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Investigation of Aflatoxin Content Using HPLC to Ensure the Sustainability of Rice Products in Jakarta City, Indonesia

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ABSTRACT

Aflatoxin exposure in foodstuff is a public health problem because it causes acute and chronic carcinogenic effects. Countries with hot and humid climates, aflatoxin exposure is a serious problem, and rice product is one of the foodstuffs that have the potential to be contaminated. Indonesia is a country that consume rice as a staple food. To ensure the sustained availability of safe rice products, initial identification of the aflatoxin content is necessary. This study aims to trace the aflatoxin content in rice obtained randomly from traditional market and a logistic agency in the Jakarta area, Indonesia. The number of samples is 20 types of rice grouped by price, area, and location category. Rice samples were tested for aflatoxin exposure by using a HPLC method in a laboratory that has been accredited by the Indonesian National Accreditation Committee. Results show that 95% of the samples have aflatoxin levels below 0.50 mg/kg as the limits of international standards and regulations for AFB_1, AFB_2, AG_1, AG_2, and AFT. However, 5% of samples show that AFB_1 content is 2.45 mg/kg and AFT content is 2.62 mg/kg. These contents are higher than the limits regulation (maximum 2.0 mg/kg). The results indicate that there is a risk of aflatoxin contaminations in rice traded in the market within Jakarta area, Indonesia. It is important to maintain post-harvest processes with good procedures, including distribution, storage, and packaging to minimize the appearance of aflatoxin.

1. INTRODUCTION

Mycotoxin contamination is a major food safety problem in agricultural products. Mycotoxins generally arise, within agricultural products, in countries with tropical and subtropical climates. In these countries, with high temperatures and humidity, this condition can encourage the growth and proliferation of *Aspergillus spp.* [1]. Mycotoxins are secondary metabolites produced from fungi [2]. Types of mycotoxins are very diverse; more than 400 types of mycotoxins have been identified from several types of fungi [3]. The World Health Organization (WHO) reports that aflatoxin is a type of mycotoxin that is harmful to humans [4].

Aflatoxins are produced by *Aspergillus* fungi [5, 6], particularly *Aspergillus flavus* and *Aspergillus parasiticus* [2]. Aflatoxins can contaminate food, especially those from agricultural products [7], for example, aflatoxins can be found in peanut, corn, and cotton seeds [8]. Aflatoxin's contamination can occur due to the presence of *Aspergillus* species which are commonly found in soils [9, 10], and in post-harvest processes [11]. Aflatoxins are relatively stable in nature at various temperature conditions and environmental pH. These conditions promote aflatoxins accumulation in food ingredients along supply chains [1].

Rice (*Oryza sativa L.)* is an important source of protein, energy, fibres, minerals, and bioactive compounds in several countries in the world. Worldwide, more than 769.9 million tonnes are produced, of which 80% are destined for human consumption. Currently, rice is a food crop that is widely consumed in many countries covering two-thirds of the world's population. The largest consumption of rice is in the Asian region, where most of the calorie needs are obtained by consuming rice [12]. The main producers of rice in the world today are mostly located in China, India, Indonesia, Bangladesh, Myanmar, Thailand, and Vietnam [13].

Rice is a strategic commodity as well as a political commodity that concerns basic needs for 95% of the Indonesian people, so its availability must always be monitored both at the central and regional levels. In line with the government's program for a sustainable rice selfsufficiency program, the availability of rice needs to be followed by consistent quality to meet consumer needs or trade needs, both for export and domestic. Almost all rice is grown by irrigation systems in areas with high temperature and relative humidity. These conditions are optimal for the growth and reproduction of fungi, which are then brought to the warehouse with the rice. *Aspergillus*, *Penicillium*, and *Fusarium* are the main fungi causing losses in stored cereals because they produce mycotoxins. So that, rice has the potential to be contaminated by aflatoxins.

Aflatoxin contaminations in rice have been reported in several studies [1, 5, 13-16]. Aflatoxin B_1 (AFB_1) is the most dangerous type of aflatoxin produced by the fungi *Aspergillus flavus* [17]. Based on the classification of the International Agency for Research on Cancer (IARC), AFB_1 is included in the class of carcinogenic substances (potentially causing cancer). The mechanism of AFB_1 toxicity in a human body is to cause DNA *adducts* which are the trigger of the formation of cancer cells [10]. AFB_1 is also capable of causing liver toxicity, growth disorders [10], and fertility disorders [18]. Although rice is not immediately considered a high-risk commodity, there are substantial evidence showing that aflatoxin contaminations have been detected in rice varieties in various countries including the United States, United Kingdom, Egypt, Pakistan, Malaysia, Philippines, India, Nepal, Iran, and China [1].

As a form of risk management, some countries set limits on the content of aflatoxins in agricultural products. Regulators as authorities that regulate limits for aflatoxin content in various countries have set tolerance limits for aflatoxins ranging from 0 to 50 g/kg to control levels in food supply [19]. While specifically in Indonesia, there is regulation that controls the limits of aflatoxins in agricultural products concerning the safety and quality of fresh food from plants. In this regulation, the limit of aflatoxin contamination is set at 15 mg/kg for AFB_1 and 20 mg/kg for total aflatoxin in corn, corn flour, peanut, and pepper products. Meanwhile, for rice products, the limits for aflatoxin contamination have not yet been regulated.

