

Isolation And Characterization Of Yeast And Mold From The Khamir Kapang Number Test (AKK) On Lemang And Air Pollution Test In The Original Lemang Production Room Of Tebing Tinggi City

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui nilai Angka Khamir Kapang (AKK) pada lemang mengetahui nilai cemaran udara pada ruang tempat produksi lemang dan mengetahui genus dari khamir dan kapang yang diisolasi dan dikarakterisasi pada lemang dan ruang tempat produksi lemang asli Kota Tebing Tinggi. Penelitian ini dilakukan dengan metode eksperimental yaitu menguji 3 sampel lemang dan ruang tempat produksi lemang yaitu lemang 01, lemang 02 dan lemang 03. Hasil penelitian menunjukkan bahwa pada 3 sampel lemang mempunyai nilai AKK yang masih dalam batas aman untuk dikonsumsi yaitu kurang dari 1×10^5 dan 3 sampel udara ruang tempat produksi lemang masih dikategorikan batas aman ruang terkontaminasi, setelah dilakukan karakterisasi morfologi pada lemang 01 ditemukan genus *Penicillium*, pada lemang 02 ditemukan genus *Candida* dan *Penicillium*, dan pada lemang 03 ditemukan genus *Aspergillus* dan *Penicillium*. Sedangkan, pada ruang tempat produksi lemang 01 dan lemang 02 ditemukan genus *Aspergillus* dan *Penicillium*, sedangkan pada lemang 03 ditemukan genus *Aspergillus*, *Alternaria*, dan *Trichoderma*.

Kata Kunci : Lemang, Ruang Tempat Produksi Lemang, Khamir dan Kapang

ABSTRACT

This study aims to determine the Yeast and Mold Count (YMC) value in lemang, assess air pollution levels in the lemang production facility, and identify the genera of yeast and mold isolated and characterized in lemang and the lemang production facility in Tebing Tinggi City. This study was conducted using an experimental method, testing three samples of lemang and the lemang production room, namely Lemang 01, Lemang 02, and Lemang 03. The results showed that the three lemang samples had AKK values within safe limits for consumption, i.e., less than 1×10^5 , and the three air samples from the lemang production room were still categorized as safe, even though they were contaminated. After morphological characterization, the lemang 01 sample was found to contain the genus *Penicillium*, in lemang 02, the genus *Candida* and *Penicillium* were found, and in lemang 03, the genus *Aspergillus* and *Penicillium* were found. Meanwhile, in the production room of lemang 01 and lemang 02, the genus *Aspergillus* and *Penicillium* were found, while in lemang 03, the genus *Aspergillus*, *Alternaria*, and *Trichoderma* were found.

Keywords: Lemang, Lemang Production Room, Yeast and Mold

I. PENDAHULUAN

1. Latar Belakang

Lemang is a food made from glutinous rice and coconut milk cooked by burning in bamboo. Lemang from Tebing Tinggi City does not contain preservatives, so it can only last up to 3 days if stored in bamboo containers. As a semi-wet food product with a moisture content of 55.55% and water activity (a_w) between 0.65-0.90, lemang is susceptible to mold and yeast growth. Lemang deterioration is characterized by dark color, sour smell, mushy and slimy texture, which is caused by mold growth. A good lemang has a bright color, the right texture (not too hard or mushy), fragrant aroma, and non-acidic taste. Lemang can also be contaminated with microorganisms from the production environment, which can affect its quality and safety. This microbial contamination can occur during the production process. The microbiological test is done by testing the mold and yeast count (AKK), where the maximum limit for mold and yeast in similar products, such as lontong and coconut milk-grilled rice, is 1×10^5 CFU/g. The lemang production space required an air contamination test in the production space using the settle plate method as a parameter of cleanliness of the production space free from microbial contamination, namely mold and yeast.

2. Perumusan Masalah

The problem formulation of this research are: whether the value of mold and yeast count (AKK) in lemang in Tebing Tinggi City exceeds the specified limit, whether the air contamination test in the lemang production room shows the room is free from microbial contamination, what are the genus of yeast and mold found in lemang and production room, and whether there are differences in the genus of yeast and mold between lemang and lemang production room.

