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To cite this article: A H Mulyati *et al* 2021 *J. Phys.: Conf. Ser.* **1882** 012115

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## Determination of the Aflatoxin levels in Corn (*Zea mays*, L.) during storage process

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**Abstract.** Aflatoxin contaminant in corn being a serious problem, due to its toxicity. This contamination was influenced by the storage and treatment process. During storage, the aflatoxin level was influenced by the water content of corn seed. In this study, the water content were performed by gravimetric method, while the aflatoxin contents have been carried out by ELISA and HPLC methods. The corn seed samples were obtained from Tangerang, Cikande, and Cirebon. The results showed that the aflatoxin content were increased in fourth-week storage. The analysis of the aflatoxin content using the ELISA method showed that corn seed obtained from Tangerang, Cikande, and Cirebon were 74,06 ppb; 9.33 ppb; 28.67 ppb respectively, while the HPLC method showed that the aflatoxin content were 75.02 ppb; 9.83 ppb; 28.43ppb respectively. The results also showed that the water content in the corn seed obtained from Tangerang, Cikande, and Cirebon were 10.64%; 10.17%, 10.25% respectively on fourth-week storage. In conclusion, the aflatoxin content in corn seed from Cikande and Cirebon were in accordance with SNI standards, while corn seed from Tangerang contain aflatoxin was above 50 ppb.

**Keywords:** corn seed, aflatoxin, water content, ELISA, HPLC

### 1. Introduction

Corn is a type of cereal plant that has been strategic in agricultural development [1]. Corn is an important commodity for humans and animals. Corn is the world's most important food crop besides wheat and rice as the main food in Indonesia. In addition, corn is also widely processed as food ingredients, including corn starch, popcorn, soup, mixed ingredients in making bread and cakes,. Corn is a commodity that has problems during postharvest handling, which are characterized by high contamination aflatoxins [1]. Aflatoxin contamination in corn kernels causing health problem and also lost income in many developing countries [2]. Aflatoxins and cause liver cancer in humans and also cause severe economic losses for farmers [3]. Aflatoxin is insoluble in water, nonpolar, stable to heat, physical treatment, and chemical treatment, therefore it is very difficult to remove if the corn kernels have been contaminated by the aflatoxins. Most of the aflatoxin problems are caused by *Aspergillus flavus*. This fungus infects corn kernels since the corn crop is still in the field and peaks after harvesting and also during storage process [4]. Management strategies should be designed immediately for use at the pre-harvest stage Maize in Indonesia generally contains high levels of aflatoxins [5].



Aflatoxin is a secondary metabolite compound from *Aspergillus flavus* and *Aspergillus parasiticus* which can contaminate food or feed ingredients so that it is dangerous for animal and human health. Contamination of aflatoxin-producing molds is found in food and feed derived from agricultural products. Corn is an agricultural product that is easily contaminated by *Aspergillus* [6]. There was a significant correlation between the contamination level of *Aspergillus flavus* and the level of aflatoxin in corn kernels. There was a significant grain relationship between the proportion of grain groups with defects (burnt, sprouted, burned and mouldy grain and total injury) and the estimated level of aflatoxin contamination in the maize sample [8]. From various research results in Indonesia, aflatoxins are the main mycotoxins to pollute maize and animal feed ingredients [9]. The aflatoxin content in maize will increase because aflatoxins can be produced by fungi from planting to storage and are supported by mycotoxin properties that are stable to the environment, and are not easily damaged by various processing. Several physical, chemical and biological methods can be used to remove some or all of these toxins from food and ensure food safety and consumer health concerns [10].

Storage of corn kernels is an important factor that can trigger the growth of *Aspergillus flavus*, the main producer of aflatoxin B1. The storage conditions for corn kernels such as storage time and temperature and humidity during storage greatly affect the growth of these aflatoxin-producing fungi. The potential use of Vis-NIR spectroscopy to identify agricultural commodities contaminated with aflatoxins due to fungal infections will be carried out in the future [11]. Aflatoxin levels can be determined by testing using the ELISA and HPLC methods. A sensitive and robust HPLC method with fluorescence detector (FLD), photochemical reaction device (PHRED) was developed to determine Aflatoxins (B1, B2, G1, and G2) and Ochratoxin A in animal feeds. Immunoaffinity columns (IAC) was used to purify the samples [12]. Meanwhile, the moisture content was carried out by using the gravimetric oven drying method by calculating the weight of water lost during drying.

## 2. Research Methods

### 2.1. Material

The materials used were dry shelled corn, methanol, acetonitrile, aquadest, and aquabidest. The tools used are the Aflatoxin AgraQuant Romer ELISA Kit (AgraQuant® Total Aflatoxin Assay 4/40) which consists of a standard solution of aflatoxin 0-40 ppb, conjugate antiaflatoxin HRP (Horseradish Peroxidase), TMB (Tetra Methyl Benzidine) substrate, and stop a solution in the form of a strong acid such as HCl (Appendix 2). Other tools are Whatman No. filter paper. 1, ELISA reader (Opsys MR DYNEX Technologies), Agilent Technologies 1120 Compact LC Pump, Agilent Technologies 1200 Series Detector, Orbital shaker GFL 3005 series, Memmert oven UFP 600, and balance sheet Kern EWB 620-2M series.

