

Visual Method for Detecting Contaminant on Dried Nutmeg Using Fluorescence Imaging

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Abstract. Traditional practice of nutmeg sun-drying causes some fungi such as *Aspergillus flavus* to grow. One of the secondary metabolites of *A. flavus* named aflatoxin (AFs) is known to be carcinogenic, so the dried nutmeg kernel must be aflatoxin-free in the trading. Aflatoxin detection requires time and costly, make it difficult to conduct at the farmers level. This study aims to develop a simple and low-cost method to detect aflatoxin at the farmer level. Fresh nutmeg seeds were dried in two ways; sundried everyday (continuous), and sundried every two days (intermittent), both for around 18 days. The dried nutmeg seeds are then stored in a rice sack under normal conditions until the fungi grow, then they were opened and the images of kernels were captured using a CCD camera, with normal light and UV light sources. Visual observation on images captured in normal light source was able to detect the presence of fungi on dried kernels, by 28.0% for continuous and 26.2% for intermittent sun-drying. Visual observation on images captured in UV light source was able to detect the presence of aflatoxin on dried kernels, indicated by blue luminance on kernel, by 10.4% and 13.4% for continuous and intermittent sun-drying.

1. Introduction

Indonesia is one of the world's largest nutmeg exporting countries with the largest export destination countries are Vietnam (22%), Netherlands(16%), the United States (13%), India (9.4%), and Germany (6.8%) [1]. In some periods, the nutmeg (*Myristica fragrans* Houtt) exported by Indonesia to the European Union was rejected due to the contamination of *Aspergillus flavus* (*A. flavus*) fungus. This type of fungus is known to release secondary metabolites known as aflatoxin that can cause liver cancer in humans and livestock when consumed in certain quantities. Some other fungi that produce aflatoxin are *A. parasiticus*, *A. nomius*, and *A. tamaraii*. In addition to nutmeg seeds, *A. flavus* can also be found on other products such as corn, peanuts, milk and derivative products [2]. Some countries in the European Union apply strict regulations to the limits of aflatoxin content in imported foods of 3 to 5 ppb [3], the US Food and Drug Administration (FDA) sets a limit of 20 ppb for aflatoxin content in food and feed [4], while Indonesia categorizes nutmeg as a spice product with the aflatoxin content limit of 15 ppb [5]. Fungal contamination may develop from improper postharvest handling especially drying and storage stages. To reduce contamination, drying should be done properly, for example on a shelf with a distance of about 1 meter above the ground to avoid direct contact with the soil that can be a source of fungus spread. Storage should be done in a fairly dry place and packed with sacks or closed cans [6].



Several methods have been developed to measure aflatoxin levels such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and enzyme-linked immune assay (ELISA) [7] which require time, costly, and destructively. So a method that can detect aflatoxin levels quickly, does not require high cost, non-destructively and easily to apply especially at farmers level, is needed. From several literatures, aflatoxin is known to fluoresce with blue and green luminescence intensities under UV light with emission wavelengths of 420 - 450 nm and excitation wavelengths of 350 to 370 nm [2]. The objective of this research was to develop a contaminants detection nutmeg kernels visually through images of nutmeg kernels captured using a CCD camera with normal light source to detect a presence of fungi and UV light source to detect aflatoxin on dried nutmeg kernels.

2. Materials and methods

The material used in this research were fresh nutmeg seeds obtained from farmers in Siau Island, Sitaro District, North Sulawesi Province, then sent directly to Bogor by sea carrier. Equipment used in the experiment includes image recording device consisting of a CCD camera (charged couple device), a set of PC (personal computer), camera buffer, board to place the samples, four pieces ordinary Philips 5 watts lamp as a normal light source, and four pieces UV lamps SANKO DENKI brand with wavelength of 352 nm as the source of fluorescent light, as well as a set of tools for measurement of moisture content such as oven, analytical scales, cups, and desiccators. Analysis of moisture content was done based on AOAC standard (1999).

