

Diversity of Contaminants Fungi in Coffee Beans Stored Using Polystyrene and Gunny Sacks in South OKU Regency (Indonesia)

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Abstract: Robusta coffee is a coffee type found in South OKU Regency in Indonesia, and its storage process is highly susceptible to the growth of various fungal species, including fungi that produce Ochratoxin A (OTA). Therefore, this study aims to examine the fungal contamination contained in storage using polystyrene (PS) and gunny sacks for 20 and 30 days. Data were obtained from surveys, interviews, and samples from 30 farmers which stored coffee beans using PS and gunny sacks in May-July 2020. The results showed that the moisture content was higher in storage using gunny sacks than in PS sacks, at $16.94 \pm 0.878\%$ and $15.99 \pm 1.33\%$, respectively. Furthermore, 16 fungal species were present in gunny sack storage, while 14 fungal species were observed in PS sacks. The percentage of OTA-producing fungi *A.niger* and *A.ochraceus* in the two stores yielded 100% and 63.3%, respectively.

Keywords: coffee beans, fungi, Ochratoxin A, storage

Abstrak (Indonesian): Kopi Robusta merupakan jenis kopi yang terdapat di Kabupaten OKU Selatan Indonesia, dan proses penyimpanannya sangat rentan terhadap pertumbuhan berbagai jenis jamur, termasuk jamur penghasil Ochratoxin A (OTA). Oleh karena itu, penelitian ini bertujuan untuk mengkaji cemaran jamur yang terkandung dalam kopi robusta dengan penyimpanan menggunakan polistirena dan karung goni selama 20 dan 30 hari. Data diperoleh dari survei, wawancara, dan sampel dari 30 petani yang menyimpan biji kopi menggunakan karung PS dan karung goni pada bulan Mei-Juli 2020. Hasil penelitian menunjukkan bahwa kadar air pada penyimpanan menggunakan karung goni lebih tinggi dibandingkan dengan penyimpanan karung polistirena, yaitu sebesar $16,94 \pm 0,878\%$ dan $15,99 \pm 1,33\%$, masing-masing. Selanjutnya, 16 spesies jamur ditemukan dalam penyimpanan karung goni, sedangkan 14 spesies jamur diamati dalam karung polistirena. Persentase jamur penghasil OTA *A. niger* dan *A. ochraceus* di kedua toko tersebut masing-masing menghasilkan 100% dan 63,3%.

Kata kunci: biji kopi, jamur, penyimpanan, Ochratoxin A

1. Introduction

Postharvest, which involves the process of storing coffee beans in warehouses, often experiences a decrease in quality and quantity because of the interaction between biotic and abiotic factors. Biotic factors constitute the main causes of damage to coffee beans during storage, specifically, insects and microorganisms. The microorganisms contaminate coffee berries and beans during all stages of development, harvest, preparation, transportation, and storage [1][2].

The presence of ochratoxin A-producing fungi may cause health problems. According to [3][4], OTA in coffee maybe potentially nephrotoxic and nephrocarcinogenic in both animals and humans. OTA also has immunosuppressive properties, inhibits the

process of gluconeogenesis in the kidneys, nephropathy, kidney tumors, and carcinogens.

Storage in this context is an activity to manage the supply of coffee beans safely in a room during a certain period which influences its packaging [5] and shelf life. According to [6], packaging plays an important role in protecting products from environmental conditions to enhance their shelf life. During the storage period, coffee beans are highly susceptible to mycotoxin contamination which is also influenced by the packaging used during the storage period. Therefore, this study aims to examine fungal contamination, including OTA-producing fungi, on coffee bean storage using PS sack packaging. There are 2 methods of storage that are usually carried out in OKUS Regency, namely using PS sack and gunny sacks. The use of PS sacks is the most widely used

because the selling price is quite economical and easy to obtain. Meanwhile, the use of gunny sacks is only very small because burlap sacks have begun to be abandoned due to their relatively expensive prices and difficult to obtain. The PS sack is made of PS yarn and gunny sack is made of strong fiber, namely jute fiber or natural fiber with a rough texture.

