

IDENTIFICATION OF ASPERGILLUS SP MUSHROOMS IN WHEAT FLOUR SOLD OPENLY IN CIROYOM MARKET AS RAW MATERIALS FOR HALAL AND MUNGLE-FREE

Fitri Rahmi Fadhilah¹, Ally Kafesa², Sopi Napita³

Institut Kesehatan Rajawali Bandung

Correspondence Author: ffitirahmi@gmail.com

Abstract: *Wheat flour is used as the main ingredient of food which is often used by the wider community, one of which is as an ingredient for making various kinds of food products. Wheat flour is included in food products safe and halal for consumption. The wheat flour which is sold openly is packaged in transparent plastic. With the easy-to-open packaging, it is easy for microorganisms to enter the flour, one of them is fungus. This study was aim to determine the types of Aspergillus sp in wheat flour which is sold openly in the Ciroyom Market. **Method** The research used is descriptive by using 30 samples of wheat flour which are sold openly in the Ciroyom Market. This research was conducted in the laboratory of the Rajawali Health Institute, Bandung. **Result and Discussion:** The results of this study showed the presence of Aspergillus flavus (50%), Aspergillus fumigatus (50%), Aspergillus niger (16.7%), Aspergillus terreus (3.3%) and negative (26.7%) Aspergillus sp. **Conclusion:** From this research, it can be concluded that the species of Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger and Aspergillus terreus were found so we can conclude that the wheat flour that is sold openly is still a halal product but it is not mushroom-free products.*

Keyword: *Wheat flour, Aspergillus sp, Sold Openly, Ciroyom Market.*

Abstrak: Tepung terigu dijadikan sebagai bahan utama makanan yang sering digunakan masyarakat luas salah satunya sebagai bahan pembuat aneka olahan atau berbagai macam produk makanan. Tepung terigu termasuk ke dalam produk makanan aman dan halal untuk dikonsumsi. Adapun tepung terigu yang dijual secara terbuka dikemas oleh plastik transparan sehingga dengan kemasan yang mudah terbuka mengakibatkan mikroorganisme mudah masuk kedalam tepung salah satunya jamur. **Tujuan** Penelitian ini untuk mengetahui adanya jenis-jenis *Aspergillus sp* pada tepung terigu yang dijual terbuka di pasar ciroyom. **Metode** penelitian yang digunakan adalah deskriptif. Dengan menggunakan 30 sampel tepung terigu yang dijual secara terbuka dipasar ciroyom. Penelitian ini dilakukan dilaboratorium Institut Kesehatan Rajawali Bandung. Hasil dari penelitian ini menunjukkan adanya *Aspergillus flavus* (50%), *Aspergillus fumigatus* (50%), *Aspergillus niger* (16,7%), *Aspergillus terreus* (3,3%) dan negatif (26,7%) *Aspergillus sp*. **Kesimpulan:** Dari penelitian ini dapat disimpulkan bahwa, ditemukannya jenis *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* dan *Aspergillus terreus* sehingga dapat disimpulkan bahwa tepung terigu yang dijual secara terbuka masih merupakan produk halal tetapi bukan produk bebas jamur.

Kata Kunci: *Tepung Terigu Aspergillus sp, Dijual Secara Terbuka, Pasar Ciroyom.*

INTRODUCTION

Wheat flour is the main raw material used in the manufacture of various food products, such as: (bakery), noodles (noodles), spaghetti, pizza, etc. Wheat flour is rich in carbohydrates, but very little in vitamins and minerals. To enrich the nutrient content, several food additives are often added as a fortification of wheat flour.

Decree of the Minister of Health Rep. Indonesia No. 962/Menkes/SK/VII/2003 concerning Wheat Flour Fortification states that flour produced, imported or circulated in Indonesia must contain fortifications, which include: iron (Fe), zinc (Zn), vitamin B1, vitamin B2, and folic acid.

In terms of halal, wheat flour is relatively no problem. However, various added ingredients and improving agents are susceptible to various contaminations of illicit substances. For example, vitamin B1 (thiamine), vitamin B2 (riboflavin), and folic acid (folic acid) which are sourced from halal plants are consumed. These vitamins change status to non-halal when they are produced microbiologically using non-halal media.