This study analyses rice for several types of aflatoxins, namely total aflatoxin (AFT), aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2). Data obtained in this study are compared with some internationally permissible limits for aflatoxin contamination in rice. From the comparisons, results show that most of the analysed samples exceed AFB1 and AFT levels (2 and 4 g/kg, respectively) as tolerated in European Community regulations for cereal products [20]. For comparison, aflatoxin contamination of various rice tested by study [1] in Saudi Arabia, complies with EU and other international standards. Another study concluded that the average concentration of AFB_1 in rice from Mashhad, is significantly different from the limits set by the EU and Iranian standards [21]. Meanwhile, a study resulted in the findings of total aflatoxins (B_1, B_2, G_1, and G_2) in 18 of 20 rice samples detected containing aflatoxins [22]. The total aflatoxin content was found to be below the EU tolerance level. Since the limit for aflatoxin contamination in rice has not been set and approved internationally, regulations in several countries vary, for example, in India the maximum B_1 limit is 30 mg/kg, Japan has a maximum B_1 of 10 mg/kg, South Korea has a maximum of $B_1$10 mg/kg, Malaysia has 35 mg/kg for total aflatoxin, Thailand with a limit of 20 mg/kg for total aflatoxin, Taiwan with a limit of 10 mg/kg for total aflatoxin [23]. In Asia, rice is considered contaminated at > 20 mg AFs/kg [1].

2. MATERIALS AND METHODS

2.1 Research location

The largest rice stock was found in producer and consumer households for about 51.5% (3.9 million tons), Logistic Agency at 17.8% (4 million tons), milling at 16.5% (1.3 million tons), traders 9.6% (734 thousand tons), hospitality 4.1% (315 thousand tons), and others Cipinang Rice Market (PIBC) and Community Food Barns (LPM) only 0.4% and 0.1% (Figure 1), so that the rice sampling in this study is obtained from both traders and Jakarta Logistic Agency.

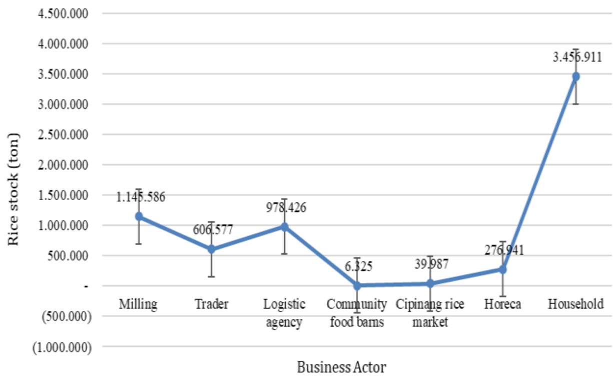
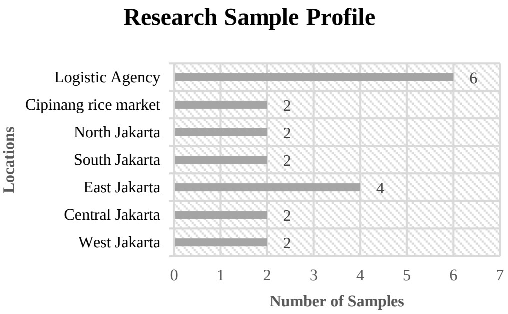


Figure 1. Comparison of rice stocks (in ton) for each business actor at the research sample location [24]

The sampling is selected so that conclusions from this study is statistically conclusive. In this study, assumptions are set for the level of homogeneity of the circulating rice. Meanwhile, the types or varieties of rice are not considered, because the type of variety does not affect the moisture content of rice [25] which is a critical factor for the growth of *Aspergillus spp.* which produces mycotoxins [26]. Several things that affect the moisture content are the methods used at post-harvest such as threshing, handling, cleaning, drying, and milling [27].

The samples variation is based on the price of rice, namely the lowest price and the medium price at the sampling location (Figure 2). The categorization of rice is based on the Regulation of the Ministry of Trade, Republic of Indonesia, concerning the highest retail price of rice [28]. The categories are (1) premium (high) rice prices (in the range of Rp. 10901 - 13900), (2) medium rice prices (in the range of Rp. 9000 - 10900), and (3) low rice prices (below Rp. 9000). The rice used in this study was collected randomly from traditional markets in East Jakarta, West Jakarta, Central Jakarta, North Jakarta, South Jakarta, and Jakarta Logistic Agency, with a total of 20 rice samples taken. The sampling location selected is a large-scale traditional market to ensure that many buyers are transacting in that market. The weight of the rice for each sampling is 1 kg to provide adequate mass for analysis and to make purchasing easier. Representative sampling was carried out in containers where rice was sold by taking the top, middle and bottom areas. Then the three parts are mixed until they become homogeneous so that all positions can be represented. The rice is put in a special food plastic packaging to prevent contaminations from outside. Samples are then stored at room temperature before being tested and analysed. Finally, the rice samples are prepared and taken to an accredited laboratory.



(a)

(b)

Figure 2. The number of samples (a) and sampling locations in DKI Jakarta Province (b) were carried out in proportion to the stock of rice in each region

2.2 Sample testing

Sample testing performed at the laboratory which has been accredited by national accreditation committee (KAN) for the content of aflatoxins in rice. Accreditations based on the general requirements for the competence of testing and calibration laboratories are given with the aim of increasing confidences in the operation of the laboratory. With the recognition of accreditation, the laboratory can operate reliably, and is able to provide valid results [29]. Tests are carried out to detect the content of AFB_1, AFB_2, AFG_1, AFG_2, and AFT using a High-Performance Liquid Chromatography (HPLC) method. The HPLC is one of the most widely used chromatographic techniques for determining food contaminants, including contaminants belonging to the mycotoxin group [1].