Coffee formers in the BPR RT sub-district have a habit of storing dry coffee beans for 20 to 30 days, this is because to see the market price where the coffee price is getting cheaper at that time, usually people will keep saving until the market price improves. In addition, storage is also carried out to maintain the existence of coffee until the next harvest season.

2. Materials and Methods

2.1. Materials

A sampling of Robusta coffee beans was carried out at the farmer level in Tanjung Setia Village, BPR Ranau Tengah District, OKU Regency in May-July 2020. This village was chosen based on previous research on the process of drying coffee beans using a tarpaulin [7]. The samples were then analyzed for moisture content, species, and percentages of fungi in the Microbiology Laboratory of FMIPA Sriwijaya University. Furthermore, the coffee beans been dried in the sun were then stored in 3 PS sacks and 3 gunny sacks for 20 days by each farmer. The samples of coffee beans from each sack were homogenized afterward and taken as a composite of as much as 1000 grams from PS sacks and 1000 grams from gunny sacks. The samples were separated using the Sample Divider, to analyze water content. Meanwhile, 100 grams of sample from the PS sacks and 300 grams from the gunny sacks were obtained for analysis of fungal species and their percentages. After 30 days of storage, sampling of coffee beans in each package was also carried out.

2.2. Methods

2.2.1. Water content analysis (SNI:01-2907-2008)

The determination of water content involves calculating the difference between the weight of the coffee sample before and after drying in the oven. This was carried out in three replications.

$$\text{Calculation: Water content} = \frac{W1 - W2}{W1 - W0} \times 100\%$$

W0 : Cup and lid weight (grams)

W1: Initial sample weight (grams)

W2 : Final sample weight after drying (grams)

2.2.2. Analysis of fungal species in coffee beans

The analysis of fungal species was performed by using direct plating methods as described by [8]. Coffee beans from each sample were disinfected with 1% sodium hypochlorite for one minute and rinsed with sterile distilled water for 1 minute, with 2 replications. They were then dried in a Petri dish covered with 2 sheets of sterile filter paper. Then, a total of 10 beans were placed in a Petri dish (9 cm in diameter) containing a PDA medium with 0.01% chloramphenicol. The beans were inoculated when the medium was frozen and then incubated at 28^oC temperature until there was fungal growth on the medium (4-5 days). The beans were observed macroscopically by paying attention to the characteristics of each fungus. Furthermore, the fungal isolates were grown on MEA (Malt Extract Agar) medium and observed microscopically. Identification was carried out by using key fungal identification tables or matching the descriptions by [9],[10] and [11]. The percentage of fungi was calculated by the formula:

$$\text{Percentage \%} = \frac{\text{Number of Fungus Attacted Coffee Beans}}{\text{Number of Coffee Beans}} \times 100\%$$

2.3. Data Analysis

The data consisting of water content was analyzed using the non-parametric one-way Wilcoxon test, followed by the Mann-Whitney test. The fungal percentage was analyzed using the Kruskal Wallis test.

3. Results and Discussion

3.1. Water Content in Coffee Beans

According to the 2008 Indonesian National Standard, the water content of coffee beans prior to storage was 12.5%. The measurement after 20 days showed an increase after being stored in the gunny sack which was higher, namely 17.50 ± 0.82%, higher than the water content in the PS sack 16.63 ± 1.172% (Table 1).