### 2.2. Method

The samples tested came from Tangerang (sample A), Cikande (sample B), and Cirebon (sample C). Samples were stored at 25°C and were analyzed total aflatoxin and moisture content at baseline (0 week), 1 week, 2 weeks, 3 weeks, 4 weeks after storage. Determination of water content was carried out by gravimetric methods.

### 2.3. Analysis of Aflatoxin Levels by ELISA

500 grams of shelled maize sample was mashed using a blender, it was sieved with a 20 mesh sieve. ± 20 grams into 250 ml Erlenmeyer and homogenized with 100 ml methanol 70% by rotating in an orbital shaker for 5 minutes of 200 rpm. The homogenized sample was filtered using filter paper Whatman No.1. The filtrate is ready to be analyzed by ELISA. Put the sample in a dilution microplate (200µl + 100 µl standards/samples, homogenized and then put 100µl into the coating plate, incubated for 15 minutes (25°C). Plate washed by water for 5 replicates. Add 100 µl substrate solution into the dried plate, incubated for 5 minutes. Add 100 µl stop solutions, and measured the optical density value by ELISA reader at a wavelength of 450 nm.

#### 2.4. Analysis of Aflatoxin Levels with HPLC

25 grams of shelled corn samples and  $\pm 5$  grams of NaCl powder into a blender, added with 125 mL methanol 70% then blended for 1 minute. The solution is filtered with filter paper, 15 mL filtrate homogenized with 30 mL of water. 15 mL filtrate is processed by the immunoaffinity column with a flow rate 1 mL/minute and washed by 10 mL water at a flow rate 2 mL/minute. After all the liquid has dropped, the air is removed using a syringe, and the stored liquid is removed. 1 mL of methanol is passed to the immunoaffinity column and the droplets are collected into the amber vial. Derivatization of Samples was done by evaporating standard or sample in methanol using nitrogen gas and adding 100  $\mu$ L of TFA (tri fluoro acetic acid), homogenized by vortex for 30 seconds, incubated for 15 minutes (25°C), added 900  $\mu$ L of acetonitrile: water (1: 9), vortexed for 30 seconds. The sample is injected into the chromatography system the Agilent Technologies 1120 Compact LC Pump instrument, Agilent Technologies 1200 Series Detector, Acetonitrile Phase: Methanol: Aquabidest (1: 3: 6) with a flow rate of 1 mL / minute, fluorescence detector at excitation wavelength 365 nm and wavelength. 450 nm of emissions. The column used was Rp-18 Lichrospher with a length of 250 mm, a diameter of 4 mm and a particle size of 5 $\mu$ m.

### 3. Results and Discussion

Aflatoxins can be formed during the planting period, the harvest period (during the harvesting process), and at the time of storage. The samples used in this study came from Tangerang (Sample A), Cikande (Sample B), and Cirebon (Sample C) (figure 1).



**Figure 1.** Corn seed samples from (A) Tangerang, (B) Cikande, (C) Cirebon

All samples of corn kernels generally looked good physically (figure 1). Fungal growth was not physically visible in all samples, to determine the presence of aflatoxin contamination, analyze the aflatoxin content using ELISA and HPLC methods [11].

#### 3.1. Water content

The increase in water content can be affected by conditions at storage, such as humidity, temperature, and high rainfall, especially in tropical countries such as Indonesia. The result showed that moisture content in shelled corn (feed) increased for 4 weeks of storage. The highest water content in sample A from Tangerang is 10.64%. Based on SNI 01-4483-1998, the water content maximum of shelled corn is 14%. The water content for all samples is still safe, according to SNI 01-4483-1998.

**Table 1.** Moisture Content of shelled corn (%).

Sample	Storage Time (week -)				
	0	1	2	3	4
A	10.60	10.61	10.62	10.62	10.64
B	10.13	10.13	10.14	10.15	10.17
C	10.22	10.23	10.23	10.24	10.25

### 3.2. Aflatoxin levels using the ELISA method

The results showed that aflatoxin levels increased during storage for 4 weeks. The highest aflatoxin content is sample A (74.06 ppb) and the lowest aflatoxin content was sample B (9.33 ppb). Based on SNI 01-4483-1998, the total aflatoxin content maximum for corn as feed is 50 ppb. The results showed that total aflatoxin content in samples B and C was still in safe (accordance with SNI 01-4483-1998) are 9.33 ppb and 28.67 ppb, respectively (table 2). While sample A exceeds the limit recommended is 74.06 ppb.