Research begins with sun-drying nutmeg seeds in two different methods, daily sun-drying (continuous sun-drying) and sun-drying every two days (intermittent sun-drying) for 18 days. Dried nutmeg seeds then were stored in normal condition (room temperature was 25 °C) and packed in wooden type plastic rice sack until the fungi grow. The dried seeds then were cracked and the images of kernels were captured using a CCD camera with normal light and UV light sources. Visual observation on images was conducted in the room and chemical analysis for aflatoxin level was conducted by HPLC method in a laboratory at SEAMO BIOTROP.

3. Results and discussion

3.1. Nutmeg seed drying

The fresh nutmeg fruits were harvested by farmers in Siau Island, Sitaro District in North Sulawesi and opened to obtain the fresh seeds, the packed and shipped to Bogor directly. It took eight days to reach the laboratory, with an initial moisture content of 33.3%. All the nutmeg kernels were sundried with daily temperature as shown in figure 1. Drying of nutmeg seeds is usually done to produce the aroma of nutmeg kernels.

Temperature and relative humidity vary according to the air condition during sun-drying of nutmeg seeds. The occurrence of variations or fluctuations in air temperature was normal and assumed to be last for eight hours period when the sun-drying took place. This fluctuation was closely related to the process of energy exchange that takes place in the atmosphere [8]. The day 1 and day 3 were the lowest temperature of 30 °C during sun-drying. The highest temperature during the drying process was 41 °C, occurred on the day 8. In the sun-drying of nutmeg seeds, the recommended temperature for maintaining high-quality nutmeg kernels should be not more than 45 °C. High drying temperatures can cause crack or wrinkle on nutmegs kernels due to the melting of fat content. Besides causing wrinkled on kernels, high drying temperatures can reduce the distinctive aroma of nutmeg kernels [6], thereby decreasing the quality of nutmeg kernels in trading.

