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Characterization of Contaminants Molds In Smoked Fish Coated In Chitosan

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ABSTRACT

Smoked fish is one of the traditional fish processing through the process of open heat smoking. The water content in smoked fish is quite high. The high water content will facilitate the growth and development of decaying microbes. Some studies have reported that smoked fish after being stored for several days will be overgrown at any time, even smoked fish that have been coated with chitosan. Research objectives to analyze the characteristics of contaminants in smoked fish treated with chitosan. The research was conducted from November to December 2020 at FPIK UNIPAS Morotai Laboratory. The growth medium of the glyph uses potato extract (PDA), the isolation of the glyph from smoked cob fish, as well as the characterization of the glyph which includes macroscopic and microscopic characters. The data obtained is analyzed descriptively. The result is isolation was found five isolates of any time, namely isolates KU1, KU2, KU3, MG1, and KS1. Based on macroscopic and microscopic characterization, isolate KU1 such as *Fusarium oxysporum*, isolate KU2 such as *Aspergillus niger*, isolate KU3 such as *Penicillium* sp., isolate MG1 such as *Aspergillus flavus*, and isolate KS1 such as *Acremonium* sp.

Keywords: characterization, chitosan, contaminant, mold, smoked fish

ABSTRAK

Ikan asap merupakan salah satu hasil pengolahan ikan secara tradisional melalui proses pengasapan panas secara terbuka. Kandungan air pada ikan asap cukup tinggi. Kandungan air yang tinggi akan mempermudah tumbuh dan berkembangnya mikroba pembusuk. Beberapa penelitian telah dilaporkan bahwa ikan asap setelah disimpan beberapa hari akan ditumbuhi kapang, bahkan ikan asap yang telah dilapisi kitosan. Tujuan Penelitian untuk menganalisis karakteristik kapang kontaminan pada ikan asap yang diberi perlakuan kitosan. Penelitian dilakukan pada bulan November sampai Desember 2020 di Laboratorium FPIK UNIPAS Morotai. Media pertumbuhan kapang menggunakan ekstrak kentang (PDA), isolasi kapang dari ikan tongkol asap, serta karakterisasi kapang yang meliputi karakter makroskopik dan mikroskopik. Data yang diperoleh dianalisis secara deskriptif. Hasil isolasi ditemukan 5 isolat kapang yaitu isolat KU1, KU2, KU3, MG1, dan KS1. Berdasarkan karakterisasi makroskopis dan mikroskopis, isolat KU1 seperti *Fusarium oxysporum*, isolat KU2 seperti *Aspergillus niger*, isolat KU3 seperti *Penicellium* sp., isolat MG1 seperti *Aspergillus flavus*, dan isolat KS1 seperti *Acromonium* sp.

Kata Kunci: karakterisasi, kitosan, kontaminan, kapang, ikan asap

INTRODUCTION

Smoked fish is a byproduct of traditional fisheries processing through the means of smoking. The smoking method aims to impart longevity and a distinct flavor to fish through natural fuels (Wibowo, 2000). Smoked fish is commonly referred to as *fufu* fish in North Maluku. Along with North Maluku, North Sulawesi is also listed in South Sulawesi. Smoked fish is referred to as *asar* or selling fish in Maluku.

Smoked fish contains a significant amount of water. According to Alinti et al. (2017), smoked fish has 58.2 percent water. Additionally, Kaban et al. (2019) say that the water content of smoked fish is between 55 and 62.5 percent. Microorganisms can grow and develop in smoked fish due to the high water content. Along with the water content, the protein content of fish meat aids in chemical and biological deterioration. Bawinto et al. (2015) discovered that the existence of *Aspergillus* sp. and *Penicillium* sp. Bitung City smoked fish according to Montiel et al. (2012), fungal growth in fish can alter the texture and create rancid odors. According to Sopandi and Wardah (2014), the presence of whenever affects the quality and safety of food products. It is because certain types of Timex are capable of producing mycotoxins.

Losses incurred from microbial contamination are critical, as these forms of time can be directly pathogenic (Buckle et al., 2013). Additionally, some other forms of time may create toxins (mycotoxins) during their growth and food decay processes. Mycotoxin compounds have been linked to a wide range of health problems, including death (Pitt and Hocking, 1997). Frivad et al. (1998) argue that the *Aspergillus* community is economically significant due to its ability to target various foodstuffs.