The selection of wheat flour is also very important as a food raw material that is safe for consumption. Like low protein wheat flour, it is wheat flour that is commonly used to make cookies, cakes, pastries and fried foods. So that the texture of the cuisine becomes more crunchy. The protein composition contained in wheat flour is about 8% to 11%. This flour is also durable and not easily moldy while so far there are some wheat flours that are sold without packaging, especially if they are stored openly like in traditional markets

According to WHO, there are more than 50% of diarrheal diseases caused by contaminated food, causing 550 million people to experience illness and death as many as 230,000 people (WHO, 2015). Meanwhile, according to BPOM, food poisoning in Indonesia in 2014 was 974 cases and decreased to 697 cases in the following year. In 2016 food poisoning again increased to 791 cases and the morbidity rate due to food poisoning was the highest case (BPOM, 2017). One of the causes of poisoning is *Aspergillus* sp.

Aspergillosis is a disease caused by the fungus *Aspergillus*. *Aspergillus* sp is a eukaryotic microorganism that has the widest distribution area and is abundant in nature, often found in various habitats, but generally saprophytes in the soil. *Aspergillus* sp. has a very small and light spore size so that it is easily spread by the wind (Utami et al., 2012). Room temperature conditions of 20-25°C as well as organic materials such as carbohydrates, proteins, and fats are needed by *Aspergillus* to grow and reproduce. In addition, *aspergillus* is able to live on materials that have a high degree of acidity and starch content (Alvarez et al, 2010).

Aspergillus sp has species namely *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus terreus*. In addition, this fungus can produce mycotoxins, which are called aflatoxins. Mycotoxins are part of secondary metabolites in fungi that can contaminate food and cause poisoning in humans. If a person consumes food contaminated with *Aspergillus* sp. continuously for a long period of time, it will cause diseases such as liver cancer, central nervous system disorders, leaver and hepatitis (Andoro A, 2011).

Wheat flour is used as the main ingredient of food which is often used by the wider community, one of which is as an ingredient for making various processed or various kinds of food products. Wheat flour comes from wheat seeds that are finely ground into flour. The wheat seed used to process flour is only the inner part called the wheat endosperm (Separinta, 2017). The content contained in flour is 8% protein content, 12% water content, 1.3% ash content, 1.5% fat, 2.5% fiber, and starch with a range of 60-68% (Sunarsi, et al 2011).

Ciroyom Market is one of the biggest markets in the city of Bandung, located right at the Ciroyom terminal, there are several stalls and shops as a place to sell. Ciroyom market is listed as one of the largest waste-producing places in Bandung city, so that garbage builds up in temporary dumps. The condition of the market is muddy, there is a chicken slaughter place not far from the trade where the rinse water of the slaughtered chicken does not go directly to the water disposal but is scattered into the streets, and there is garbage that is dumped near the store so that it can trigger the growth of fungi where the spores can be carried away by the wind (Nano, 2018).

The wheat flour which is sold openly is packaged in transparent plastic. Coupled with the easy-to-open packaging, it is easy for microorganisms to enter the flour, one of which is fungus. Factors that can affect the occurrence of contamination in wheat flour are packaging factors, environmental factors, and storage duration factors (Pudatiati, 2018). One level of damage during storage is air circulation in storage, the humidity of the storage room temperature is 25-27°C. The presence of microorganisms that grow in a food ingredient greatly affects the quality of the product (Mizana D, 2016).

According to research conducted by Wiwik Pujiati in 2018 with the topic of *Aspergillus sp.* on wheat flour which is sold openly in the Legi Jombang market, the results obtained were 71.40% *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* obtained 14.30% with the same percentage. According to research conducted by repertoire in 2013 with the topic of the effect of storage time on the diversity of mold contaminants in wheat flour, it was found that the presence of *Aspergillus sp.*, *Penicillium funiculosum*, *Eupenicillium hirayamae*, *Cladosporium sphaerospermum*, and *Rhizopus*. To identify *Aspergillus sp.*, namely macroscopically and microscopically. Based on the description above, the authors are interested in conducting an examination of "Identification of the fungus *Aspergillus sp* in wheat flour which is sold openly in the Ciroyom Market".

METHOD

A. Tools Sterilization

1. The tools to be used are prepared in advance.
2. The tool is washed and dried
3. The tool is put into an autoclave with a temperature of 121°C (Murniana, 2011).

B. Making SDA Media (Sabourand dextrose agar)

1. SDA media was weighed as much as 40.3 grams. using a digital scale and inserted into the Erlemeyer.

2. Add 620 ml of distilled water into the Erlenmeyer.
3. SDA media is heated above the spirit with a tripod.
4. The media is homogenized with a stirring rod until it boils.
5. Erlenmeyer is closed with a cotton plug.
6. The medium was sterilized using an autoclave at 121°C.
7. The medium is allowed to stand until it becomes solid.