Table 1. Comparison of Water Content in Coffee Beans in PS and Gunny Sack Storage for 20 and 30 Days

Variable	Water Content in 20 Days of Storage		<i>p</i> value
	PS Sack (%)	Gunny Sack (%)	
Water Content in Coffee Beans			
Mean ± SD	16.63 ± 1.172	17.50 ± 0.82	0.000
Min - Max	12.78 - 17.56	14.32 - 17.82	
Variable	Water Content in 30 Days of Storage		<i>p</i> value
	PS Sack (%)	Gunny Sack (%)	
Water Content in Coffee Beans			
Mean ± SD	15.99 ± 1.33	16.94 ± 0.878	0.000
Min - Max	11.87 - 17.82	13.84 - 17.75	

Mann Whitney Test, $\rho = 0.05$

Table 1 above shows that there was a difference in mean water content between the use of gunny and PS sacks (*p*-value 0.000) for 20 days of storage. According to [12][13], this high water content leads to susceptibility to mold growth and toxin production in coffee beans. [14] and [15] stated that the increase in water content with storage using gunny sack was due to its permeability to water, steam, and surrounding gases. This permeability causes respiration in coffee beans thereby releasing heat, water, and CO₂ gas. Furthermore, there was a condensation process on the surface of the coffee beans because it was cooler than the surrounding environment and caused the water vapor to stick to the surface. The droplets were then absorbed by the beans thereby increasing the water content.

On the 30th day, the average water content in the coffee beans decreased. A greater decrease occurred in the storage using gunny sacks, namely 16.94 ± 0.878%, while storage using PS sacks was 15.99 ± 1.33%. The process of absorbing water vapor from the surrounding air will continue until the water content of the coffee

beans reaches an equilibrium. Therefore, a decrease occurred on the 30th day of storage. According to [16], the beans have hygroscopic and equilibrium properties similar to sponges which can store the absorbed water until it is balanced with surrounding conditions.

3.2. Fungal Diversity in Coffee Bean Storage

The percentage results of the fungal infestation (Table 2) showed that all coffee beans stored with both gunny and PS sacks were attacked by *Aspergillus niger* after 20 days. *A. ochraceus* (60% vs 63.3%), *Aspergillus wentii* (23.3% vs 36.7%), *Aspergillus fumigatus* (83.3% vs 93.3%), *Fusarium semitectum* (16.7% vs. 23.3%), *Rhizopus oryzae* (6.7% vs 13.3%), *Mucor javanicus* (26.7% vs 50.0%), *Lasiodiplodia theobromae* (73.3 vs 76.7), and *Penicillium notatum* (36, 7% vs 53.3%) were more common in gunny sack storage than PS sack storage. Therefore, the percentage of fungal contamination was higher in the storage of gunny sacks than in PS sacks (Table 2).

Table 2. Percentage of Contaminant Fungal Attack on Coffee Beans Stored in PS and Gunny Sacks for 20 Days

Fungal Species	PS Sack for 20 Days		Gunny Sack for 20 Days	
	The Number (%) of Samples Attacked	The Number (%) of Samples Attacked	The Number (%) of Samples Attacked	The Number (%) of Samples Attacked
<i>A. niger</i>	30 (100.0)		30 (100.0)	
<i>A. ochraceus</i>	18 (60.0)		19 (63.3)	
<i>A. flavus</i>	27 (90.0)		27 (90.0)	
<i>A. wentii</i>	7 (23.3)		11 (36.7)	
<i>A. fumigatus</i>	25 (83.3)		28 (93.3)	
<i>Fusarium oxysporum</i>	2 (6.7)		2 (6.7)	
<i>F. semitectum</i>	5 (16.7)		7 (23.3)	
<i>F. acuminatum</i>	2 (6.7)		2 (6.7)	
<i>Lasiodiplodia theobromae</i>	22 (73.3)		23 (76.7)	
<i>Mucor javanicus</i>	8 (26.7)		15 (50.0)	
<i>R. oryzae</i>	2 (6.7)		4 (13.3)	
<i>Penicillium notatum</i>	11 (36.7)		16 (53.3)	

Furthermore, the fungi *Aspergillus flavus* (90%), *Fusarium oxysporum* (6.7%), and *Fusarium acuminatum* (6.7%) were observed to be equal in both storage methods after 20 days. The fungi percentage comparison results between PS and gunny sacks for 20 days using the Mann Whitney test and the Independent

T. Test showed that there was a difference in the percentage of *A.niger*, *A.ochraceus*, and *A.flavus* between the storage of PS and gunny sacks after 20 days. Meanwhile, for the other fungi, there was no difference in the percentage in both PS and gunny sacks after 20 days.