Over time, contamination of fishery products, especially smoked fish, can be overcome by adding natural preservatives such as chitosan. Chitosan is a preservative that is applied by coating. Among the benefits of using chitosan as a food coating are its antimicrobial properties, nontoxicity to humans, low cost, and ease of biodegradability (Wang, 1992; Muzzarelli, 1996; Paul et al., 2013). According to some research, chitosan has antimicrobial properties and can be used in foods (Odu et al., 2012; Chamanara et al., 2015; Suseno et al., 2015). However, when smoked fish is coated with chitosan, the shelf life of the smoked fish is extended to four days, at which point the smoked fish is overgrown with something. As a result, this research was conducted to determine the pollutants' characteristics in smoked fish treated with chitosan.

MATERIALS AND METHOD

This experimental study in which smoked fish is immersed in a chitosan solution and then develops during the storage period is isolated. The analysis took place between November and December 2020. The period was quantified at Universitas Pasifik Morotai's FPIK Laboratory.

Instruments and Materials

Microscopes, glass pieces, glass covers, Petri disc, pumpkin Erlenmeyer, measuring gourds, digital scales, knives, cup glasses, pans, autoclaves, drip pipettes, micropipettes, spiritus burners, inoculum needles, boxes, and plastic containers were all utilized in this research. Potatoes, aqua, starch, dyes, spiritus, chitosan, and smoked cob fish are used as ingredients.

Procedures

1. Synthesis of chitosan solutions
   Chitosan was used at concentrations of 0.5; 1; 1.5; and 2%. To make a 2% chitosan solution, weigh 2 grams of chitosan into an Erlenmeyer flask and add acetic acid at a concentration of 0.5 percent, stirring uniformly until a suspended solution forms. The same procedure is followed for chitosan concentrations of 0.5, 1, and 1.5 percent.
2. Smoking fish
   The fish is split open, and the gills and stomach contents are discarded; the contents of the fish are then cleaned and drained for 15 minutes. After that, the fish is placed on top of the para-para (smoking device) and smoked for 3-4 hours or until cooked.
3. Using chitosan to coat smoked fish
   The smoked fish is then coated with chitosan following the smoking procedure. Chitosan is used at concentrations of 0.5; 1; 1.5; and 2%. Fish were immersed in a chitosan solution for 5 minutes for each treatment. The fish is drained after immersion.
4. Production of media
   Potatoes are thoroughly peeled and cut into small pieces. 250 g of potatoes are boiled in 500 ml of aqua until tender, then filtered and poured into a cup. The extract is combined with 25 g granulated sugar and 14 g agar, and the mixture is heated until it dissolves and the mixture is homogeneous. The media is then sterilized and placed in an Erlenmeyer flask. The aseptically sterile medium is then poured into a petri dish.
5. Protection against smoked fish at any time
   Castor is isolated from smoked fish by series dilution and aerobic planting (distribution technique). Ten grams of mashed smoked fish is suspended in 90 milliliters of the sterile aqua vortex to homogeneous (dilution of 10-1). The suspension is taken at a concentration of 1 ml and suspended in 9 ml sterile aqua to dilute 10-2 and vortex to homogeneity. Each dilution suspension was spread on PDA media with 0.1 ml of inoculation, flattened, and incubated at room temperature (30°C) for seven days. Separate colonies in growth were relocated and then purified using the single colony method (Cappucino and Sherman, 1999).
6. Characterization of mold
   Observation of isolates of the mold is carried out macroscopically and microscopically. Macroscopic characteristics are determined using pigmentation and colony characteristics (colors of the colony's upper and back surfaces), colony edges, textures, and concentric circles. Vesicles, phialides, conidia, conidiophores, and hyphae are all microscopic characteristics. At any time, microscopic observations are made using a microscope with magnifications of 18x40 and 18x100. Identification is based on the publications Barnet and Hunter (1998), Gandjar et al. (1999), Samson et al. (1999), and Watanabe (2002).