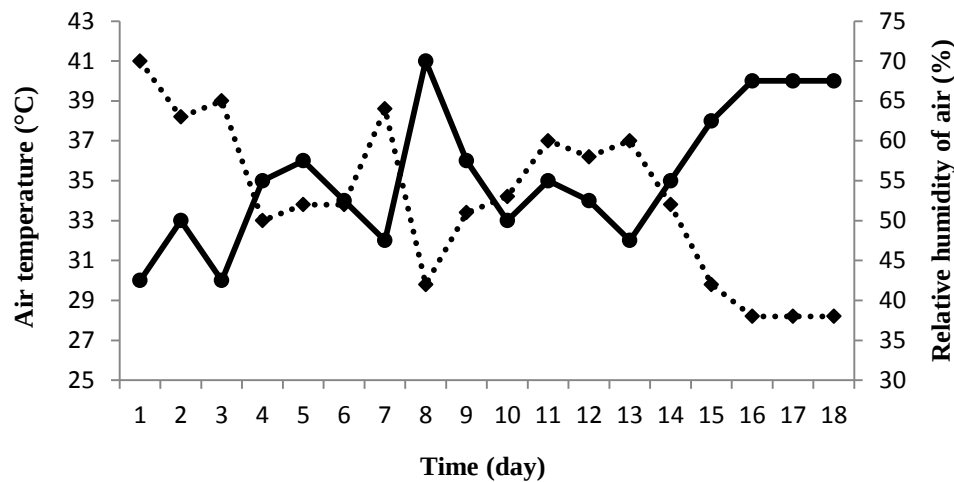


Figure 1. Temperature (●) and relative humidity (◆) of air during drying

The initial moisture content of nutmeg seeds was 33.3%. In general, the chart of changes in the moisture content of nutmeg seed with continuous sun-drying and intermittent sun-drying show the same pattern, which increased after the first day of sun-drying and then tends to decrease until the end of sun-drying process. figure 2 shows changes in the moisture content of nutmeg seeds that were sundried everyday. Sun-drying was discontinued at day 17 because the water content had reached 13.5% and would not decrease anymore because it reached the equilibrium moisture content, known from constant weight of nutmeg seeds. The moisture content of nutmeg seeds which were sundried every two days decreased until the sun-drying was stopped when it reached 10.7% (figure 3) at day 16 of drying, when constant weight of nutmeg seeds was observed. This method takes a shorter time compared to daily sun-drying. This is related to the characteristics of the material that is less exposed to high temperatures due to drying then the evaporation will become faster when the material is dried. The moisture content of nutmeg seeds at the request of the European Spices Association (ESA) is 12% wet basis. Meanwhile, according to SNI (No. SNI 01-0006-1993) the maximum moisture content of nutmeg seeds on nut quality standard requirements of nutmeg is 10% [6].

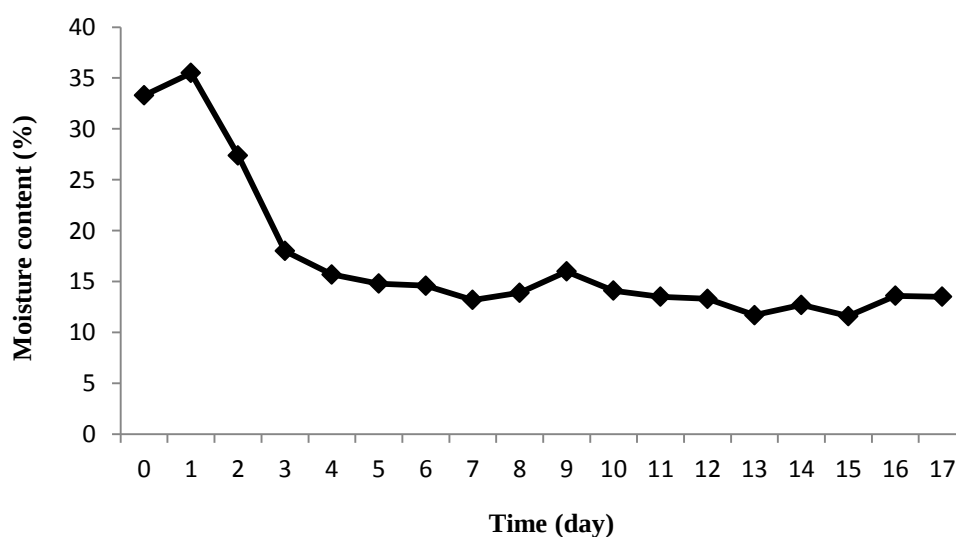


Figure 2. Changes in the moisture content of nutmeg seeds sundried every day (continuous sun-drying)

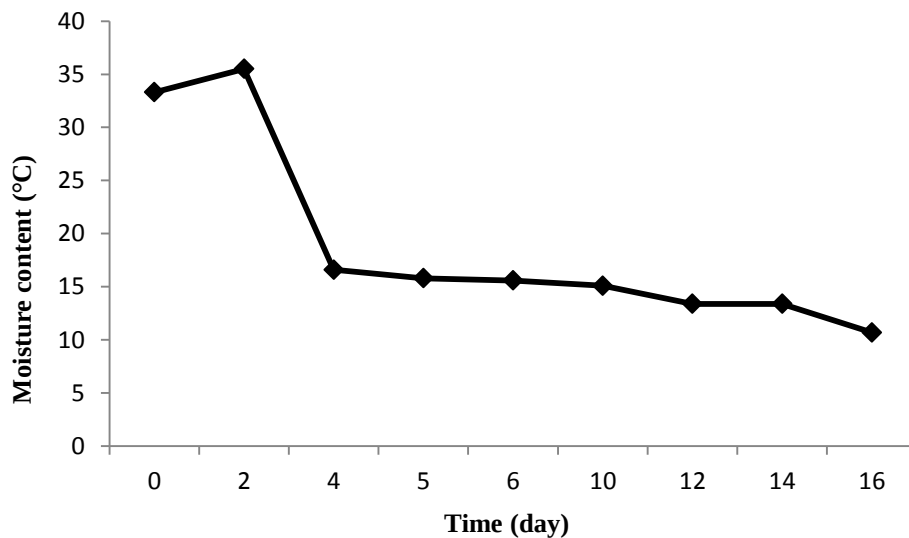


Figure 3. Changes in moisture content of dried nutmeg seed every two days (intermittent drying)