In determining aflatoxins, the analytical procedure using HPLC has been widely reported by previous researchers [1, 5, 13, 14, 30-33]. HPLC is an analytical technique for identifying substances or compounds by separating and measuring their amounts in a mixed solution [34]. This HLPC technique has many advantages, including having a very good detection limit to determine the aflatoxin content in food up to mg/kg units [31, 35], requires only a small sample size, the test can be modified depending on the level of quantification required and can provide specific identifications [36].

In this study, HPLC conditions and sample preparation were carried out based on a method that has been published and validated by GazioĞlu and Kolak [37] which is a modification of the AOAC 991.31 method. The chromatography conditions in this method use a column RP C18 (4.6×250 mm, 5 µm) with a fluorescent detector at wavelengths and emissions were 365 and 435 nm and in column temperature of 45℃. The mobile phase used is a mixture of water, methanol, and acetonitrile in volume ratios (54:38:8) by adding 0.2 g potassium bromide and 360 µL HNO_3 (in 1 L solution) with a flow rate of 1.0 mL/min and an injection volume of 100 µL. Rice samples were extracted with 75% methanol solution for 30 minutes, the extraction result was filtered through Whatman No. 1 filter paper and 15 mL filtrate was diluted with 30 mL distilled water.

In addition, to determine the level of correlations between moisture content and fungal growth in rice products, testing on moisture content parameters are also carried out based on national standard of Indonesia (SNI). The moisture content test was carried out by the oven method. This moisture content has the principle of losing weight at 105℃ and is considered as the composition of water contained in a sample. The testing procedure is that a number of samples with a mass of 1-2 grams are dried in an oven at a temperature of 105℃ for 3 hours. Then, the mass loss in calculated from the sample is considered as the moisture content of the sample.

3. RESULTS AND DISCUSSION

3.1 Visual characterization of rice sample

Based on the SNI, definition of the integrity and fracture of the shape of rice, basically the shape of rice does not affect the safety of rice consumption. The form factor and appearance of rice have more influence on the aesthetic value of the product and the social level of its consumers. To know the rice is still in good condition or fit for consumption, it is necessary to know how to test or identify the rice. Testing can be done simply by observing the senses.

Determination of the color of rice is done simply and quickly with the sense of sight. Characterized by the color and cluster of normal rice, normal white for perfectly milled rice and brownish red to black for red or black pigmented rice. The shape of the rice must be whole. If a lot of it is broken, crushed and a lot of crumbs (flour) stuck to the palms of the hands, it indicates that the rice has been stored for a long time and should not be consumed. Determination of rice texture is done by the sense of taste (kinaesthetic). Good rice texture is still hard, not easily broken. Good rice still tastes like rice. If it starts to taste bitter, then the rice has been indicated to be unfit for consumption. Determination of the presence of musty, sour, rancid, or other odors is carried out by the sense of smell which is characterized by a distinctive odor. Determination of the presence of bran, or a mixture of other foreign objects (gravel, metal, rice stalks) is done by visual observation.

Rice products circulating in the market have various types of production quality to meet the various needs of consumers with different types of consumption. Grading on rice functions in classifying levels of rice products based on the same quality, both qualitatively (visual inspection) and quantitatively (based on quality parameters). The laboratory plays an important role in determining the quality of rice by conducting tests in accordance with the grading parameters that have been set in the standard. The parameters are polishing grade, moisture content, head rice, broken grains, groats, red grains, yellow grains, lime grains, foreign matter, and grain grains [38].

This grading is useful for farmers, suppliers, and consumers in terms of determining prices, types of production processes, product variations and consumer choices when making purchase. This study uses a grading sample with a low to middle price variable at each sampling location. This variable was chosen with the consideration that the price of rice is positively linearly correlated with the quality of rice [39]. Initial analysis was carried out by visually observing the sample which included colour, grain shape, and storage conditions also varied (Table 1). In addition, these classes of rice are the most consumed rice in Jakarta area. The rice samples for this research are divided between samples with low prices and middle prices, with the condition of the sample being mostly white rice (80%) and the condition of whole rice being more than broken rice in each sample (60%).

The sampling locations consisted of two main locations, namely traditional markets, and warehouses for the Logistics Agency. The characteristics of the rice samples are that most of the sample storage mats use wooden pallets (60%), dry storage conditions (70%) and the product display area is almost evenly distributed between using wooden box and plastic box and is slightly dominated by plastic sack.