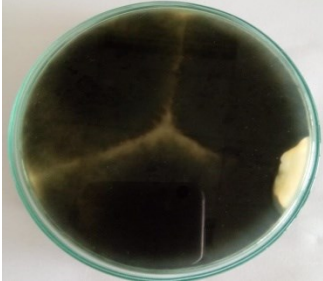
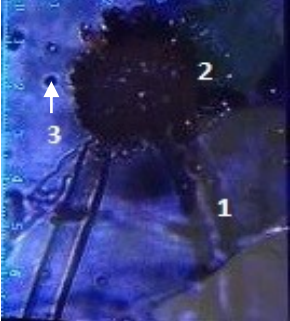

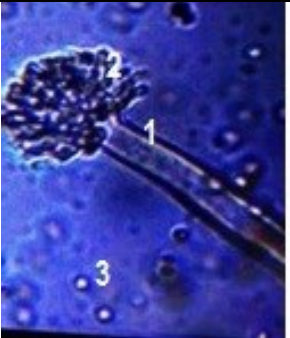
Macroscopic		Microscopic	
Medium MEA	Description		Description
	<ul style="list-style-type: none"> - Growth: Fast/4 days - Colony color: White with a black center - Diameter: + 4.7 cm 		<ol style="list-style-type: none"> 1. Hyphae 2. Conidia 3. Vesicles
<i>Aspergillus niger</i>			
	<ul style="list-style-type: none"> - Growth: Slow/7 days - Colony Color: Yellow - Diameter: + 2 cm 		<ol style="list-style-type: none"> 1.Hyphae 2.Conidia 3.Vesicles
<i>Aspergillus ochraceus</i>			

Figure 1. Macroscopic and Microscopic Characteristics of Fungi with the Potential to Produce OTA in Coffee Beans

Aspergillus niger according to [11][17] is a cosmopolitan type of fungus (found everywhere) and is dominant in coffee beans, followed by *Aspergillus flavus* and *Aspergillus ochraceus*. This agrees with [18][13][19] which stated that the *Aspergillus* genus is very dominant in storage using gunny sacks compared to PS sacks.

This was due to the influence of the moisture content of coffee beans which increased after storage in the gunny sack by $17.50 \pm 0.82\%$, higher than that of the PS sack by $16.63 \pm 1.172\%$ (Table 1). With the humid conditions of the warehouse and packaging as well as the high water content prior to storage, the fungi performed metabolic activity properly, therefore their growth was more optimal. Based on the observations, the average relative humidity (Rh) of the warehouse was 75.6%. According to [12][20], storage conditions cause coffee beans to absorb water from the air, which increases the water content to 20%. Consequently, the coffee beans utilize oxygen from the

air for the respiration process as well as produce carbon dioxide (CO₂) and heat. Research by [19] showed that the moisture content of $10 \pm 12\%$ with $50 \pm 70\%$ RH is the recommended condition for safe storage of coffee beans without loss of quality.

Improper and less hygienic storage methods lead to the growth of ocratoxin A-producing fungi, namely *A. niger* and *A. ochraceus* species (Figure 1). [21][10] stated that *A. niger* and *A. ochraceus* are known to have the potential to produce ochratoxin A. Furthermore, [23] observed that both *A. niger* and *A. ochraceus* species are sources of ochratoxin A contamination in coffee bean food.