RESULTS AND DISCUSSION

The aching that develops in smoked fish with chitosan coating is isolated and purified based on the colony's appearance. Purification findings obtained five isolates of mold: isolates KU1, KU2, KU3, MG2, and KS1. Isolates KU1, KU2, and KU3 were first isolated and classified from smoked fish by coating shrimp skin chitosan and are the 1st, 2nd, and 3rd isolates. Isolate MG2 was first isolated and described from smoked fish by cooking oil coating and is the 2nd isolate. Isolate KS1 was first isolated and characterized from smoked fish by coating the chitosan fish scales and is the 1st isolate. The isolate is then identified by making observations on macroscopic and microscopic characters. The observed macroscopic characters include the upper color of the colony, the color behind the colony, texture, edges, concentric circles, radial

lines, elevations, and colony shapes. While the microscopic characters observed include hyphae, conidiophore, phialides, and vesicle.

Isolate KU1

The macroscopic characteristics of isolate KU1 are the color of the upper white colony (Fig. 1a). In contrast, the color behind the yellow colony of brownish (Figure 1b) shows no concentric and radial lines, shaped like cotton, and the edges of filamentous colonies. Microscopic characteristics include interested hyphae, having conidia, chlamydospore, and phialides. Macroscopic and microscopic features of ku1 isolates after comparison with identification guidelines showed features such as *Fusarium oxysporum*.

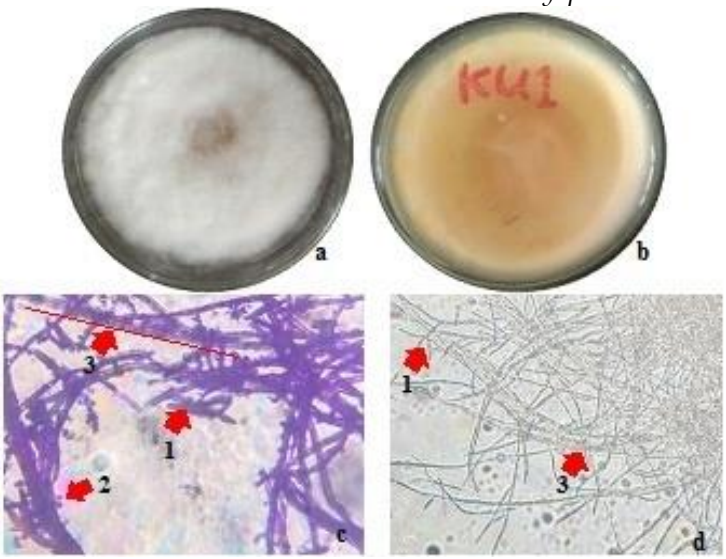

Figure 1. Morphology of isolate KU1. a) the upper surface of the colony; b) the surface behind the colony; c) microscopic with dye (magnification 720 times); d) microscopic without dyes (magnification 180 times) (magnification 180 times). 1) chlamydiospores; 2) konidia; 3) hyphae. Petri dish diameter = 6 cm; bar = 10 m.

Fusarium oxysporum has been described as having long macroconidia with a spiky tip (fusion), crescent-shaped, slender, and prickly septa, frequently found in pairs or groups, sealed, and colorless mycelium (Putri et al., 2014). Additionally, Sari et al. (2017) confirmed that the color of the *Fusarium* spp. white in color and colonial, such as cotton. Additionally, *F. oxysporum* has macroconidia in the form of a crescent moon, and macroconidia have multiple bulkheads. Microconidia are oval or kidney-shaped, with single or branched phialides. Klamidospora is a spherical, thick-walled fungus that forms at the end or middle of the hyphae.

Isolate KU2

Isolates KU2 have white and black colonies with a brownish-yellow bottom color (Figure 2b), flat colony development, thin fiber-shaped, cotton-like, and granular textures, filamented edges, and no concentric or radial lines. Colonies are white when they are young but darken to black as conidia form. Isolate KU2 has a round vesicle, smooth-walled yet unbranched conditions, and hyphae (Figure 2c), and around and prickly conidia shape. Macroscopic and microscopic properties of KU2 isolates, as compared to classification manuals, indicate that they are *Aspergillus niger* molds.