C. Isolation of Fungi on SDA (Sabouraud Dextrose Agar) Media Using the Seedling (Sow) Method

1. The sample is first weighed as much as 1 gram, then fix the periphery of the petri dish by passing it in the spirit, open the petri dish and then sprinkle the flour on top of the media that has been solid until smooth.
2. Fixation of the petri dish that has been planted with wheat flour samples and then incubated for 3-5 days at a temperature of 27 C
3. Observations were made macroscopic by looking at the color of the colony, the shape of the colony on the media.

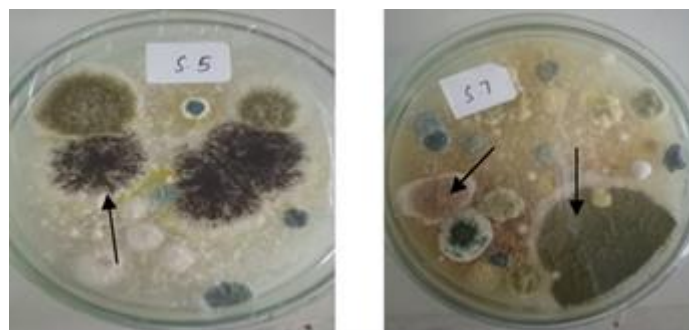
D. Microscopic examination

1. The tools to be used are prepared in advance.
2. A few drops of distilled water are placed on the object glass.
3. The spores are fixed above the spiritus.
4. Colonies are taken and then placed on a glass object that has been given distilled water and mixed.
5. LPCB (Lactophenol cotton blue) was dripped as much as 50 m and covered using a glass deck.
6. Observed under a microscope with a magnification of 40x

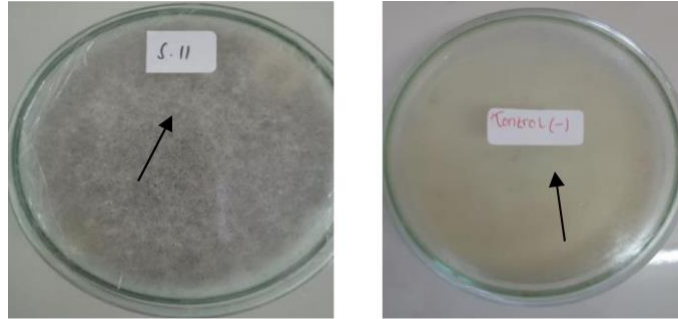
RESULT AND DISCUSSION

This research was conducted at the Microbiology Laboratory of the Rajawali Health Institute, Bandung in June 2021. This research began with sampling at the Ciroyom market and inoculation, then continued with the identification of *Aspergillus* in wheat flour that was sold openly.

4.1. Identification Results of *Aspergillus* in Wheat Flour Sold Commercially Open at Ciroyom Market



- 1) *Aspergillus Niger*, 2) *Aspergillus flavus*,
3) *Aspergillus fumigatus*, 4) *Aspergillus terreus*



- 5) Negatif *Aspergillus sp* 6) Negative control

Figure 4. 1 Makroskopik *Aspergillus sp*
Source : Self Documentation, 2021

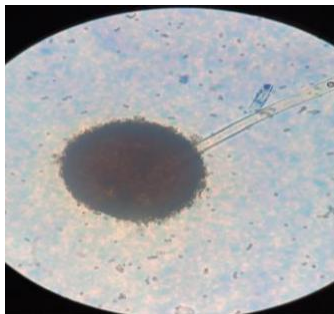
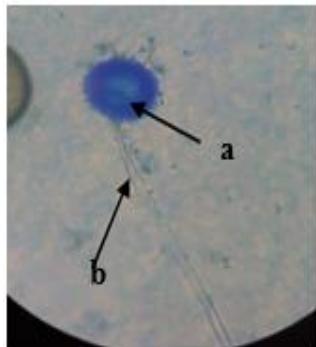
Table 4. 1 Macroscopic Observation

Hyphae Colour	Colony		Eksudat	Suspect
	Edge	Texture		
			Drop	Species
1. Black	White	Cattony	No	<i>Aspergillus Niger</i>
2. Green yelowish	White	Velvety	No	<i>Aspergillus Flavus</i>
3. Dark green	White	Velvety	No	<i>Aspergillus Fumigatus</i>
4. Light brown	White	Velvety	No	<i>Aspergillus Terreus</i>

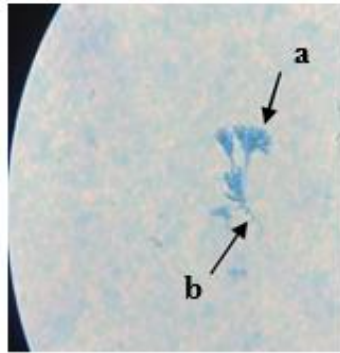
Identification of the fungus *Aspergillus* sp. in wheat flour that is sold openly was carried out macroscopically and microscopically including color, colony edges, texture, drop exudate, conidia and hyphae. The results of macroscopic observations can be seen in the table below.