**Table 2.** Aflatoxin levels using the ELISA method.

Sample	Storage Time (week -) (ppb)				
	0	1	2	3	4
A	73.94	73.95	73.96	74.00	74.06
B	9.21	9.23	9.25	9.30	9.33
C	28.57	28.59	28.61	28.65	28.67

Aflatoxin content is influenced by water content. The water content of all samples was in the range of 10% (table 1). The aflatoxin content of sample A exceeds the limit allowed by SNI even though the water content is in accordance with SNI, it is possible that the aflatoxin content contained in sample A was formed during the planting period. The aflatoxin content during storage will not decrease, it can even increase or remain [13].

### 3.3. Aflatoxin levels using the HPLC method

Analysis of aflatoxin levels using the HPLC method was carried out only at the fourth week of storage. The analysis showed that the highest total aflatoxin levels were in sample A (75, 02 ppb) and the lowest in-sample B was 9.83 ppb. Table 3 showed that the aflatoxin content of sample A exceeds the standard threshold allowed by SNI 01-4483-1998 (max. 50 ppb), while sample B and C are still in accordance with SNI.

**Table 3.** Aflatoxin levels using the HPLC method.

Sample	Aflatoxins (ppb)				
	B1	B2	G1	G2	Total
A	70.48	4.54	ND	ND	75.02
B	8.96	0.88	ND	ND	9.83
C	25.43	2.90	ND	ND	28.43

ND = Not detected

### 3.4. Effect of Water Content on Aflatoxin Content

The results showed that total aflatoxin content in shelled corn (feed) increased during 4 weeks of storage is affected by water content. Aflatoxin production is influenced by water content. At water content (7.79%) aflatoxin B1 content was 2.93 ppb and aflatoxin B2 was 1.54 ppb. After storage, the increase in water content increased the aflatoxin B1 content [14]. High water content greatly affects the growth of *Aspergillus flavus* and the formation of aflatoxins, corn storage should be done at low water content. In temperate countries, the ideal moisture content is <13% for storage more than 9 months, while for short storage, the moisture content can be as high as 14%. Whereas for tropical countries with high temperature and humidity, the ideal water content ranges from 7-9% for more than three months [15].

### 3.5. Results of Aflatoxin Analysis using ELISA and HPLC Methods

The results of the analysis of aflatoxin content were carried out by the ELISA and HPLC method. The highest aflatoxin content using the ELISA method was sample 1 from Tangerang, amounting to 74.07 ppb. Whereas with the HPLC method, in sample 1 the result was 75.02 ppb and it exceeded the maximum standard threshold. 50 ppb allowed SNI 01-4483-1998. The water content in sample 1 of 10.64% is still in accordance with SNI 01-4483-1998. The aflatoxin content for the other two samples originating from Cikande and Cirebon fulfills the SNI requirements, while the water content for all samples of Tangerang, Cikande, and Cirebon is in accordance with the SNI requirements maximum 14% (table 4).

**Table 4.** Comparison of Aflatoxin ELISA and HPLC Levels.

Sample	Aflatoxin levels (ppb)		Water content (%)
	ELISA	HPLC	% water
A	74.06	75.02	10.64
B	9.33	9.83	10.17
C	28.67	28.43	10.25

There are differences in the results obtained for both methods. This difference has been affected by the sensitivity of the analysis methods. The ELISA method is widely used, accurate, easy, and fast. It is based on the antigen superimposed on the test plate which is a specific antigen in the sample, which could minimize positive error. Besides the ELISA method, aflatoxin analysis can be carried out by High Performance Liquid Chromatography (HPLC). Since 2004, the aflatoxin analysis method has used HPLC with a fluorescence detector, because it is a high sensitivity, high-resolution column, and automatic processing. HPLC analysis is widely used because it has accurate quantification value and is easy to operate [16, 17]. The ELISA method has several advantages that are accurate, easy, fast, sensitive, and cheap. The HPLC method can detect Aflatoxin B1, B2, G1, and G2 with the calibration using aflatoxin standards [17].

### 4. Conclusion

The moisture content increased during 4 weeks of storage but still safe accordance with SNI 01-4483-1998, a maximum of 14%. The highest water content in the sample from Tangerang was 10.64%. The increase in water content affects the increase in aflatoxin levels during storage. In the fourth week, the aflatoxin levels using the ELISA method were 74.06 ppb, 9.33 ppb; 28.67 ppb on sample from Tangerang, Cikande, and Cirebon, respectively. The Aflatoxin levels using the HPLC method were 75.02 ppb; 9.83 ppb; 28.43 ppb on sample from Tangerang, Cikande, and Cirebon, respectively. The sample from Tangerang exceeds the threshold allowed by SNI No. 01-4483-1998, which is a maximum of 50 ppb, while the Cikande and Cirebon samples are still safe.

### 5. Acknowledgements

This research has been conducted with students from the chemistry department of Universitas Pakuan, (Riana Septyaningrum) to produce information that is very useful for Indonesian society.

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