Samson et al. (1999) described *Aspergillus niger* colonies as having a thick and dark layer of conidiophore solid brown or black with a white or yellow basal patch. Additionally, Dewi (2018) reported that the isolate of *Aspergillus niger* has a black colony with a brownish-yellow

colony lower color, and no concentric or radial lines were observed. The colony had grown to fill the petri dish by the third day. When the colony is young, it is white and will eventually turn a dark brown color upon conidia development.

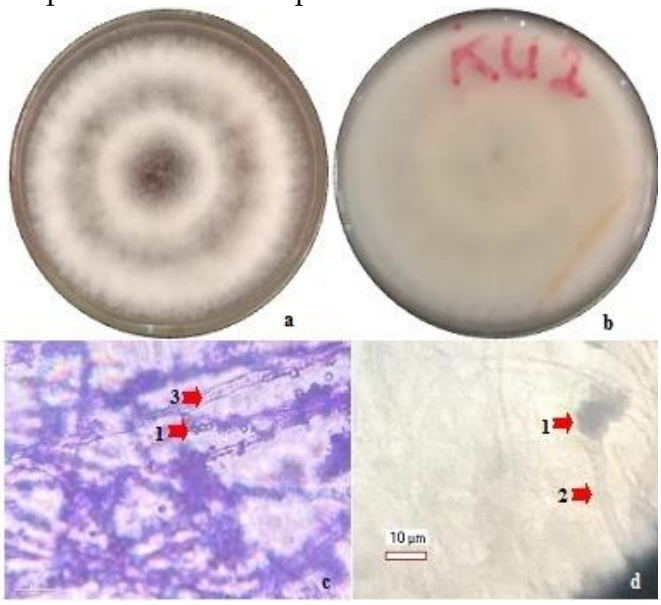

Figure 2. Isolates of KU2 with varying morphologies. a) the colony's upper surface; b) the colony's underside; c) microscopic with dye (magnification 720 times); d) microscopic without dyes (magnification 720 times). 1) konidia; 2) condidiosfor; and 3) hyphae. Petri dish diameter = 6 cm; bar = 10 m.

Isolate KU3

The macroscopic morphology of isolate KU3 colonies was initially white. However, it quickly changed to a bluish-green color with a robust and velvety texture (Figure 3a) and the color behind the yellow/beige colony (Figure 3b). Microscopic examination reveals the presence of conidiophores with fine, branched walls, metula, phialides, round conidia, have septa, and hyaline hyphae. ku3 isolates are identical to *Penicillium* sp. based on these macroscopic and microscopic characteristics.

According to Domsch and Gams (1980), *Penicillium* sp. produces colonies that are brownish-orange, bluish-green, yellowish-green, and yellow. The colony's surface is smooth, has a velvet-like texture, and sometimes resembles cotton, allowing for yellow or hyaline exudate removal. In the end, conidiospores from vesicles; the sum varies according to the type; has phialides, metula, have septa hyphae; the number of conidia is one; conidia are round or elliptical. Additionally, Singh and Mathur (1991) stated that the *Penicillium* sp. colony changes from white to greenish-gray, turquoise, olive-gray, and sometimes yellow or reddish, the color behind the pale yellow colony. Additionally, the colony is stringy, with a velvety, woolly, or cotton-like texture.

According to Akmalasari et al. (2013), the colony of *Penicillium* sp. has a bluish-green top surface color, which is the opposite of the yellowish-brown colony, yellow colony pigmentation, radial colony growth type, smooth colony surface texture, interested hyphae, phialides, metula, conidiophore, and conidia that are one cell in size and round. Singh and Mathur (1991) also recorded that *Penicillium* sp. has a conidiophore that grows upright from the mycelium and branches at its end, with a group of phialides, hyaline hyphae (transparent), round, and unicellular conidia at the end.