3. Tujuan Penelitian

The objectives of this study were: To determine the value of mold yeast count (AKK) in lemang in Tebing Tinggi City exceeds the specified limit, to determine the air contamination test in the lemang production room shows the room is free from microbial contamination, to determine what genus of yeast and mold are found in lemang and production room, and to determine if there are differences in the genus of yeast and mold between lemang and lemang production room.

4. Manfaat Penelitian

The benefits of this research are: For the researcher, to add insight into how to test for mold yeast count (AKK) in food, test for air contamination in food production rooms, and characterize the morphology of the isolated yeast / mold. For academics, this study provides baseline data to formulate or update production standards to ensure lemang is produced according to safety and quality criteria. For the public, this study provides information on the quality of lemang in Tebing Tinggi City based on the existing AKK.

II. METODE PENELITIAN

This study was conducted experimentally where microbiological examination was carried out. This study includes the calculation of mold and yeast count (AKK), air contamination test in the production room, as well as isolation and characterization of macroscopic and microscopic morphology of mold and yeast found through direct experimentation in lemang and lemang production room.

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The tools and materials used are Laminar Air Flow, newspaper, plastic, vortex, isopad, petri dish, goblet, measuring cup, erlenmeyer, cotton swab, microtip and tip, test tube, bunsen lamp, incubator, autoclave, analytical balance, aluminum foil, plastic wrap, durham tube, microscope, cover glass, object glass, tube, and haemocytometer. The materials used in this study were lemang produced by 3 lemang entrepreneurs in Tebing Tinggi City, sterile distilled water, 0.1% Buffered Pepton Water (BPW), Saborraud Dextrose Agar (SDA), 70% alcohol, 0.9% NaCl, bromothymol blue, 1% sugar, Lacto Phenol Cotton Blue (LPCB), chili plants, and chloramphenicol.

This research procedure began with sterilization of tools using an autoclave. SDA (65 g in 1 liter of distilled water) and BPW 0.1% (10 g in 1 liter of distilled water) media were prepared and autoclaved, then chloramphenicol dissolved in NaCl 0.9% was added. for SDA. For the yeast mold number (AKK) test, 25 g of lemang samples were mixed with 225 mL of 0.1% BPW solution, homogenized, and diluted to 10^{-5} . The dilution was repeated twice and placed on a petri dish with SDA media poured in the petri dish, with three replications per sample. The dishes were incubated at 20-25°C for 5 days to count the fungal colonies. For the air contamination test, petri dishes were opened in the production room for 30 minutes at a predetermined point, then incubated at 25°C for 4 days. Controls were used to ensure media sterility. Isolation was carried out by transferring hyphae to SDA media and incubated for 3-7 days to observe colony morphology macroscopically and also microscopically with LPCB staining. Glucose fermentation test was conducted to test gas production in yeast. Spore density was calculated by mixing the fungal culture in distilled water, diluted to 10^6 , and counted using a haemocytometer.

Pathogenicity tests were conducted following Koch's postulates to ascertain whether fungal isolates cause leaf spot on plants. This test uses cayenne pepper (*Capsicum annum*) leaves as the host. Fungal isolates were inoculated on the wounded leaves and covered with tape. Observations were made for 7 days to see symptoms of leaf spot, which indicates the pathogenic nature of the fungus.

III. HASIL DAN PEMBAHASAN

Yeast mold count (AKK) test on lemang

The results of the mold Khamir Number (AKK) test on three lemang samples in Tebing Tinggi City (lemang Ajo, lemang Batok, and lemang Bundo Widya) showed AKK values that still meet BPOM standards, which are less than 1×10^5 CFU/g, and safe for consumption. The average AKK value in lemang was less than 10 colonies, which does not meet the calculation requirements according to PPOMN (2006). This is due to the cleanliness of raw materials and production equipment that are well maintained. This study is in line with the results of Enny Sholichah et al (2020) which showed that the AKK of lemang without preservatives on day-0 was 3.33 CFU/g, which is still safe for consumption.

Air Pollution Test in Lemang Production Room

The results of the air contamination test in the lemang production rooms in Tebing Tinggi (lemang Ajo, lemang Batok, and lemang Bundo Widya) showed that the air contamination value still met the limit set for a contamination-free room, with an average air contamination of less than or equal to 5 colonies/30 minutes. All three production rooms are class B, with an average of 1.6 colonies/30 minutes (lemang Ajo), 1.5 colonies/30 minutes (lemang Batok), and 3 colonies/30 minutes (lemang Bundo Widya) according to CPOB 2018. This study shows that air contamination in the lemang production room is

categorized as low, with values less than 50 CFU/m³, in accordance with BPOM (2018) standards and the air quality category of non-industrial homes or workplaces.