3.2. Visual appearance of nutmeg seeds with normal light source

Figure 4 is a visual appearance of the fungus on dry nutmeg kernels. Nutmeg kernels are the original Indonesian herbs and are highly susceptible to fungal infestations. The fungus can spread through soil and air by two main factors that support contamination such as temperature and humidity [3]. Through visual observation, total fungus infestation on nutmeg seeds with continuous sun-drying reached 28% with 3.2% high infestation, 7.2% medium infestation, and 17.6% low infestation. The nutmeg seed dried every two days resulted in a total infestation of 26.2% with high infested seeds of 12.2%, medium infestation of 4.3%, and low infested of 9.8% (table 1). The maximum number of fungi in the general requirements of nutmeg quality according to SNI (No. SNI 01-0006-1993) is 8% [6].

Daily sun-drying in the open air allows more fungi to contaminate nutmeg seeds compared with nutmegs that are dried in every two days, but contamination does not develop and spread because the nutmeg was exposed to higher temperatures longer as well as lower humidity does not support the growth and development of the fungus. While the sun-drying done every two days allows the fungus infestation to grow and spread faster so that more infestations found in high category. This phenomenon occurred because when nutmeg seeds were not dried then the nutmeg directly interact with the higher relative humidity and lower temperature of the room which is very supportive for fungus growth.

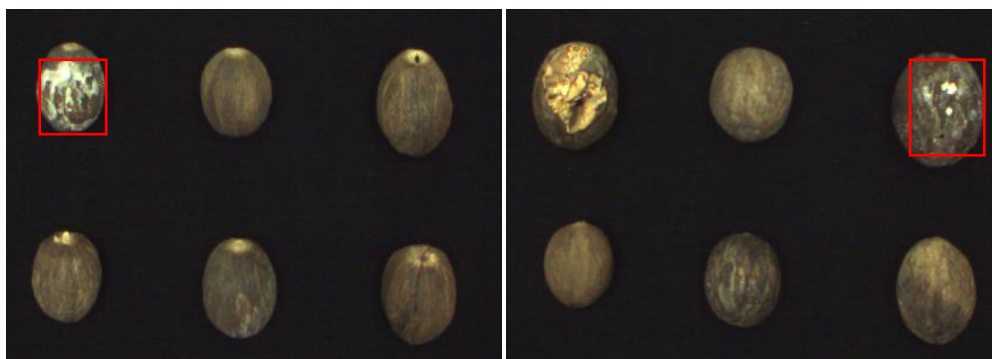


Figure 4. Image of nutmeg seeds infested by fungus observed using a CCD camera with normal light source

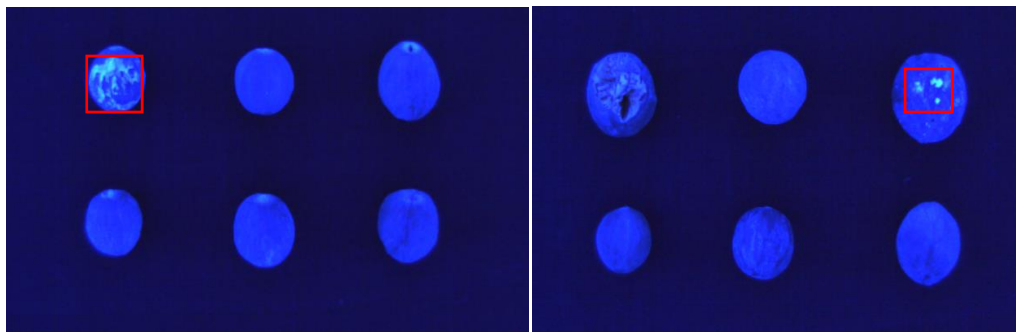
Table 1. Presence of fungus on nutmeg kernels observed on images captured using CCD camera with normal light source

