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# Detection of Mycotoxigenic Fungi on Food from Markets with the Polymerase Chain Reaction Method for Halal and Thoyiban Food

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## Abstract

Secondary metabolites of filamentous mold are mycotoxins, which in some situations can develop on foods derived from plants or from animals. *Fusarium*, *Aspergillus*, and *Penicillium* are the most common types of mold that produce mycotoxins and also often contaminate human food and animal feed. Aflatoxins including aflatoxin B1, B2, G1 and G2 are produced by *A. flavus* and *A. parasiticus*. M1 and M2 aflatoxins are found in dairy products. In this study, we used PCR to detect and identify mycotoxigenic fungi material in foods from traditional markets and supermarkets in Surabaya, Indonesia. Samples of chicken meat from a traditional market and from a supermarket (10 pieces each) were placed in a conical tube and crushed in PBS. The crushed samples were centrifuged and the supernatants were cultured on potato dextrose agar (PDA) media and observed using a reverse microscope. DNA was isolated from cultured samples and subjected to PCR with primers specific for genes encoding aflatoxins. Interestingly, we revealed that the Polymerase Chain Reaction (PCR) analysis showed *A. flavus* and *A. ochraceus* were present on chicken meat sold at traditional markets and supermarkets. In sum, enhanced precautions may be needed to ensure that foods sold in traditional markets and supermarkets are free from molds that have the potential to produce mycotoxins. Further studies are needed to detect and identify the prevalence of mycotoxins in the food supply.

***Keywords:*** *Aflatoxin B, mycotoxins, traditional market, supermarket.*

## Introduction

Secondary metabolites of filamentous mold products and in some situations are present on foods derived from plants or animals. *Fusarium*, *Aspergillus*, and *Penicillium* which are the most common types of mold that produce mycotoxins, often contaminate human food and animal feed. These molds grow on food or feed ingredients, both before and during harvest or when foods are improperly stored[1,2]. Mycotoxins cannot be detected by smell or taste, but can significantly reduce livestock production performance and pose a threat to human health[1].

Aflatoxin B1, B2, G1 and G2, are the main types of mycotoxins produced by *A. flavus* and *A. parasiticus*[3]. The presence of mycotoxins has expanded at all levels of the food chain. In terms of food security and its implications for human health and the economy, mycotoxins are an important contaminant of the food chain[4]. In this study, we used PCR to detect and identify the presence of mycotoxigenic fungi in human foods purchased from traditional markets and supermarkets in Surabaya, Indonesia.

## Material and Methods

### Experimental Site:

Whole chicken samples were taken from several traditional markets and supermarkets in the city of Surabaya, Indonesia (10 pieces each).

**Material and Research Tool:**

PBS (Thermo Scientific), potato dextrose agar (PDA) media (Himedia), DNA extraction kit (Qiagen), PCR reagents (Qiagen), reverse microscope (Olympus, type OPTO-EDU A12.2602-T), PCR machine (Bio-Rad).

**Research Procedure and Data Collection:**

Each sample was placed in a conical tube and crushed in PBS. The mixtures were centrifuged and the supernatants were cultured on PDA media and observed using a reverse microscope. DNA isolation is done using materials from the Qiagen factory. 900 µL cell the lysis solution is put in a 1.5 tube mL and 300 µL of culture sample were added. The tube is turned 5-6 times so that the solution can mixed up. The tube is incubated for 10 minutes at room temperature (during incubation flip the tube 2-3 times), then centrifuged with a speed of 13,000-16,000 rpm for 20 seconds at room temperature. The supernatant discarded, and vortexed for 10-15 seconds. After that, added to the tube 300 µL nuclei lysis solution and solution pipette 5-6 times to break the wall mushroom. Then add 100 µL of protein precipitation solution, and divortex for 10-20 seconds. The supernatant was transferred to the tube 1.5 mL of new sterile that has been previously filled with 300 µL isopropanol. The centrifuge tube with a speed of 13,000-16,000 rpm for 3 minutes at room temperature. The solution stirring by turning the tube up to visible fine white threads of DNA. Centrifuged tube with a speed of 13,000-16,000 rpm for 3 minutes at room temperature, so you can see the pellet on the bottom of the tube. The supernatant was removed, and also added 300 µL 70% ethanol. The tube is turned over to make the pellet and the solution is mixed then centrifuged with speed of 13,000-16,000 rpm for 3 minutes at room temperature. Slowly ethanol is removed with a pipette. The tube is reversed on absorbent paper and dried pellets for 10-15 minutes. Last added 50 µL DNA rehydration solution into the tube. DNA is stored overnight at 4 ºC, then DNA is stored at 2-8 ºC. PCR was carried out in two stages 2 (two) different pairs of primers. To identify the presence or absence of mycotoxigenic genes pyrG using PCR we designed the following primers:

1) Af.pyrg.far.F, (5′-CCTCAAACAATGCTCTTCACCC-3′); Af.pyrg.R (5′-CATTCCCTATCAACTCCCCCTC-3′)
2) flank.pyrg.F3 (5′-TATGGCTTCTCTCGGCTCAG-3′); flank.pyrg.R3 (5′-CCACGGTTGTCTTCTTGGTGTAG-3′)
3) Af.pyrg.R (5′-CATTCCCTATCAACTCCCCCTC-3′); flank.pyrg.F1 (5′-GCTCTATTCAACGGACTTCAACC-3′)

PCR amplifications were performed 20 pmol of primers were added and mixed to obtain 50 µL final volume of the PCR mix. PCR was carried out using 30 cycles of denaturation at 94 °C for 30 sec, annealing at 58 °C for 30 sec, and extension 68 °C for 150 sec. PCR products were resolved by electrophoresis on an agarose gel, which was stained with ethidium bromide and documented on a UV box (Gel Ninja equipped with a camera).

## Results And Discussion

Based on this study, *A. flavus* and *A. ochraceus* were present in chicken samples purchased from traditional markets and supermarkets, respectively (Figures 1 and 2). Traditional markets have 2 sample positive and supermarkets have 1 sample positive. These results suggest that supervision of foods in both traditional markets and supermarkets is needed. *Aspergillus* spp. such as *A. flavus* and *A. parasiticus* can be found on various substrates, including soil, fruit leaves, and seeds, which are all typical ingredients for manufacture of animal feed products from agricultural commodities[5,6]. Molds found in animal feed can produce aflatoxins as secondary metabolites that are known to have carcinogenic, mutagenic, teratogenic[7,8], hepatotoxic and immunosuppressive properties[9].

The results of the present study are similar to those of Irdawati *et al*. who found that *Aspergillus* contamination was present in traditional markets located at Pasar Raya, Padang, Indonesia[10]. In addition, a study by Handajani *et al*. showed that shrimp patties have a 10-fold greater risk of contamination with various aflatoxins[11]. Aflatoxins are mycotoxins that are mainly produced by the fungi *A. flavus* and *A. parasiticus*[12], as well as by several other fungi such as *A. ochraceoroseus*, *Aspergillus* SRCC 1468, *Emericella astellata*, and *Emericella* SRCC 2520 species[13]. Relative to other mycotoxins, aflatoxins have higher toxicity and higher potential to cause disease[11]. According to the International Agency for Research on Cancer (IARC), aflatoxin B1 can cause cancer in humans, particularly liver cancer (hepatocellular carcinoma)[14].

Aflatoxins have the potential to be carcinogenic, mutagenic, teratogenic and immunosuppressive[12,15,16]. As such, precautions must be taken to ensure that the food supply is not contaminated by fungi that produce mycotoxins.