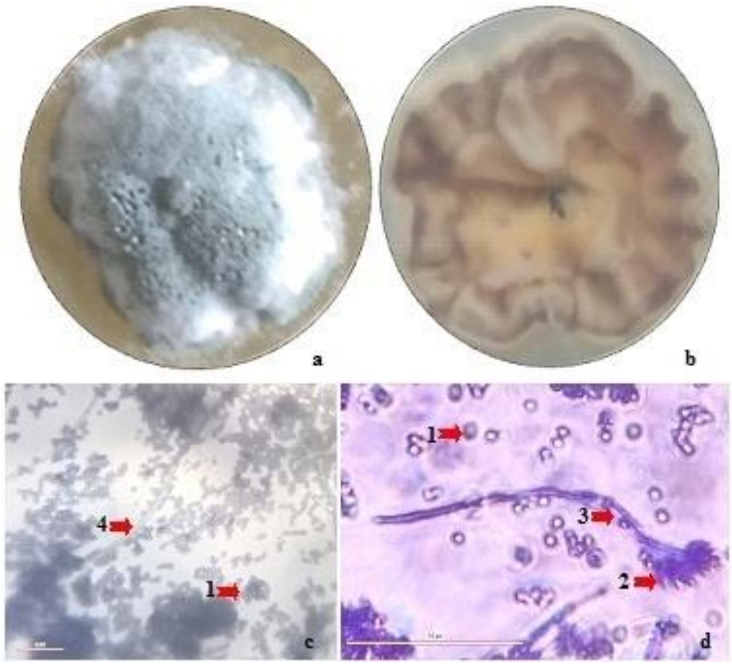

Figure 3. Illustrates the morphology of isolates KU3. a) the colony's upper surface; b) the colony's underside; c) microscopic without dyes (magnification 180 times); d) microscopic with dyes (magnification 720 times). a. conidia; b. phialid; c. Conidiophore; d. hyphae. Petri dish diameter = 6 cm; bar = 10 m.

Additionally, Simanjuntak et al. (2015) clarify that *Penicillium* has orange, bluish-green, and yellowish-green colonies with smooth or tapered colony edges. The colony has a rough, cotton-like texture that is dense and thin. The microscopic character is shaped like a bottle or a long cylinder with a pointed neck, cylindrical metula, greenish-colored conidia, round, semi-round, or ellipsoid shape smooth and hyaline condiment.

Isolate MG2

Macroscopic features of isolates MG2 are the color of the yellow to green colonies (Figure 4a), the color behind the beige colony (Figure 4b), the flat grain-shaped colony when the time is young, and there is a radial groove. Colonies are white at first but quickly turn bright yellow to green with age. Microscopic features include a radiating round conidia head, black conidia, long and colorless conidiophore. Isolate MG2 is covered with whole vesicles, and phialide, round and long vesicles, smooth surface, hyphae are not crossed. Based on macroscopic and microscopic characteristics, the MG2 isolate character (Figure 4) is similar to the *Aspergillus flavus*.

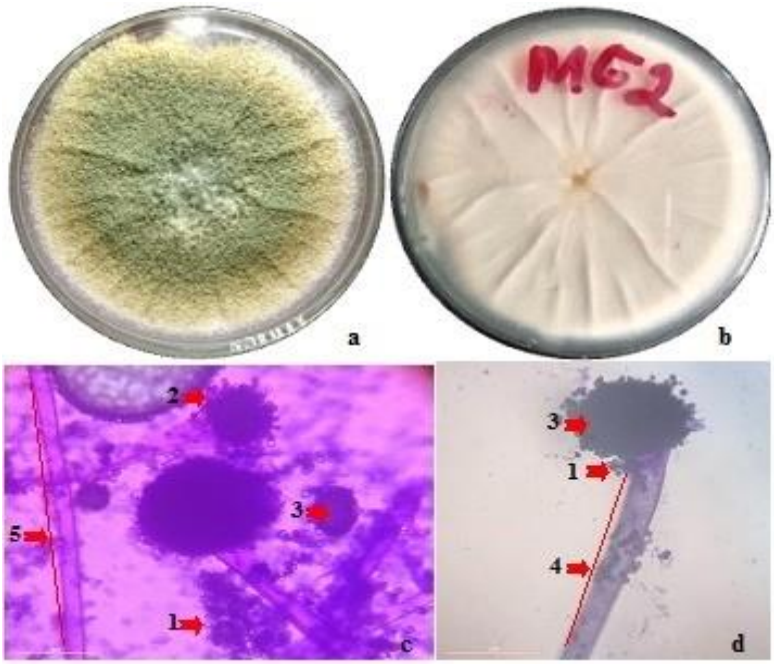

Figure 4. Morphology of isolates MG2. a) the upper surface of the colony; b) the surface behind the colony; c) microscopic without dyes (magnification 720 times); d) microscopic with dyes (magnification 720 times). 1) conidia; 2) phialide; 3) vesicles; 4) conidiophore; 5) hyphae. Diameter of petri dish = 6 cm; Bar = 10 µm.