After being observed macroscopically, then microscopic observations were made to identify what type of fungus *Aspergillus* sp. grows on wheat flour that is sold openly by looking at the characteristics. The results of microscopic observations can be seen in the table below.

Table 4. 2 Microscopic Observation

No	Microscopic	Characteristic Features	Suspect species
1		<p>a. Conidia: dark brown until black, shaped round, hyaline smooth-walled</p> <p>b. Hyphae: Transparent and not aspirated</p> <p>(P:40X)</p>	<i>Aspergillus niger</i>
2		<p>a. Conidia: blue colored, shaped round sized small, hyaline insulated.</p> <p>b. Hyphae: Transparent, and not aspirated</p> <p>(P:40X)</p>	<i>Aspergillus flavus</i>

3

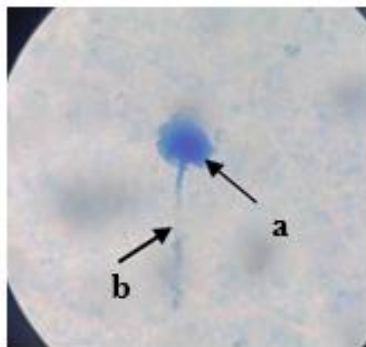


a. Conidia:
blue colored, *Aspergillus*
shaped like leaves. *fumigatus*
small size

b. Hyphae:
Transparent, and
not aspirated

(P:40X)

4



a. Conidia:
Aspergillus color *Aspergillus terreus*
blue, *Terreus*
shaped
like a fan.

Conidiophores:
hyaline and walled
fine.

(P:40X)

Based on table 4.2, it is known that there are types of *Aspergillus* that grow in several samples, namely *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus terreus*. Below are data from 30 samples of wheat flour which are sold openly in the Ciroyom market.

Table 4. 3 Result from 30 Samples

Sample	<i>A.Niger</i>	<i>A.Flavus</i>	<i>A.Fumigatus</i>	<i>A.terreus</i>	Keterangan
1	-	+	-	-	<i>Aspergillus sp</i>
2	+	-	+	-	<i>Aspergillus sp</i>
3	-	-	-	-	-

4	-	-	+	-	<i>Aspergillus sp</i>
5	+	+	+		<i>Aspergillus sp</i>
6	-	+	+	-	<i>Aspergillus sp</i>
7	-	+	+	+	<i>Aspergillus sp</i>
8	-	+	+	-	<i>Aspergillus sp</i>
9	-	-	-	-	-
10	-	-	+	-	<i>Aspergillus sp</i>
11	-	-	-	-	-
12	-	+	-	-	<i>Aspergillus sp</i>
13	-	-	-	-	-
14	-	+	+	-	<i>Aspergillus sp</i>
15	+	+	-	-	<i>Aspergillus sp</i>
16	-	+	-	-	<i>Aspergillus sp</i>

17	+	-	+	-	<i>Aspergillus sp</i>
18	-	-	-	-	-
19	-	-	-	-	-
20	-	+	+	-	<i>Aspergillus sp</i>
21	-	-	-	-	-
22	-	+	+	-	<i>Aspergillus sp</i>
23	-	-	+	-	<i>Aspergillus sp</i>
24	-	-	+	-	<i>Aspergillus sp</i>
25	+	+	+	-	<i>Aspergillus sp</i>
26	-	+	-	-	<i>Aspergillus sp</i>
27	-	+	-	-	<i>Aspergillus sp</i>
28	-	-	-	-	-

29	-	+	-	-	Aspergillus sp
30	-	-	+	-	Aspergillus sp
Negative control	-	-	-	-	-

Viewed from table 4.3, 22 positive samples of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus* were obtained. and 8 negative samples of *Aspergillus sp*. Then the percentage of fungi that grow and do not grow in the table below.

Table 4. 4 Percentage of *Aspergillus sp*

Identification Result	N	Amount	Presentase
<i>Aspergillus niger</i>	30	5	16,7%
<i>Aspergillus flavus</i>	30	15	50%
<i>Aspergillus fumigatus</i>	30	15	50%
<i>Aspergillus terreus</i>	30	1	3,3%
Negatif	30	8	26,7%

Explanation : N : Amount of wheat flour examined

Based on table 4.4 that half of them have *Aspergillus flavus*(50%), *Aspergillus fumigatus* (50%), a small proportion of *Aspergillus niger* (16.7%), *Aspergillus terreus* (3.3%) and almost half were negative (26.7%) *Aspergillus sp*.

