



Presence of Ochratoxin in a Green Coffee Beans Processed Naturally by Smallholder Farmers in Jember



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Abstract

Moulds growth and contaminated green coffee beans, produced mycotoxins as secondary metabolites such as Ochratoxin A. Ochratoxin A have been reported carcinogen in human health. The aim of this research was determined the occurrence of Ochratoxin A in green coffee beans using C18 SPE cartridges as alternative clean-up method. Samples were collected from Argo puro mountain areas, Jember Indonesia during the coffee year. Samples were prepared with extraction, clean-up, separation, and quantification. The parameter of OTA was determined by HPLC-FL Detector fitted with C18 HPLC column. The result shown 9 of 10 samples were detected OTA with the highest concentration was 0.4319ppm and the lowest concentration was 0.0146ppm.

Keywords: Jember green coffee beans; Ochratoxin A; C18 SPE Cartridges

Introduction

Mycotoxins are contamination toxin produced by filamentous fungi as second metabolite produced. OTA has been reported in coffee beans [1-3]. International Agency for Research of Cancer has classified OTA in group 2B as a possible human carcinogen [4]. Clean-up is the step called for enrichment OTA, usually used liquid-liquid and solid-solid extraction [5]. Immunoaffinity SPE cartridges are high cost, for the main reason more economic alternatives are required. C18 SPE cartridges known as recommended applications for aflatoxins determination. The aims of this study were determined the amount of Ochratoxin A in green coffee beans produced by smallholder farmers in Jember using C18 SPE cartridges as the economic clean-up separation.

Material and Methods

Sample collection

A total of 10 green coffee beans collected during coffee year.

Sample preparation

100mg of chloroform and 12.5mL of phosphoric acid (0.1M) added into Erlenmeyer flask with 10g of ground coffee. The mixture was stirred and filtered. The filtrate was transfer into 500mL separatory funnel, 50mL of the lower layer transferred to round-bottom flask and evaporated to dryness. The residue was dissolved

in 5mL of hexane and 5mL of methanol/ water mixture (1:1, v/v), filtered and the filtrate were transferred into 50mL separatory funnel. The lower layer was collected into 25mL Erlenmeyer flask, and the upper phase extracted twice with 5mL methanol/ water mixture (1:1, v/v) to rinse the round bottom flask containing the residue.

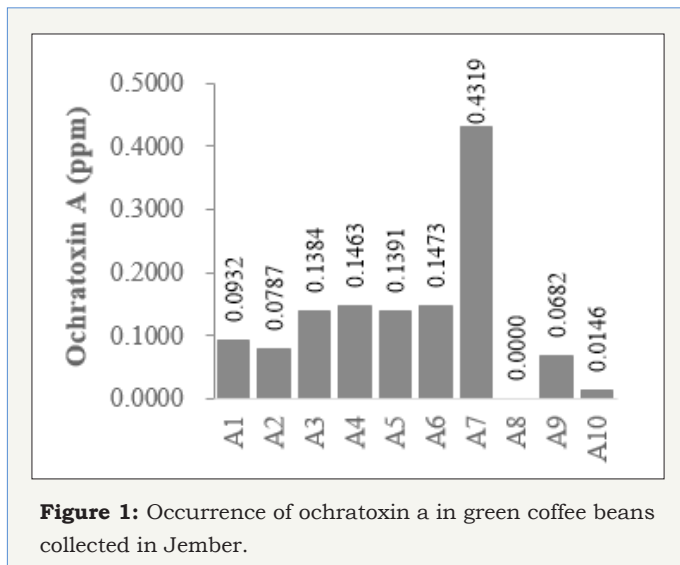
Solid phase extraction (SPE)

The C18 SPE (Inert Sep VRA-2 6mL) conditioned with 5mL of methanol and 5mL of methanol/water mixture (1:1, v/v). 5mL sample were loaded into C18 cartridges. The cartridges washed with 5mL methanol/water mixture (3:1, v/v). OTA was eluted from the cartridges with 10mL of methanol/formic acid mixture (98:2, v/v). The solution evaporated under nitrogen stream for 2min, 45 °C. The solution was added with Trifluoro Acetic Acid 0.1mL and stirred about 15 min, added with 0.9mL of Acetonitrile/water mixture (1:9, v/v) and injected into HPLC-FLD.

HPLC injection

HPLC fitted with C18 HPLC column (Inert Sustain swift, C18/ 50m, 4.6x250mm). Mobile phase solution was Acetonitrile: Glacial acetic acid: Distilled water (5:1:4, v/v). Flow rate was 0.700, temperature control 45 °C, Ex 333nm, and Em 450nm. Samples were loaded 20µL with micro insert.

Result and Discussion



The results of the Ochratoxin A analysis of 10 samples were shown in Figure 1. A7 was the highest Ochratoxin A with concentration of 0.4319ppm. And A10 was the lowest Ochratoxin A with concentration of 0.0146ppm. A8 was not detected as Ochratoxin A contamination in coffee sample. The difference of the concentration shown in Figure 1 caused by many factors such different location of the coffee growth and the process after harvesting the coffee beans. The lack of clear difference in the incidence and concentration of mycotoxins in agricultural commodities is affected by many factors, such as environmental and geographic location where crops are grown. In addition, harvest and the storage conditions may influence the mycotoxin production [6,7]. C18 column as SPE clean-up could be determined OTA as immunoaffinity column. This alternative could reduce high


cost because of high demand of immunoaffinity column. C18 SPE is more efficient and more precise than immunoaffinity cartridges. C18 much lower than the price of immunoaffinity cartridges [4].

Conclusion

The determination of ochratoxin A with HPLC-FL Detector method fitted with C18 SPE (Solid Phase Extraction) cartridges as clean-up method give best result of 10 ground coffee samples. The amount of difference Ochratoxin A caused by many factors. It depends on the location of the coffee growth, the moulds that contaminated, climate, and quality of the process used by farmer.

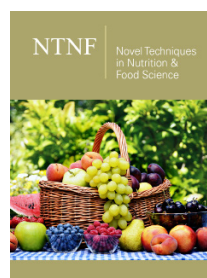
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