Table 1. Visual characteristic of rice samples

Sample Code	Rice Category	Rice Price (Rp)	Rice Characteristics		Sampling Site Conditions		
			Colour	Grain	Storage Pad	Storage Condition	Product Display Area
JB-M-0306	Low price	8.000	white	mixed (whole = broken)	sack pad	dry	wooden box
JB-T-0306	Middle price	9.000	white	mixed (whole > broken)	sack pad	dry	wooden box
JP-M-0306	Low price	9.000	white	mixed (whole = broken)	sack pad	moist	wooden box
JP-T-0306	Middle price	10.000	white	mixed (whole > broken)	sack pad	moist	wooden box
JS-M-0306	Low price	8.000	white	mixed (whole > broken)	sack pad	moist	wooden box
JS-T-0306	Middle price	8.000	white	mixed (whole > broken)	sack pad	moist	wooden box
JU-M-0306	Low price	8.500	white	mixed (whole < broken)	wooden box	dry	plastic box
JU-T-0306	Middle price	11.000	white	mixed (whole = broken)	wooden box	dry	plastic box
JT-M-0306	Low price	7.000	yellowish	mixed (whole < broken)	wooden pallet	dry	plastic box
JT-T-0306	Middle price	9.000	white	mixed (whole = broken)	wooden pallet	dry	plastic box
JT-80-0306	Low price	8.000	greyish white	mixed (whole < broken)	wooden pallet	moist	plastic box
JT-85-0306	Low price	8.500	white	mixed (whole > broken)	wooden pallet	moist	plastic box
BC-M-0306	Low price	7.600	yellowish	mixed (whole > broken)	wooden pallet	dry	plastic sack
BC-T-0306	Middle price	9.000	white	mixed (whole > broken)	wooden pallet	dry	plastic sack
BU-DN-2018	Middle price	9.000	white	mixed (whole > broken)	wooden pallet	dry	plastic sack
BU-DNB-2018	Middle price	8.500	yellowish	mixed (whole > broken)	wooden pallet	dry	plastic sack
BU-DN-2020	Middle price	9.500	white	mixed (whole = broken)	wooden pallet	dry	plastic sack
BU-LNI-2018	Low price	8.500	white	mixed (whole > broken)	wooden pallet	dry	plastic sack
BU-LNV-2018	Middle price	10.000	white	mixed (whole > broken)	wooden pallet	dry	plastic sack
BU-LNT-2018	Middle price	10.000	white	mixed (whole > broken)	wooden pallet	dry	plastic sack

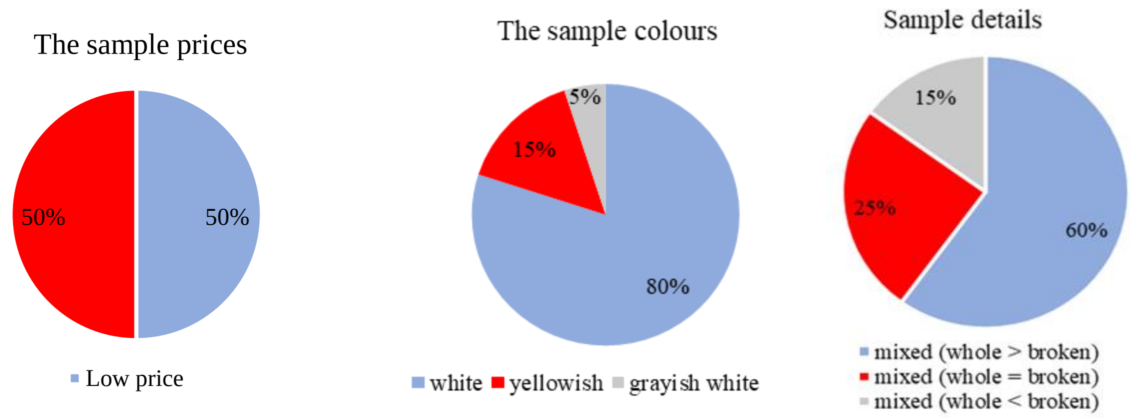
Note: Each sample is coded according to the sampling location. The sample is divided into three price categories (low, middle, and high) in Rupiah (Indonesian). Sample characteristics are differentiated based on color and grain, while sample conditions are divided into 3 categories, namely storage pad, storage condition and product display area.

Based on the rice category, all rice in the middle price category has a white colour, while the rice in the low-price category has various colours, namely yellowish, greyish white, and white (Figure 3). Based on the sampling obtained, there are differences in rice quality (Figure 4) and storage methods (conditions) used (Figure 5). Rice with the middle price and rice taken from Logistic Agency have almost the same characteristics, namely most of the rice colour in both categories is white. This white colour is because the condition of the rice is still in good condition. While the rice with low price, there is rice that has changed its colour to yellowish and greyish white. In addition, for the characteristics of grain shape, rice with middle price and rice taken from Logistic Agency, have similar conditions in term of their grains. This condition indicates that the rice is in good condition. As for the category of rice with low prices, broken rice grains have more percentage content than the good grain within the whole rice grains.

Differences in the characteristics of rice into yellow or damaged grains (not whole) are the result of physical processes or microorganism activities. Based on SNI 6128:2015 regarding rice, it contains quality requirements for the premium and medium rice categories (quality I, quality II, and quality III). In premium rice, no yellow grains are allowed. As for the quality requirements of the premium rice, the allowed medium yellow grain rice content is up to 2%, 3% and 5% respectively for medium quality I, II and III. Yellow/damaged grain measurement is done manually by using direct observation by the research team. Observations were made with adequate lighting so that the difference in colour between each rice was able to be distinguished. If the percentage of yellow/damaged grains does not meet the standard requirements, it is possible that improvements can be made at the stages of harvesting, drying, grain storage and rice storage.

