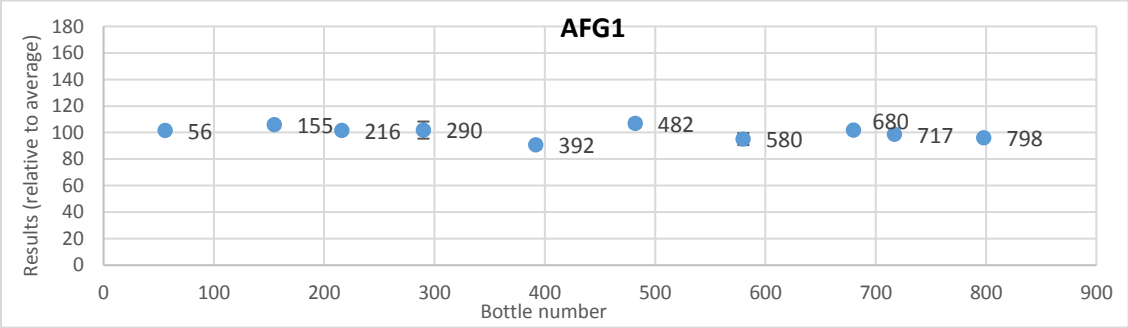

Figure 4.3. AFG1, Homogeneity plot

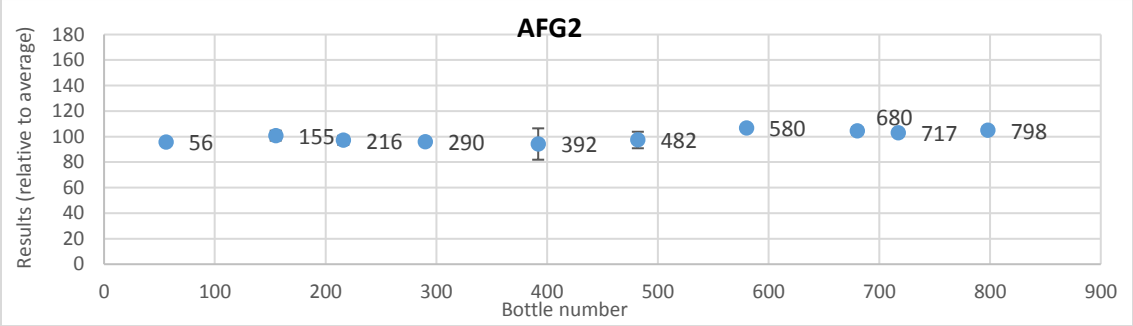

Figure 4.4. AFG2, Homogeneity plot

5. Short term stability study STS

The stability was studied under the storage and transport conditions including controlled low and high temperature. Stability studies were performed with isochronous design which is cited in ISO Guide 35. Two different temperatures (25˚C and 45˚C) and 4 time points (0, 1, 2, and 4 weeks) were tested. 16 samples were selected by TRaNS. Two samples analyzed in replicates at each time point and each temperature.

Figure 5. The workplace- Reference materials laboratory at TÜBİTAK UME

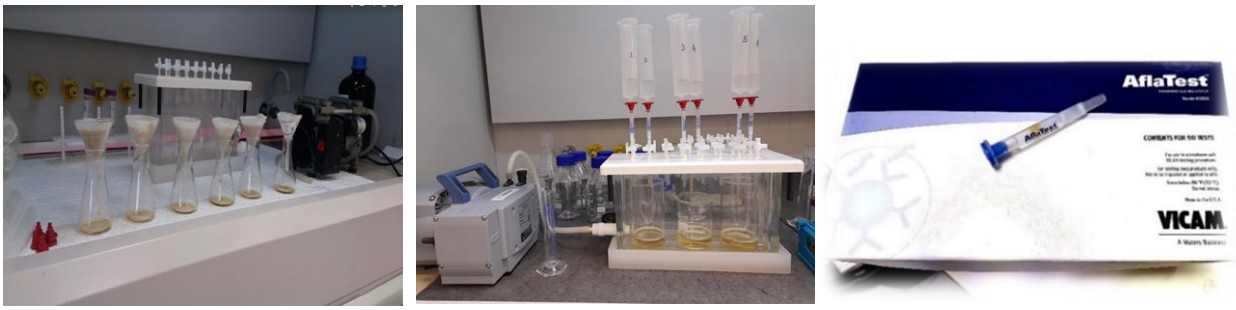

Figure 6. Filtration and Extraction stage

The graphs of the short term stability results are as follows;