Sample	N	n (%)	Infestation (%)		
			low	medium	High
Continuous Drying	125	35 (28,0%)	22 (17,6%)	9 (7,2%)	4 (3,2%)
Intermittent Drying	164	43 (26,2%)	16 (9,8%)	7 (4,3%)	20 (12,2%)

N=total number of nutmeg kernels; n=number of contaminated nutmeg kernels

3.3. Visual appearance of nutmeg seeds with UV light sources

The presence of aflatoxin observed visually on nutmeg kernels were characterized by blue luminescence on the monitor screen (figure 5). The high percentage of *A. flavus* contamination in dried nutmeg kernels indicates the risk of aflatoxin formation. The amount of *A. flavus* contamination in the dried food can be affected by the conditions and the duration of drying and storage. Drying and storage conditions play an important role for growth of *A. flavus* during storage [9]. The results (table 1) showed that overall the total nutmeg kernels contained aflatoxin by 10.4% and 13.4%, respectively, for nutmegs dried everyday and dried every two days. The percentage of aflatoxin on continuous sun-drying nutmeg were 8.0% for the low category, 0.8% medium category and 3.2% high category. The percentage of aflatoxin on nutmeg kernels with intermittent sun-drying were 6,09% for low category, 3.05% medium category, and 4.27% high category using the same sample as in table 1.

**Figure 5.** Luminescence of aflatoxin on nutmeg seed using CCD camera with UV light source**Table 2.** Presentation of luminescence on nutmeg using CCD camera with UV light sources

Sample	N	n (%)	Infestation (%)		
			low	medium	High
Continuous Drying	125	13 (10,4%)	10 (8,0%)	1 (0,8%)	2 (1,6%)
Intermittent Drying	164	22 (13,4%)	10 (6,1%)	5 (3,1%)	7 (4,3%)

N=total number of nutmeg kernels; n=number of contaminated nutmeg kernels

In general, more than 10 types of aflatoxin have been identified, but only aflatoxin B1, B2, G1, and G2 that have important roles due to their toxicity and are often found in food and feed products [2]. Several methods to detect aflatoxin have been developed include high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and enzyme-linked

immune assay (ELISA) [7]. Nutmeg kernel samples with continuous sun-drying method and intermittent sun-drying have been tested for its aflatoxin content of B1, B2, G1, and G2 by HPLC (High Performance Liquid Chromatographic) method and overall aflatoxin contamination is less than the limit that can be detected by the tools of <0.45 ppb for aflatoxin B1 (AFB1), <0.43 ppb AFB2, <0.48 ppb AFG1, and <0.54 ppb AFG2 (table 3). However, in visual observation through camera with UV light source, aflatoxin can be seen by the presence of blue luminescence in the area of the fungus on the nutmeg kernels. Based on physical features, aflatoxin compounds when exposed to high-energy light will emit fluorescence light with specific wavelengths [10].

Table 3. Total aflatoxin content in nutmeg kernels by ELISA method

Sample	Aflatoxin content (ppb)			
	B1	B2	G1	G2
Continuous Drying	<0,45	<0,43	<0,48	<0,54
Intermittent Drying	<0,45	<0,43	<0,48	<0,54

4. Conclusions

Daily drying (continuous drying) and drying in every two days (intermittent drying) of nutmeg seeds in ambient temperature varying between 30-41 °C and 38-70% RH result in different final nutmeg seeds moisture content. In continuous drying, the nutmeg seeds could reach a moisture content of 13.5% within 16 days while in intermittent drying, the nutmeg seeds could reach a moisture content of 10.7% within the same drying time.

CCD camera with normal light could only produced images of nutmeg kernels with the fungus, while the same camera with UV light as a source for fluorescence effect could detect the existence of aflatoxin indicating by blue luminescence on the images produced.

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