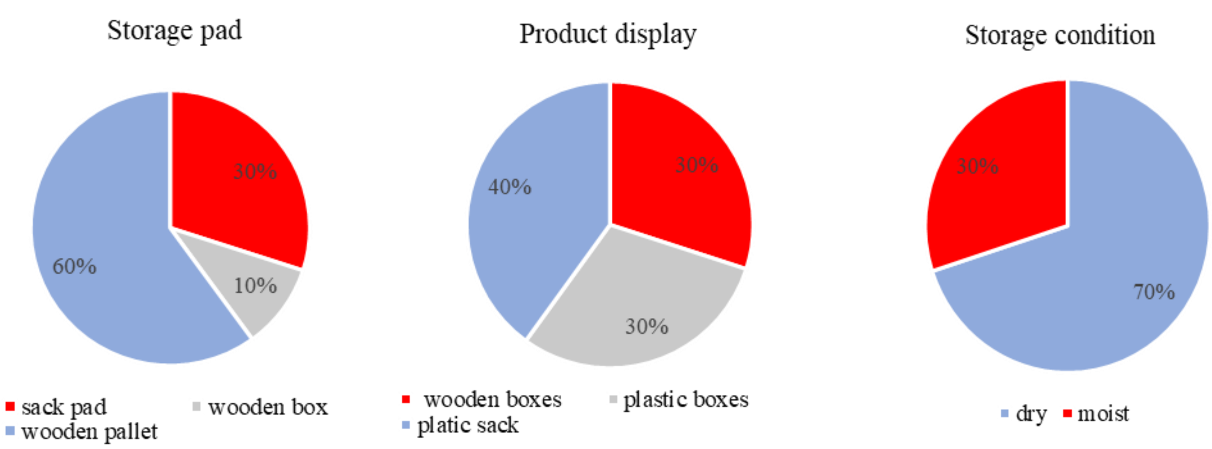
(a) (b) (c)

Figure 3. Visual condition of the rice samples obtained, divided into 3 categories namely (a) white rice (b) greyish rice (c) yellowish rice, which indicate a measure of quality



(a) (b) (c)

Figure 4. Characteristics of the rice sample consist of (a) sample price, (b) sample colour, and (c) sample detail condition



(a) (b) (c)

Figure 5. Characteristics of sampling storage consist of three criteria, namely (a) storage pad, (b) product display, and (c) storage condition

3.2 Test results

Based on the results of experiments on the characteristics and storage conditions of rice, the moisture content and aflatoxin levels in the tested rice samples are obtained (Table 2). Result shows that moisture content in each sample varies within a range from 11.9% to 14.7%. Based on SNI 6128:2015, the moisture content in rice is required to be a maximum of 14% for premium rice, medium quality rice I and medium quality rice II. Meanwhile, for medium class III rice, the maximum required moisture content is 15%. Hence, the results of testing the moisture content of the rice samples meet the requirements of SNI. There are four samples which are categorized as medium quality III rice in the experiments.

If grouped according to the category (low price, middle price, and Logistic Agency), the results of moisture content in each category will form a certain pattern (Figure 6). This pattern shows the suitability of the moisture content of the tested rice based on the requirements in the Indonesian national standard. Figure 6 also illustrates the pattern of moisture content in rice. The moisture content of low-priced rice is read higher than the moisture content of medium-priced rice. Some of the possible causes are the drying treatment carried out and the use of storage facilities [40]. This is supported by the moisture content data shown by rice at the Logistic Agency which is relatively stable due to good storage facilities with the support of periodic observations.

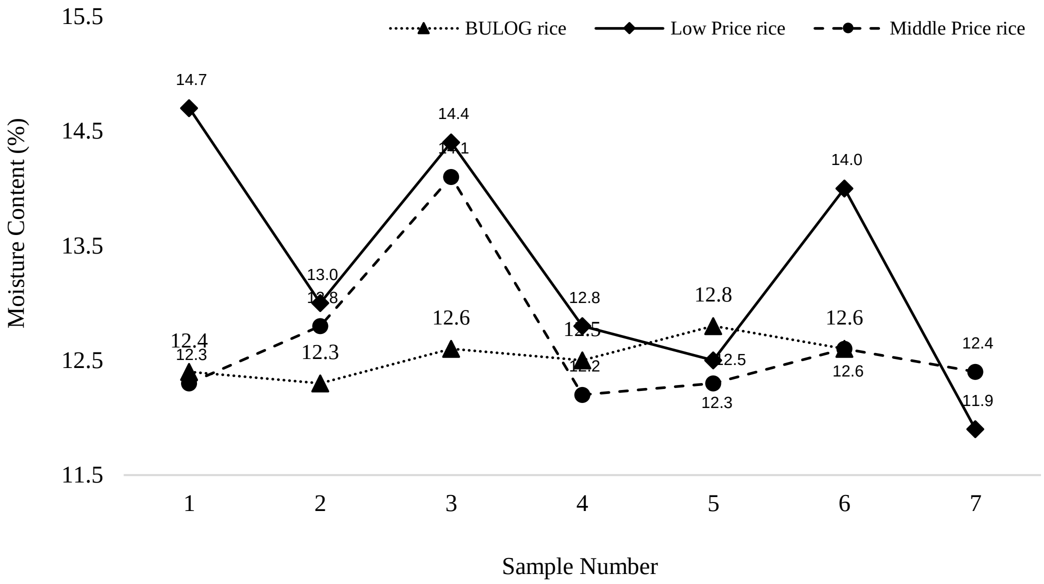


Figure 6. Comparison of moisture content of rice samples

Table 2. The test results of moisture content and aflatoxin content in the rice sample