Table 3 below shows that after 30 days of storage, all of the farmers' coffee beans were contaminated by *Aspergillus niger* in both PS and gunny sacks. *Aspergillus ochraceus* (63.3%), *Fusarium oxysporum* (60%), and *Rhizopus arrhizus* were observed to be equal in both storage methods after 30 days. Furthermore, *Aspergillus flavus* (86.7%)

vs 93.3%), *Aspergillus fumigatus* (73.3% vs 76.7%), *Aspergillus wentii* (20% vs 36.7%), *Mucor javanicus* (46.7% vs 60%), *Penicillium notatum* (36.7% vs 63.3%), *Rhizopus oryzae* (16.7% vs 26.7%), and *Lasiodiplodia theobromae* (50% vs 66.7%) were more commonly found after 30 days on gunny sack storage compared to PS sack. *Fusarium acuminatum* (43.3%

vs 33.3%), *Fusarium moniliforme* (60% vs 50%), *Fusarium solani* (40% vs 13%), and *Endomyces fibuliger* (6.7% vs 5%) were more commonly found after 30 days in PS sack storage compared to gunny sack, while *Fusarium semitectum* was only found in gunny sack storage.

Table 3. Percentage of Fungal Attack on Coffee Beans stored in PS and Gunny Sacks for 30 days

Fungal Species	PS Sack for 30 Days		Gunny Sacks for 30 Days	
	The Number (%) of Samples Attacked		The Number (%) of Samples Attacked	
<i>A. niger</i>	30	(100.0)	30	(100.0)
<i>A. ochraceus</i>	19	(63.3)	19	(63.3)
<i>A. flavus</i>	26	(86.7)	28	(93.3)
<i>A. wentii</i>	6	(20.0)	11	(36.7)
<i>A. fumigatus</i>	22	(73.3)	23	(76.7)
<i>Fusarium oxysporum</i>	18	(60.0)	18	(60.0)
<i>F. acuminatum</i>	13	(43.3)	10	(33.3)
<i>F. Moniliforme</i>	18	(60.0)	15	(50.0)
<i>F. Solani</i>	12	(40.0)	13	(13.0)
<i>Endomyces fibuliger</i>	2	(6.7)	5	(5.0)
<i>Lasiodiplodia theobromae</i>	15	(50.0)	20	(66.7)
<i>Mucor javanicus</i>	14	(46.7)	18	(60.0)
<i>Rhizopus arrhizus</i>	5	(16.7)	5	(16.7)
<i>R. oryzae</i>	5	(16.7)	8	(26.7)
<i>Penicillium notatum</i>	11	(36.7)	19	(63.3)
<i>F. semitectum</i>	0	(0.0)	12	(40.0)

Farmers' habit of re-drying coffee beans causes pre-harvest fungi to reappear in storage for 30 days. Re-drying also affects the percentage decrease in several fungal species as it is associated with a decrease in the moisture content of the coffee beans, although slightly. This agrees with a study by [1] which reported the growth of the fungus *Fusarium* sp. and *Lasiodiplodia theobromae* in coffee bean samples after storage. The growth of pre-harvest fungi is due to re-drying at the level of the coffee farmers before being sold to collectors at the subdistrict level.

From Table 3 above, the fungi percentage increased in the storage of gunny sacks for 30 days. This is because, prior to storage, the coffee beans contain moisture that exceed the SNI standard (2008) of 12.5%, which causes an increase in shelf-life of 20-30 days. The growth of fungus on coffee beans during the shelf life according to [24][18] involves several important prerequisites, including high moisture content, packaging type, and the environmental conditions of the storage room.

According to [20][25], the use of gunny sacks

that are permeable to water, steam, and surrounding gases cause respiratory activities in coffee beans which produce heat, water, and CO₂ gas. Consequently, this increases the susceptibility to fungal growth. A study by [16] stated that there was a rapid decrease in the quality of coffee beans stored in a warehouse without any environmental control.

4. Conclusion

Coffee bean samples generally obtained from storage are infected by fungi. The contamination percentage of OTA-producing fungi *A. niger* and *A. ochraceus* in the storage of gunny and PS sacks for 30 days were 100% and 63.3%, respectively. The contamination of 16 fungal species was mainly observed in the storage process for 30 days with gunny sacks. In storage with PS sacks, 14 fungal species were found which occurred due to high water content before storage the SNI standard (2008). Finally, the presence of OTA-producing fungi *A. niger* and *A. ochraceus* in coffee beans may be an indicator of OTA contamination.

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