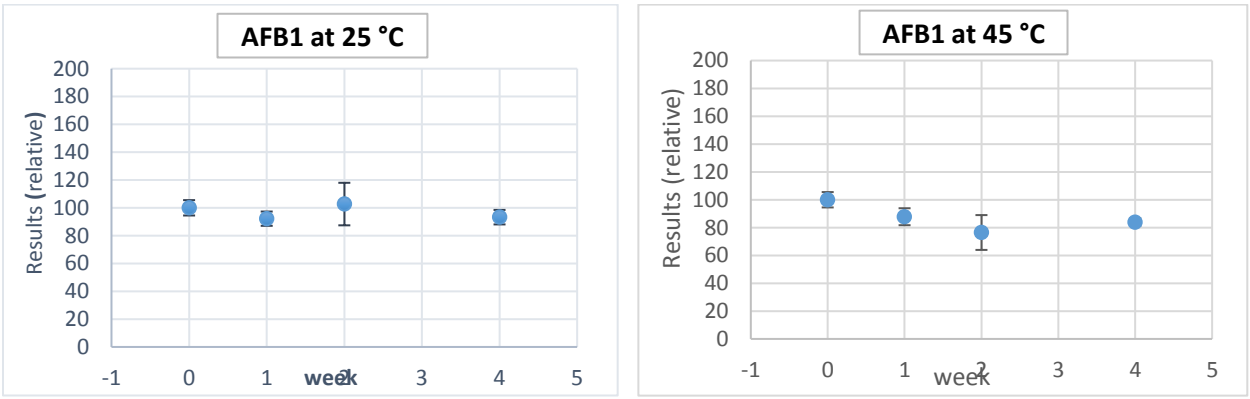

Figure 7.1. AFB1 Short Term Stability Plots

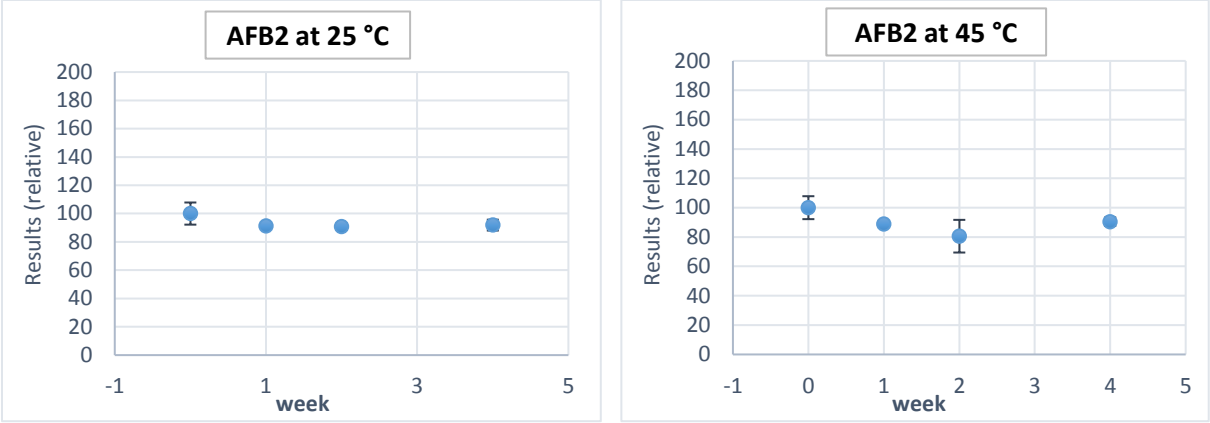

Figure 7.2. AFB2 Short Term Stability Plots

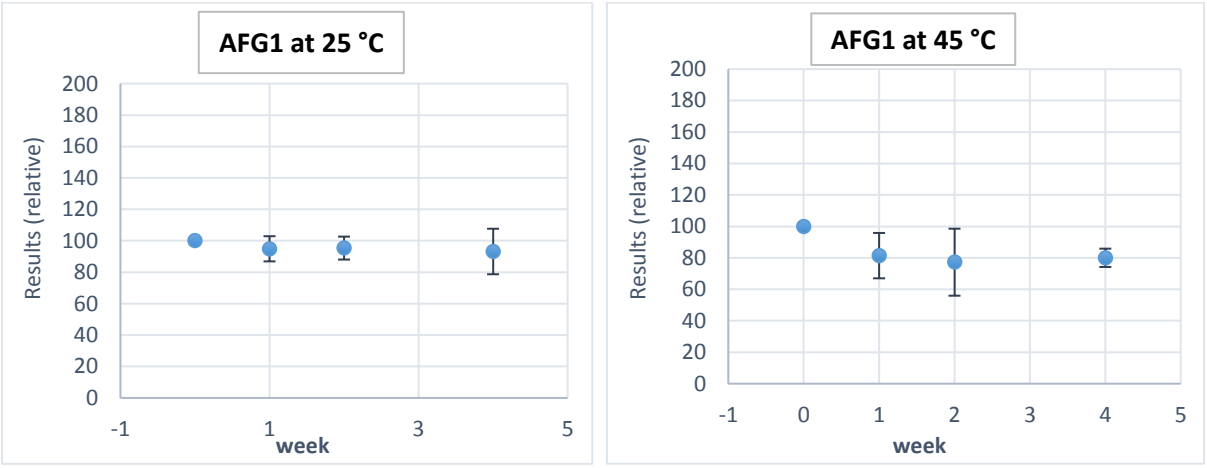

Figure 7.3. AFG1 Short Term Stability Plots

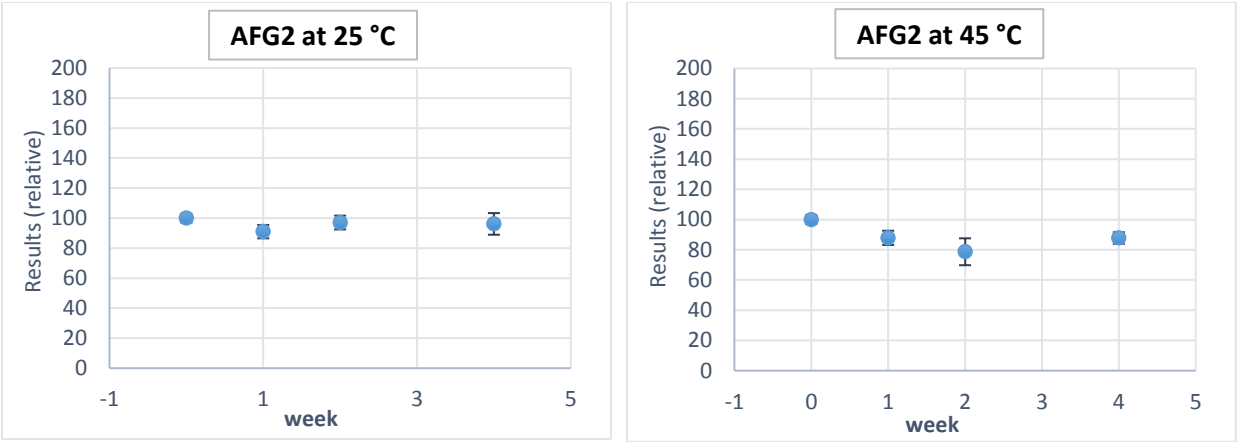

Figure 7.4. AFG2 Short Term Stability Plots

6. Instrument and chromatographic conditions

Determination of aflatoxins was performed using Agilent 1290 Series HPLC System (Agilent) Chromatographic separation was achieved using C18 column. An isocratic mobile phase consisting of water/methanol/acetonitrile (60/30/20, v/v/v) with 4N nitric acid (38.5 µL) and potassium