Sample Codes	Test Result					
	Moisture Content (%)	**AFB$_1$ (mg/kg)**	**AFB$_2$ (mg/kg)**	**AG$_1$ (mg/kg)**	**AG$_2$ (mg/kg)**	**AF$_{total}$ (mg/kg)**
JB-M-0306	14.7	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
JB-T-0306	12.3	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
JP-M-0306	13.0	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
JP-T-0306	12.8	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
JS-M-0306	14.4	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
JS-T-0306	14.1	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
JU-M-0306	12.8	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
JU-T-0306	12.2	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
JT-M-0306	12.5	2.45	< 0.50	< 0.50	< 0.50	2.62
JT-T-0306	12.3	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
JT-80-0306	14.0	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
JT-85-0306	12.6	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
BC-M-0306	11.9	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
BC-T-0306	12.4	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
BU-DN-2018	12.4	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
BU-DNB-2018	12.3	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
BU-DN-2020	12.6	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
BU-LNI-2018	12.5	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
BU-LNV-2018	12.8	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00
BU-LNT-2018	12.6	< 0.50	< 0.50	< 0.50	< 0.50	< 2.00

Note: Moisture content of rice is expressed in %, and Aflatoxin content of AFB$_1$, AFB$_2$, AG$_1$, AG$_2$, and AF$_{total}$ expressed in mg/kg

Moisture content of rice sample from Logistic Agency is relatively stable in the range of 12%. Stability of moisture content in the rice is because the storage conditions are tightly regulated and monitored, so that the moisture content in each sample shows a uniform value in the range of 12.3 - 12.8%. Because at that location use of good storage facilities supported by regular observations. The average moisture content on rice with the middle price category is also in the range of 12%. However, there is one sample that has a moisture content above 14%. This moisture content above 14% is feared to trigger the risk of the growth of fungi that may be able to produce aflatoxins.

As a result of visual sample appearance, rice will be more easily damaged that the upper part [41]. The second variation is rice storage conditions. Based on field observations, there are still sellers who store rice in moist conditions. Moist conditions are ideal conditions for microorganisms to grow, as well as fungi, that have the potential to produce aflatoxin as secondary metabolites. This condition may cause the moisture content in rice to increase. The results of testing the moisture content in rice were carried out using the test method contained in the SNI 01-2891-1992, point 5.1 that is by using an oven as a tool to dry the sample and the drying is carried out for 3 hours. A visual testing to determine quality is becoming important for consumers, including trade [42], because the visual testing can be done quickly, is relatively inexpensive and is one of the non-destructive testing techniques through observations on images of rice grains so that classification can be carried out.

Several studies reported that the contamination of grains with mycotoxins are mostly caused by inappropriate storage [14, 17, 43−46]. These studies conclude that the practice of drying in the field had a significant effect on the quality of rice, especially the recovery of head rice. This shows that the post-harvest technology used is quite good, such as grain is harvested in conditions of low moisture content or dried in the sun with good procedures and milled according to regulations [47]. The moisture content of 14% is the most stable condition for grain because the rate of absorption is very slow, so it is not easy to reabsorb water. Under these conditions, the grain is safe to store because the heat generated by the respiration of the grains and microorganisms is not sufficient to increase the temperature and humidity of the grains [48]. If the grain is harvested in conditions of high moisture content, it can increase the cost of drying. In wet condition or high moisture content, it will be more easily damaged because fungi and bacteria will be more easily appear and damage the grain or rice [49]. Rice with a high moisture content will break easily when milled the wrong way, resulting in greater weight losses. In addition, if the rice is too dry, it will reduce the weight and will cause losses. The high moisture content of rice is influenced by the moisture content of milled dry grain (GKG) [48].

Cereal grains including rice should be dried to a safe moisture level of < 14% by the one-step drying method after harvest and avoiding short-term or long-term storage in a semi-dry state. The drying method has also been shown to have a significant effect on the occurrence of aflatoxins in food commodities during storage [44]. The higher the moisture content in the rice, it indicates that the post-harvest procedure of rice is not being applied optimally. At this stage, the quality of rice decreases. it is easily contaminated and can cause losses for farmers. Moisture content is affected by humidity and temperature during storage [50].

Moisture content does not directly affect the growth of fungi on rice, but the main controlling factor is water activity (Aw). The Aw of a food is the ratio between the vapor pressure of the food itself, when in completely undisturbed equilibrium with the surrounding air medium, and the vapor pressure of distilled water under the same conditions. For example, an Aw of 0.80 means the vapor pressure is 80 percent of pure water. Water activity increases with temperature [51]. Aw which indicates the amount of free water in food that can be used by microorganisms in their growth [52]. A certain Aw is an ideal condition for microorganisms to grow [53]. The species *Aspergillus flavus* is one of the most common food spoilage fungi and is of particular concern because some species produce aflatoxins.

