Analytical Studies and Bio control of Ochratoxin A in Wheat

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Abstract

Title of the Thesis: Analytical Studies and Bio control of Ochratoxin A in Wheat.

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Ochratoxin A (OTA) has been considered a carcinogenic, teratogenic, hepatotoxic, and nephrotoxic thus reduction of OTA considered of high priority besides its economic effect on vets trading field.

A Method for the determination of OTA by HPLC-FLD has been adopted and validated. The method recoveries were (89-97%) while the limit of detection (LOD) and limit of quantification (LOQ) were (0.05-1 μg/kg), respectively. The study has concluded the efficiency and selectivity of IAC for OTA determination in wheat samples under proper selected conditions.

The method is internationally accredited by FINAS for fulfilling the requirements of ISO/IEC 17025/2017 standard.

Bio control is considered one of the most promising and more safety strategies. Five organisms were examined for activities against OTA producing *Aspergillus Steynii*, *Trichoderma harzianum*, *Trichoderma viride, and Bacillus mycodis* had no antifungal effect.

B. subtilus and *P. aeruginosa* have affected the colony.

The growth rate of *A. steynii had been* affected by different bacterial extract concentrations of both *B. subtilus* and *P. aeruginosa on YES media.* The OTA levels had been produced

by A. styenii affected by different chloroform extract concentrations of both B. subtilus and P. aeruginosa *on YES media* and stored wheat.

Keywords: OTA; LOD; LOQ; HPLC-FLD; IAC; FINAS; ISO/IEC; *Aspergillus Staynii*; *Trichoderma harzianum; Trichoderma viride; Bacillus mycodis*; *B. subtilus* and *P. aeruginosa; YES media.*

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Summary

Chapter 1:

Mycotoxins are secondary toxic metabolites produced by molds that grow on grains and cereals in the field or during storage. Mycotoxins contamination of food and feed depends mainly on the location and conditions of processing, storage, and marketing.

Ochratoxin A (OTA) mycotoxin was first detected as a fungal metabolite of *Aspergillus ochraceus* in 1965. A. Steynii is the main OTA-producing species in the section and produces 90 % of strains and toxin levels 1000 times higher than *A. Ochraceus.*

Ochratoxin A (OTA) has been categorized as one of the most poisoned mycotoxins. Toxicity studies proved that OTA is carcinogenic, teratogenic, hepatotoxic, and nephrotoxic in animals.

The European Commission (2002) declared a Regulation No. 472/2002 restrict OTA infection in unrefined cereals, counting rice, and buckwheat to 5 μg/kg.

Cereals include common OTA-contaminated feeds and foodstuffs (Maize, sorghum, wheat, rice, barley, oats, and rye).

Several analytical methods and techniques have been developed for the determination of OTA contaminant in several foodstuffs such as: IAC / HPLC-FLD, IAC / TLC, QuEChERS / UHPLC-QqLIT-MS, and BA-Nb ELISA / LC-MS/MS.

According to the high toxicity of OTA, physical, chemical, and biological detoxification methods were mainly applied to food and feed industries, but the biological methods with improved protection, flavor,

nutritional quality, organoleptic properties, availability, and cost-effectiveness were more promising than the other two classes.

Biological degradation is the method of choice for mycotoxins deactivation. Toxin binding by adsorbent materials and microbial inactivation by particular microorganisms or enzymes comprises certain degradation. Various organisms, including lactic acid bacteria, were used for this purpose. Furthermore, some enzymes were also reported to hydrolyze OTA.

Chapter 2:

Includes the experimental part which includes reagents, reference standard solution, calibration solutions, equipment's, apparatus, preparation test sample, quality control tools, HPLC components and conditions, sampling and validation process.

Includes the materials, organisms, samples, media and steps of the technique of bio control of ochratoxin A in wheat.

Chapter 3:

Results and discussion involve three parts;

1-HPLC Analysis of ochratoxin A in wheat that involves: the method adaptation which includes the following points:

- The method extraction technique.
- The method cleanup technique.
- The chromatographic method of analysis.

OTA has been separated sufficiently without matrix interferences with the isocratic mobile phase contains (water: acetonitrile: methanol: glacial acetic acid) (35: 35: 29: 1 v/v/v/v) which delivers fast OTA peak

separation within only 10 minutes. The separation has been achieved via the Agilent Eclipse Plus C18 column with a 1 mL/min flow rate.

2-This part involves also the method validation which is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use where CODEX guidelines and Eurachem guidelines were followed in performing the different validation parameters. This part can be stated in the following points:

- LOD has been 0.049 µg/Kg.
- LOQ has been 1 µg/Kg.
- Linearity which includes the standard and method linearity > 0.99.
- Method accuracy has been evidenced through:
    - Trueness (Succeeded through Several FAPAS PT rounds equal -0.5 Z-Score).
    - The bias test expressed as a relative difference (RD %) at different levels has been less than 12%.
    - The repeatability and reproducibility tests expressed as relative standard deviation have been less than 2.6% and 5.0 % respectively.
- The uncertainty estimation has been 22.8%.

3-Bio Control Reduction of Ochratoxin A in Wheat.

- Screening for bio control agent using bacteria and fungi of ochratoxigenic fungi.

Five organisms were examined for activities against OTA producing *Aspergillus Steynii*. *Trichoderma harzianum*, *Trichoderma*

viride, and Bacillus mycodis had no antifungal effect. *B. subtilus* and *P. aeruginosa* have affected the colony.

- Control of OTA production by A. staynii on media and wheat using the selected organisms.

The growth rates of A. styenii affected by different chloroform extract concentrations of both B. subtilus and P. aeruginosa *on YES media.* The concentration of 200 and 500 ppm had a very close effect. It decreased by around 12 and 23% of *B. subtilus* and *P. aeruginosa* respectively. The high reduction was observed using the concentration 1000 ppm (31 and 39% reduction for both *B. subtilus* and *P. aeruginosa* respectively).

The growth rate of A. *styenii* affected by different bacterial extract concentrations of both *B. subtilus* and *P. aeruginosa,* both bacterial extracts had a clear inhibition effect on the OTA production by *A. styenii* reaching 97% when compared with its effect on growth rate (6-25%).

The growth rates of A. styenii affected by different chloroform extract concentrations of both B. subtilus and P. aeruginosa in vitro, *B. subtilus* extract had a significant reduction effect starting from 1000 ppm, whereas only the concentrations 500 and 5000 ppm of *P. aeruginosa extract* had a reduction effect on OTA production by *A. styenii* on stored wheat.