The results of the study Sulistiani et al. (2017) showed that the colonies of *Aspergillus flavus* were initially yellow and turned greenish-yellow with age, forming coarse, thin fibers and flat colony growth. Conidia is colorless, rough, and the top is scattered, somewhat round to the column. The vesicles are rather round to rod-shaped on a small head. Dewi (2018) also reported that *A. flavus* has a yellowish-green colony color and forms a concentric line. Colonies are white when young and turn yellowish-green when forming conidia. Vesicles are round, hyphae-type is agreed, conidia is round, and has phialides.

Isolate KS1

Macroscopically, KS1 isolates feature cream-colored colonies, rough colony surfaces, and radial colony growth. Medium for microscopic observation of isolate KS1 with hyphae have septa, white conidiophore color, conidiospores has branches, generally coated chromophil, and has phialide. Based on macroscopic and microscopic characteristics, the isolate of KS1 is similar to the *Acremonium* sp. type. Macroscopic and microscopic features of isolate KS1 can be seen in Figure 5.

Acremonium sp. reported to have a white to brown colony color, the surface of the colony looks like cotton, branched conidiophores, generally coated in chromaffin, has phialides, single-celled conidia, appears somewhat clustered forming one head, conidia are elongated to round, hyphae is temperate and sometimes chlamydospore (Domsch and Gam, 1980; Gandjar et al., 1999; Akmalasari et al., 2013). It is also explained by Simanjuntak et al. (2015) that *Acremonium* sp. has a gray oval colony with choppy colony edges, solid colony texture, branched condiments, slightly bent phialide, ellipse-shaped and wavy conidia forming a slimy head.

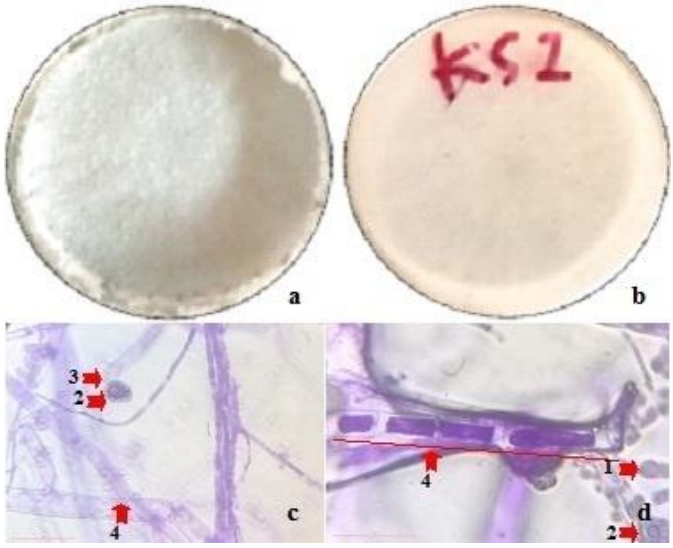

Figure 5. Morphology of isolate KS1. a) the upper surface of the colony; b) the surface behind the colony; c) and d) microscopic with dye (magnification 720 times). 1) conidia; 2) slimy head; 3) phialophores; 4) hyphae. Diameter of petri dish = 6 cm; Bar = 10 µm.

Syamsia (2016) also describes *Acremonium* sp. It has a white colony, a creamy or light brown lower surface, a rough colony surface texture, a cylindrical, rounded conidia shape, and a hyaline color. Also, *Acremonium* sp. has a smooth conidiophore surface and a hyaline color. Phialides are upright and slimy at each phialide peak, hyphae, and hyaline in color.

CONCLUSION

Based on the results of the study, it can be concluded that the results of mold isolation in smoked fish coated with chitosan found five isolates of mold, namely isolate KU1 (*Fusarium oxysporum*), Isolate KU2 (*Aspergillus niger*), Isolate KU3 (*Penicillium* sp.), Isolate MG2 (*Aspergillus flavus*), and Isolate KS1 (*Acremonium* sp.).

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