The main controller of bacterial growth is Aw [54]. *Aspergillus flavus* is a type of fungi that can grow well in air conditions with a minimum Aw = 0.8 [54], followed by its ability to continue to increase with increasing moisture content. High moisture content in rice is caused by several factors. The strongest factor is the rice storage in moist conditions. In moist conditions, this is very vulnerable and has the potential for the growth of various kinds of destructive microorganisms that can produce toxins such as aflatoxins, though visually it still looks visually good. Rice price variable affects the level of purchase and quality of rice [55]. The higher price of rice, consumers will switch or choose another brand with a lower price. This is because rice is a basic need with a high level of availability, so, the choice of quality and price varies. Meanwhile, rice with higher quality cause the price to be even higher. Quality rice is usually characterized by conformity to standards, regulations, and value-added products, such as packaging and health benefits.

Based on Table 2, the levels of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2, and AFT) found in the rice samples are mostly below the detection limit of the equipment used for testing (read values are reliable), which was below 0.5 mg/kg for aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2), and below 2.0 mg/kg for AFT. There is one sample that contained high aflatoxin. namely 2.45 mg/kg for AFB1 and 2.62 mg/kg for AFT. This high aflatoxin content is very worrying because there is one sample of rice circulating in the market with a high aflatoxin content. This aflatoxin content can cause toxicity in a human body, which triggering the formation of cancer cells [10]. Aflatoxin is also capable of causing liver toxicity, growth disorders [10], and fertility disorders [18].

Aflatoxin is a powerful hepatocarcinogen and humans can be exposed to it at any stage of life. Epidemiological studies report an association between aflatoxin intake and the incidence of hepatocellular carcinoma in several Asian countries. This condition is known as aflatoxicosis, where humans and livestock have been exposed through consumption of food or feed contaminated with high levels of aflatoxin. Aflatoxicosis is a serious problem in developing countries, especially in Asia and Africa. In developed countries such as the United States, yield losses due to aflatoxin contamination are reported to be in the millions of dollars. Hundreds of people died in Kenya from consuming corn contaminated with aflatoxins in the last 10 years, this is because aflatoxins are carcinogenic (cancer causing). mutagenic and immunosuppressive [39]. High daily rice intake with low levels of contamination still causes health problems [56]. Furthermore, aflatoxin causes liver cancer and increases carcinogenic potential with hepatitis B infection in certain individuals [23]. Aflatoxin is thought to inhibit growth (growth retardation) resulting in failure to grow or stunting [57]. Meanwhile, in the trade aspect, both national and international, each country has regulations that set limits on aflatoxin in rice (Table 3). If these regulations cannot be achieved by business actors (traders), then trade barriers will occur, and rice products will not be able to circulate in the market or country.

The high levels of aflatoxins in the rice samples are indicated by the yellowish colour of the rice (Figure 7). The yellowish colour of the rice is an indication that the rice has been physically damaged or contaminated with microorganisms which can be the *Aspergillus flavus* species. This indication is proven by the results of laboratory tests in one sample show that AFB_1 and AFT content respectively reach level 2.45 mg/kg and 2.62 mg/kg.

However, to enforce the actual conditions, it is necessary to re-confirm the relationship between moisture content and aflatoxin content in rice. The study [58] stated that rice contaminated with *Aspergillus flavus* has a greenish colour (olive green) to dark green (parrot green) depending on the level of *Aspergillus flavus* contamination in rice. *Aspergillus flavus* growth can occur at both pre-harvest and post-harvest of rice [1, 14, 59, 60]. Especially for post-harvest. temperature and humidity factors during storage are the main factors for the growth of *Aspergillus flavus* that is potentially to produce aflatoxins [17]. During storage, the control of room temperature and humidity are crucial factors to be controlled and set to correct optimal values. Even in Japan, rice is stored in warehouses at temperatures below 15℃ and relative humidity of 70-75%. These conditions prevent the postharvest contamination of rice with mycotoxins [61]. In this condition, the Equilibrium Moisture Content (EMC) or the water content is in balance, so that mycotoxins cannot or are difficult to grow [50]. The equilibrium moisture content after immersion decreased with increasing storage time. This is because the starch and protein in the rice react to form micelles, thereby preventing the entry of water into the rice. In addition, during storage. protein also undergoes oxidation to form disulphide bonds which causes a reduction in volatile sulphur compounds which will result in further inhibition of water absorption into rice [62]. Rice with moisture content less than 9% is recommended for storage periods of more than one year in plastic bags [20]. Storage of rice with moisture content less than 10% in a room free of rodents will prevent the production of aflatoxins [45].

Table 3. Limits of aflatoxin contamination in some countries

	Country	Aflatoxin Levels in Rice (mg/kg)
1.	European Union	2.0 AFB_1 and 4.0 total aflatoxin
2.	Russia	5.0 total aflatoxin
3.	China	10 total aflatoxin
4.	Japan	10 total aflatoxin
5.	USA	20 total aflatoxin
6.	Brazil	30 total aflatoxin
7.	India	30 total aflatoxin
8.	Korea	10 AFB_1 and 15 total aflatoxin
9.	Saudi Arabia	20 total aflatoxin

Source: [1]

(a)

(b)

Figure 7. Comparison of conditions and characteristics of normal rice and rice with aflatoxin. (a) normal rice is white, bright and clean, (b) while rice with aflatoxin tends to be a little dirty, and the white color fades

As shown in Table 2, aflatoxin contamination from samples taken in Indonesia are within acceptable levels set by regulations in several countries (Table 3) except for one sample (sample number 9). Sample number 9 had AFB_1 levels of 2.45 mg/kg which exceeded the allowable value by the EU is 2.0 mg/kg. This is caused by several factors, including storage, packaging and distribution of rice that is not good which allows the increase in moisture content in rice. Rice products should be packaged in special packaging for grain products, stored using pallets, not attached to walls, protected from rodents and in a conducive warehouse location. Storage is a decisive step in ensuring the availability of quality rice. During storage, rice undergoes quality and quantity shrinkage caused by physical, chemical, and biological changes [50]. Products with high moisture content will be more easily contaminated and easy for fungi and bacteria to grow [49].