bromide (13.2 mg) was used at a flow rate of 1 mL per min. The column oven temperature was maintained at 25 °C and the injection volume was 100 µL for both standards and samples. Post-column derivatization (PCD) was achieved using The KOBRA® CELL. Fluorescence excitation and emission wavelengths were set at 365 and 440 nm, respectively. Retention times of AFG2, AFG1, AFB2 and AFB1 were 5.5, 6.4, 7.6 and 9.0 min respectively. Data acquisition and processing was achieved using chromatographic software (Agilent OpenLab Chromatographic Software). Aflatoxin determination in samples was based on a five point external standard calibration curve, using a mixture of aflatoxin standards (AFB1, AFG1, AFB2 and AFG2). Calibration curves were classified as valid.

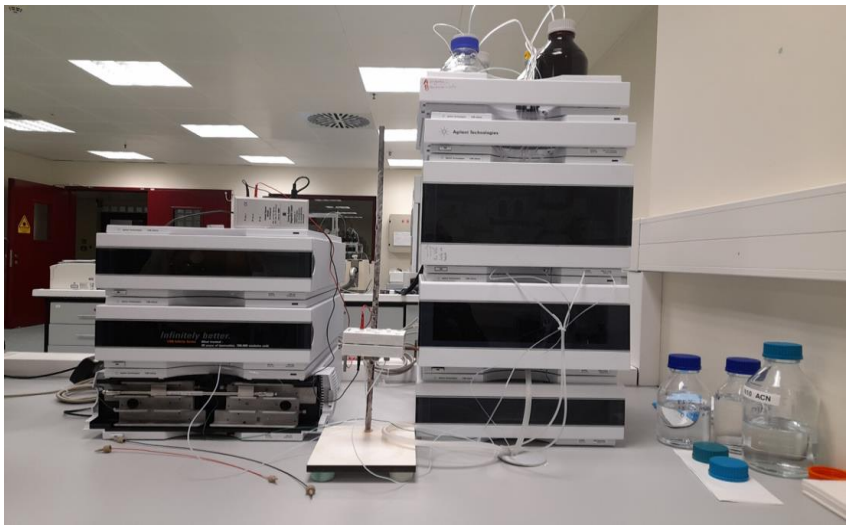

Figure 8. Measurements stage - (HPLC & KOBRA® CELL)

Conclusions and Future Work

This project aimed to learn about; how to plan RM production in compliance with ISO 17034:2016 and ISO Guide 35:2017, AFs analysis methods and method validation, applying the validated method to RM certification studies (homogeneity and stability tests), Evaluation the homogeneity and short term stability test results, how to use the HPLC techniques, processing RM and get ready to do similar preparations in the future. In Sudan we have a similar product such as peanut and its derivatives that is why we have measurement system for analysis AFs at the SSMO laboratories and we are planning to establish a chemical metrology laboratory. So participating in such project was a great opportunity for me to do the same. Furthermore during the internship I got information and I attended the practical work about; extraction of zearalenone in maize, preparation of proficiency testing sample (spiking AF M1 to milk power, spiking AF G2, G1, B2 & B1 to red pepper and spiking of poly aromatic hydrocarbons (PAHs) in baby food sample.

Over the course of reference materials production internship I also have completed the CIPM MRA e-learning course, which equipped me information about CIPM MRA mechanisms, comparisons, quality management system, calibration & measurements capabilities (CMCs), peer- review of CMCs and metrological traceability. Besides that BIPM organized CIPM MRA webinar which was an excellent learning experience and it allowed me to confirm my interest in (CIPM MRA and its requirements) as a metrologist.

Acknowledgements

I am grateful to all those whom I had the pleasure to work with. The organizers of BIPM and TÜBİTAK UME. This internship would not have been possible without the support of my managers and the organizers of SSMO. I would especially like to thank Dr. Alper İşleyen, my teacher and mentor, his support and assistance are highly appreciated. Many thanks should also go to my teacher Dr. Şükran Akkuş Özen, she has provided me extensive personal and professional guidance and taught me a great deal about scientific research and life in general. And I would like to extend my deepest gratitude to all those who gave me the opportunity to participate in such great project.