Genus of yeast and mold found in lemang and the room where lemang is produced

1. Genus of mold and yeast found in lemang

Two genus of molds, *Penicillium* and *Aspergillus*, and one genus of yeast, *Candida*, were found in lemang. *Penicillium* was the most common genus of mold found although in small quantities. The presence of this mold indicates contamination of food. Although *Penicillium* species generally do not cause disease in humans, some species can become pathogenic upon long-term exposure, especially for individuals with compromised immune systems or allergies.

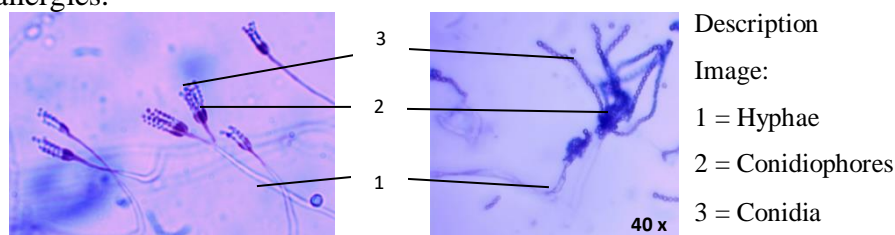


Figure 1. Microscopic characteristics of *Penicillium* sp. 40 x magnification (Source: Personal Documentation)

Aspergillus sp. was the second most common fungus found in lemang. It is a thermotolerant fungus, capable of thriving at high temperatures, with the ability to grow at temperatures between 20°C and close to 50°C. *Aspergillus* is a saprophytic fungus commonly found in foodstuffs, air, and soil, so it is easily contaminated during production. The presence of this fungus in the samples indicates that the production process supports its growth.

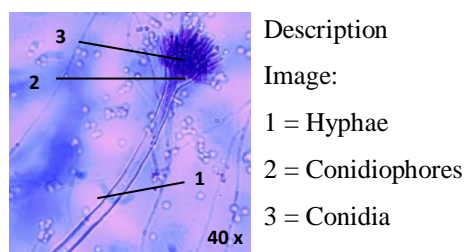


Figure 2. Microscopic characteristics of *Aspergillus* sp. 40 x magnification (Source: Personal Documentation)

Candida sp. are normal flora in the skin, mucous membranes, and gastrointestinal tract. *Candida albicans* is the most pathogenic species that often causes disease in humans, especially in immunocompromised individuals, such as diabetics, toddlers, the elderly, pregnant women, as well as those undergoing antibiotic treatment, corticosteroids, or have immunodeficiencies such as HIV/AIDS. Foods rich in sugar and carbohydrates can support the growth of *Candida*, as sugar is the main source of energy for this fungus.

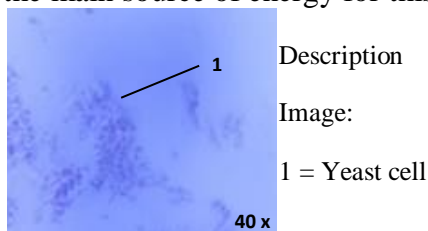


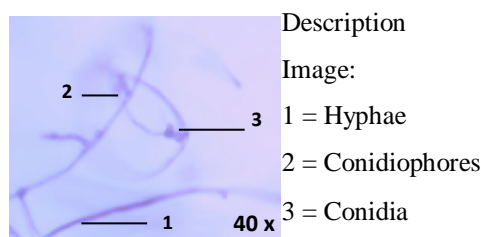
Figure 3. Microscopic characteristics of *Candida* sp. 40 x magnification (Source: Personal Documentation)

2. Genus of molds found in Lemang production room

Aspergillus is the most common mold found in the air of lemang production rooms. Although humans often inhale *Aspergillus* spores, aspergillosis generally only develops in individuals with weakened immune systems. Conditions that can result from aspergillosis include bronchopulmonary aspergillosis, aspergilloma, and invasive pulmonary aspergillosis, which can cause organ damage. Aspergillosis is not contagious between humans. *Aspergillus* is commonly found on decaying leaves, compost, trees, grain crops, and some foods and spices.