However. the quality of foodstuffs including rice during storage is strongly influenced by the initial quality of the raw materials stored. It should be considered that no exposure to any level of aflatoxin can be considered safe [63].

The growth of aflatoxin-producing microbes is usually triggered by high water content (around 85%), and is often found in tropical regions, such as Indonesia. Aflatoxin can be found in rice before and after harvest. Contamination occurs if drying/storage in high humidity conditions is delayed or disrupted. Grain stored with a moisture content of 18%, within 50 days can produce AFB_1 562 ppb [64]. In order not to cause an impact on health and the economy, the emergence of mycotoxins can be prevented by good plant cultivation and prevention procedures that have been determined. In addition, it is necessary to understand the regulations regarding mycotoxins, both regulations at the international level and SNI so that they are more aware of the importance of preventing the emergence of mycotoxins and fungi that cause them. Understanding regulations is important if agricultural business actors want to expand their product marketing, especially to export businesses. With this prevention and understanding, it is expected to improve the quality of agricultural products that are healthier. safer and free of mycotoxins.

This research has limitations in the research area, the small number of samples tested and the limited scope of sampling locations compared to the distribution of rice in the market. Further research can be carried out in other areas or locations, including using a larger covering area or nationally. Apart from that, the use of methods other than HPLC can also be used to compare results.

4. CONCLUSIONS

This research aims to investigate the aflatoxin content using High-Performance Liquid Chromatography (HPLC) to ensure the sustainability of rice products in the city of Jakarta, Indonesia. The aflatoxin content in various types of rice taken from the market and Logistic Agency in Indonesia has been tested. Through a series of experiments and analysis, various findings and conclusions can be drawn to summarize the results of this research. First of all, analysis using HPLC proved to be a very effective method for the detection and quantification of aflatoxins in rice products. Its accuracy in identifying and quantifying aflatoxins allows us to accurately assess the level of contamination in tested rice samples. These results provide a better understanding of the health risks that may be associated with the consumption of certain rice products in Jakarta. Furthermore, the findings of this study report the level of aflatoxin contamination in several brands and types of rice sold in Jakarta markets. Results show that 95% of the samples have an aflatoxin content of less than 0.50 mg/kg for AFB_1, AFB_2, AG_1, AG_2, and AFT. The samples have values that comply with international standard limits and regulations of other countries. But there is 5% sample that has a value more than that. namely the AFB_1 value of 2.45 mg/kg (exceeding the limit of EU regulations) and the AFT of 2.62 mg/kg. From these results, there is still a risk of exposure to aflatoxins in rice in the market. Factors such as farming methods, storage, and processing of rice can have a significant impact on the level of this contamination. Therefore, careful monitoring and implementation of good agricultural practices need to be strengthened to ensure the sustainability and safety of rice products. The role of the government and regulatory bodies is very important in developing regulations related to testing and monitoring rice quality. This step is necessary to protect consumers from health risks that can arise due to exposure to aflatoxin. In addition, education to farmers, traders and consumers needs to be improved to increase awareness about the dangers of aflatoxin and steps that can be taken to reduce the risk of contamination. The limitations of this research are the small number of samples tested and the limited scope of sampling locations compared to the distribution of rice in the market. However, the results of this case study can be considered valid because rice testing is carried out at a laboratory that has been accredited by a national accrediting body. This research contributes to an in-depth understanding of the quality and sustainability of rice products in Jakarta, Indonesia. The implications of these findings can provide a basis for improving agricultural practices and food quality control regulations. In this way, it is hoped that a safer and more sustainable environment can be created for rice production and consumption in Indonesia. Although there have been no reports of serious health problems due to consumption of rice containing aflatoxin, it is important to carry out regular monitoring as a measure to control food quality. The fact about the large amount of rice consumption by Indonesian people raises concerns about the accumulation of aflatoxin which has a negative impact on health.

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NOMENCLATURE

AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFT	Total Aflatoxin
AG_1	Aflatoxin G_1
AG2	Aflatoxin G_2
AOAC	Association of Official Analytical Chemists
Aw	Water activity
BULOG	Indonesian Logistic Agency
℃	Celsius degree
C18	Octadecylsilane
DKI	Daerah Khusus Ibukota
DNA	Deoxyribonucleic acid
g/kg	gram / kilogram
EU	European Union
GKI	Milled dry grain
Horeca	Hotel, Restaurant, and Catering
HPLC	High-Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
KAN	National Accreditation Committee of Indonesia
L	Liter
LPM	Community Food Barns
mg/kg	milligram / kilogram
min	minutes
mL	mililiter
mm	milimeter
nm	nanometer
pH	potential of Hydrogen
PIBC	Cipinang Rice Market
ppb	part per billion
RP	Reverse Phase
Rp	Indonesian Rupiah
SNI	national standard of Indonesia
spp	Species pluralis
WHO	World Health Organization

Greek symbols

%	Percent
μ	Micro
<	Less than
>	Greater than