In this study, the sample used was wheat flour. Wheat flour is the main raw material used in the manufacture of various food products, such as bakery, noodles spaghetti, pizza, etc. Where the sampling is in accordance with the criteria, namely wheat flour which is sold

openly and sold in the Ciroyom Market. The medium used for isolation is *Sabouroud Dextrose Agar* which is a selective medium for the growth of fungi and yeasts. This medium consists of 15 grams of agar, 40 grams of dextrose which is a fermented carbohydrate in high concentration as an energy source. There are also 5 grams of animal tissue enzymatic and 5 grams of casein enzymes, both of which provide amino acids and nitrogen compounds for the growth of fungi and yeasts. The pH contained is 5.6 acidic (Mutiawati, 2016). The media is first made, if the media is solid, the sample is isolated as much as 1 gram using the sowing method, then incubated for 3-5 days at 27°C.

According to Gandjar (2006), there are several factors that can affect fungal growth, namely optimal temperature of 27°C but there are also fungi that can grow at 12-55°C, for example *Aspergillus fumigatus*, the required pH is 5-6. Generally, fungi such as mucor or *Rhizopus* require 90% humidity while for *Penicillium*, *fusarium*, and *Aspergillus* the humidity level is 80%. In addition, the most needed factor for substrate fungi as a source of new nutrients is used after the fungus secretes enzymes and breaks down simple compounds.

After being incubated for 3-5 days, the media were observed macroscopically for color, colony edges, texture, and exudate drop, conidia and hyphae. After that, microscopic observations were carried out to ensure that *Aspergillus* sp., the staining used was LPCB (Lactophenol Cotton Blue). Lactate serves to clarify the background and sharpen the mushroom structure (Savitri, 2012).

The results obtained from the study of wheat flour which was sold openly in the Ciroyom market from 30 samples showed that almost half of them contained the fungus *Aspergillus* sp. The types of *Aspergillus* that have been identified macroscopically and microscopically are *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus terreus*. *Aspergillus niger* was obtained as much as 16.7% in wheat flour macroscopically has the characteristics of black colonies, the surface is like Cottony, there were no exudate droplets (in the form of liquid dots visible on the surface of the colony), there were no concentric circles, and there were no radial lines.

Microscopically, the conidia are dark brown to black, round in shape, large in size, hyaline with smooth walls, transparent hyphae. This fungus causes black fungus disease in onions, peanuts, grapes, certain vegetables and fruits (Refai, 2014). *Aspergillus niger* is useful for industry because it can produce galic acid, a phenolic compound that can be used in the food industry as an antioxidant and has a toxin in the form of ochratoxin. If *Aspergillus niger* is inhaled it will cause reactions such as allergic alveolitis and asthma (Handajani, 2006).

Aspergillus flavus found as much as 50% of this fungus often contaminates food can produce aflatoxins and is hepatotoxic and carcinogenic (Khasanah, 2013). Macroscopically, the colonies were yellowish green, cotton-like surface (Cottony), there were no exudate droplets (in the form of liquid dots visible on the colony surface), there were no concentric circles, and there were no radial lines. While microscopically the conidia heads were round, yellowish green, conidiophores were rough-walled, hyaline in color, and vesicles were round.

Aspergillus fumigatus can produce helvolic acid which causes liver damage (Khasanah, 2013). The results obtained were 50% which macroscopically had the characteristics of dark green colony color, like cotton (Cottony), there were no exudate droplets (in the form of liquid dots visible on the surface of the colony), there were no concentric circles, and there were no lines. Radials. whereas macroscopically small oval conidia attached to the ends of one or two rows of strigmata that are regularly coiled on the tip surface of the conidiopore called vesicles.

Aspergillus terreus was found in a small percentage of 3.3% macroscopically having the characteristics of a light brown colony color, like cotton (Cottony), there were no exudate droplets (in the form of liquid dots visible on the surface of the colony), there were no concentric circles, and there were no lines. Radials by microscopic conidia are blue, fan-shaped. conidiophores: hyaline and smooth-walled, hyphae transparent. The types of *Aspergillus* sp. were found in 30 samples of wheat flour which were sold daily.

CONCLUSION

From the research on the identification of the fungus *Aspergillus* sp. in wheat flour which is sold openly in the Ciroyom market, the results obtained were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus terreus* so we can conclude that the wheat flour that is sold openly is still a halal product but it is not mushroom-free products.

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