Penicillium is a cosmopolite mold that can be found on a variety of substrates, such as plant tissue, soil, air, foodstuffs and sea sponges. These molds can cause infections in humans, especially in individuals with reduced immunity, such as people with HIV, corticosteroid users, chemotherapy patients, or post-surgery.

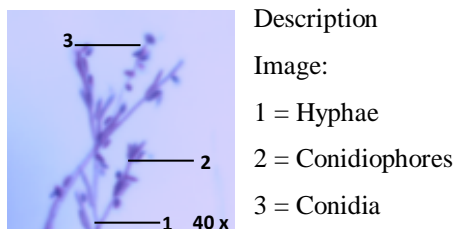
Airborne *Alternaria* spores can help optimize fungicide applications on cereals and tackle phytopathogens on crops such as cotton and potatoes. In addition, *Alternaria* spp. are a major cause of respiratory allergy in Europe and globally, with sensitivity rates to *Alternaria* allergens in Europe reaching 8.9%. Exposure to *Alternaria alternata* spores more often causes asthma, especially in children, and can trigger rhinoconjunctivitis and worsen asthma attacks.



Description
Image:
1 = Hyphae
2 = Conidiophores
3 = Conidia

Figure 4. Microscopic characteristics of *Alternaria* sp. 40 x magnification (Source: Personal Documentation)

Trichoderma is a biological fungus often used for plant pest control, especially soil-borne diseases, as well as some foliar and grain diseases. It can prevent diseases, promote plant growth, nutrient use efficiency, plant resistance, and ameliorate agrochemical pollution. *Trichoderma* is found in soil, air, plant surfaces and other ecological environments.



Description
Image:
1 = Hyphae
2 = Conidiophores
3 = Conidia

Figure 5. Microscopic characteristics of *Trichoderma* sp. 40 x magnification (Source: Personal Documentation)

Based on the results of the carbohydrate fermentation test, the yeast isolates showed a change in media color from blue to yellow and the appearance of bubbles in the Durham

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tube after 72 hours, indicating that the yeast can ferment glucose into ethanol and CO₂. The spores produced by the mold isolates ranged from 2×10^5 to 2.8×10^6 spores/ml, with variations in the number of spores between different genera. The molds in each isolate did not show pathogenicity on chili leaves, including *Alternaria* isolates which are known as pathogens. This may be due to secondary metabolite compounds in chili leaves that can inhibit the growth of diseases caused by *Alternaria* mold.

Differences in the Genus of Yeast and Mold found in Lemang and Lemang Production Room

There were differences in the yeasts and molds found in lemang and lemang production rooms. The yeast genus *Candida* sp. was found in lemang, but not in the production room, possibly because the cleaner and drier conditions of the production room did not support its growth. Meanwhile, two mold genera, *Alternaria* sp. and *Trichoderma* sp. were found in the production room but not in lemang. This could be due to molds being more adaptive to certain conditions in the production process, such as high humidity or organic matter that supports their growth. *Alternaria* is a plant pathogen that can grow on organic matter, while *Trichoderma* is often used in biocontrol. Spores of both molds can be airborne and land in production spaces through human activity or the movement of tools and materials.

IV. KESIMPULAN

Based on the research results, the conclusions are as follows: The value of mold yeast count (AKK) in three samples of lemang sold in Tebing Tinggi City (lemang Ajo, lemang Batok, and lemang Bundo Widya) did not exceed the maximum AKK limit ($<1 \times 10^5$ CFU/g), although mold was present. However, the number of mold colonies in the samples did not meet the calculation requirements because it was less than the range of 10-150 colonies. Air contamination tests in the production rooms showed that all three production rooms were class B, with an average air contamination of less than or equal to 5 colonies/30 minutes (lemang Ajo: 1.6, lemang Batok: 1.5, lemang Bundo Widya: 3). Fungi found on lemang included *Aspergillus*, *Penicillium* and *Candida*, while *Penicillium*, *Aspergillus*, *Alternaria* and *Trichoderma* were found in the production room. The spore density varied between 2×10^5 to 2.8×10^6 spores/ml, when tested for gas production on yeast the results showed positive gas but the pathogenicity test showed that all mold isolates were not pathogenic on chili leaves. The difference between lemang and production room was the presence of the yeast genus *Candida* in lemang and the mold genus *Alternaria* and *Trichoderma* in the production room which were not found in lemang.

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