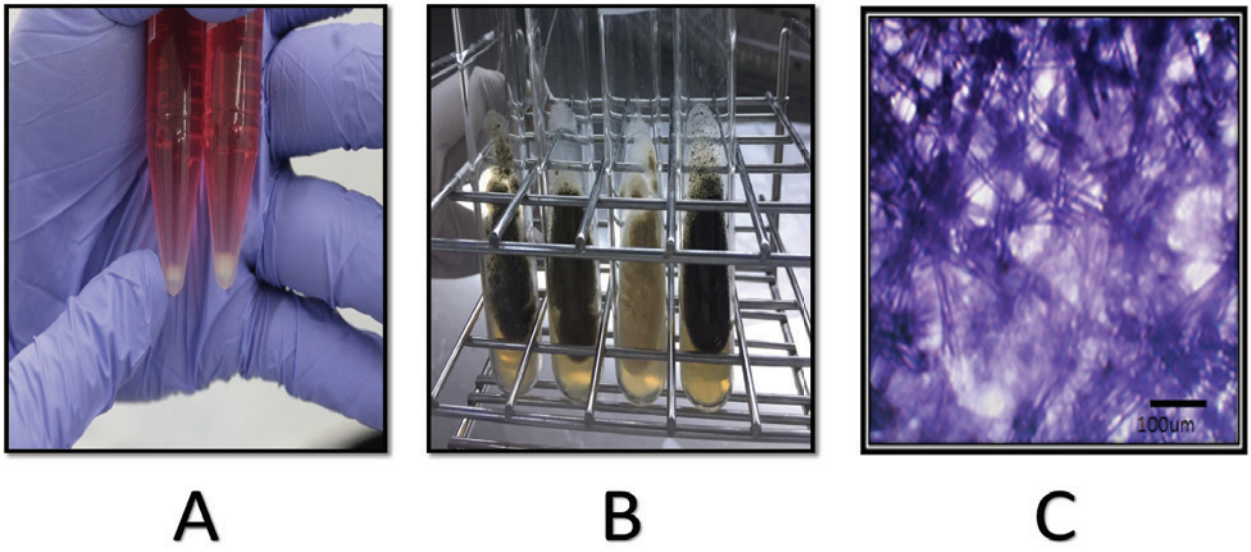


**Figure 1. Preparation of food samples for analysis. A. Sample after homogenization and centrifugation; B. Propagation of supernatants on PDA media; C. Staining and observation of culture growth by reverse microscopy with HE staining and 100× magnificient.**

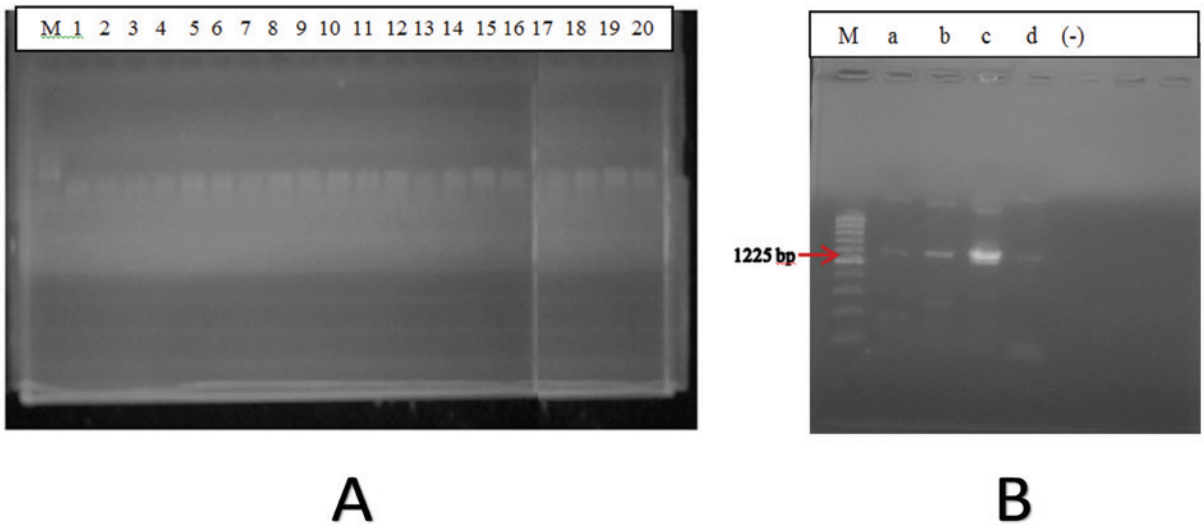


**Figure 2. PCR analysis of food samples. A. Whole genome DNA extracted from cultures of samples from traditional markets and supermarkets. Remarks M= marker; 1-20= samples; B. Analysis of PCR products amplified from samples propagated on PDA media using a primer specific for *Aspergillus* genus aflatoxin; M= marker; a sample from the traditional market (a and b); samples from supermarkets (c); control positive (d); (-) = negative control, using the Af pyrG gene.**

## Conclusion

In sum, enhanced precautions may be needed to ensure that foods sold in traditional markets and supermarkets are free from molds that have the potential to produce mycotoxins. Further studies are needed to detect and identify the prevalence of mycotoxins in the food supply.

**Conflict of Interest:** The author declare no conflict of interest.

**Ethical Approval :**

All applicable institutional guidelines for the care and use of animals were followed. This research received ethical clearance approval from Animal Care and Use Commitee, Faculty of Veterinary Medicine, Universitas Airlangga Surabaya, East Java, Indonesia.

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