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Mold Screening on Dried *Rebon* Shrimp (*Acetes* sp.) from Jargaria Market, Dobo City, Indonesia

Martha L. Wattimena^{1*}, Esterlina E. E. Nanlohy¹, Maranatha C. Pattiwaellapia¹, Raja B. D. Sormin¹, Rahman²

¹Department of Fisheries Product Technology, Faculty of Fisheries and Marine Sciences, Pattimura University, Maluku, Indonesia

²Department of Marine Science, Faculty of Fisheries and Marine Science, Pattimura University, Maluku, Indonesia

*Corresponding author: marthaluanawattimena@gmail.com

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ABSTRACT

This research aimed to study the presence of mold from dried Rebon shrimp sold at the Jargaria market in Dobo City, Maluku. The method used in this research was the descriptive method. The obtained samples of dried Rebon shrimp (Acetes sp.) were collected from Jargaria Market, Dobo City. Four samples (A1, A2, A3, and A4) were taken from four different traders, and then TPC analysis, mold analysis, water content, and salt content were carried out. The results of the research showed that samples of dried Rebon shrimp from the Jargaria market in Dobo City contained mold microorganisms, including Aspergillus sp., Fusarium sp., Penicillium sp., and Mucor sp. The highest mold colony count was found in sample A1 (2.0 \times 10² CFU/g), and the highest bacterial colony count was also recorded in sample A1 (6.1 \times 10² CFU/g). Additionally, the highest moisture content was observed in sample A2 (19.05%), while the highest salt content was detected in sample A4 (33.31%). The presence of these microorganisms was determined through a structured analysis involving total plate count (TPC), mold identification, moisture content measurement, and salt content determination. These analytical approaches provided a comprehensive understanding of microbial contamination in dried Rebon shrimp, emphasizing the importance of proper processing, storage, and hygiene to minimize mold and bacterial contamination.

INTRODUCTION

Indexed in Scopus

Shrimp is a seafood product rich in protein, vitamins, and minerals, making it highly beneficial for human health, particularly in supporting child growth and development (**Rosmiyaty**, 2013). Among various shrimp species, *Rebon* shrimp (*Acetes* sp.) is a small crustacean commonly found in Southeast Asian waters. Despite its nutritional benefits, *Rebon* shrimp is highly perishable, requiring an immediate processing to prevent spoilage. One of the most common preservation methods is drying, which reduces moisture content and extends the product's shelf life.

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In Dobo City, dried *Rebon* shrimp is widely marketed at the Jargaria market, where it is typically stored in large, transparent plastic containers and sold openly. However, improper storage conditions can lead to microbial contamination, particularly from molds. The presence of molds in dried seafood products is a significant concern, as some fungal species produce mycotoxins—harmful secondary metabolites that pose health risks to consumers (**Smaoui** *et al.*, **2023**). Mold contamination can cause deterioration in food quality and can contribute to foodborne illnesses. Additionally, mycotoxins have been associated with allergic reactions, immune system suppression, and even carcinogenic effects (**Chelkowski**, **1991**).

The growth of mold in dried seafood products is influenced by environmental factors such as temperature, humidity, and hygiene conditions during processing and storage. If drying is not conducted properly or if the product is exposed to high moisture levels, fungal contamination can occur, leading to economic losses and potential health hazards. Research has shown that molds such as *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Mucor* sp. are commonly found in dried seafood, producing mycotoxins that may affect food safety (**Pitt & Hocking, 1985**).

Given the importance of dried *Rebon* shrimp as a local seafood product, ensuring its safety for consumption is crucial. This study aimed to assess the presence of mold contamination in dried *Rebon* shrimp sold at the Jargaria market in Dobo City, identify the fungal species present, and evaluate potential health risks. The findings of this research are expected to provide insights into the microbial safety of dried seafood and offer recommendations to improve storage and handling practices, ultimately reducing the risk of contamination and enhancing product quality.

MATERIALS AND METHODS

1. Materials

The raw material used is dried *Rebon* shrimp (*Acetes sp.*). Samples were collected from four different traders (A1, A2, A3, and A4) in Jargaria Market, with each trader providing approximately 250 grams of dried *Rebon* shrimp. The selection was based on visual characteristics such as color uniformity, dryness level, and the absence of visible mold contamination. All samples were placed in sterile plastic packaging and transported under controlled conditions. The ingredients used were sodium chloride (NaCl), plate count agar (PCA), alcohol, potato dextro agar (PDA), aquades, silver nitrate (AgNO₃), potassium dichromate (K₂Cr₂O₇), and hydrochloric acid (HCl).

The equipment used consists of analytical scales, micropipettes, petri dishes, autoclaves, Erlenmeyer, Bunsen lamps, test tubes, Ose needles, measuring cups, beakers, incubators, burettes, clamps, stirring rods, ovens, saucers, desiccators, tongs, and muffle furnaces.

The collected samples were properly packed in sterile plastic packaging to prevent contamination during transportation. They were then shipped from Jargaria Market, Dobo City, to Ambon, ensuring that the storage conditions minimized exposure to moisture and microbial contamination. Upon arrival in Ambon, the samples were taken to the Fisheries Product Technology Laboratory, Faculty of Fisheries and Marine Sciences, Pattimura University, where they were stored in a controlled environment before further analysis, including total plate count (TPC), mold identification, moisture content, and salt content determination.

2. Sample analysis

The microbiological and physicochemical analyses of dried *Rebon* shrimp (*Acetes* sp.) samples were conducted in the Fisheries Product Technology Laboratory, Faculty of Fisheries and Marine Sciences, Pattimura University, Ambon. Several analytical methods were applied to assess microbial contamination, moisture content, and salt concentration in each sample.

The total plate count (TPC) was performed to determine the bacterial load in the samples. The analysis was conducted using plate count agar (PCA) as the growth medium, with incubation at 37°C for 48 hours. The bacterial colonies were counted manually and expressed as colony-forming units per gram (CFU/g) following standard microbiological procedures.

Mold contamination was analyzed using potato dextrose agar (PDA). The samples were incubated at 25°C for 5–7 days, allowing fungal growth to be observed. The mold colonies were identified macroscopically based on their color, texture, and growth patterns, followed by microscopic examination using Lactophenol Cotton Blue staining to determine the presence of *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Mucor* sp.

The moisture content of the dried *Rebon* shrimp was determined using the oven drying method, where samples were dried at 105°C for 24 hours until a constant weight was achieved. The moisture content was then calculated as a percentage of the original weight.

The salt content was analyzed using the Mohr titration method, which involved dissolving the shrimp samples in distilled water, adding silver nitrate (AgNO₃) as a titrant, and using potassium chromate ($K_2Cr_2O_7$) as an indicator to determine chloride concentration. The salt content was calculated based on the chloride ion concentration present in the sample solution.

These analytical methods provided a comprehensive understanding of microbial contamination and physicochemical properties in dried *Rebon* shrimp, which are crucial for ensuring food safety and maintaining product quality.

RESULTS AND DISCUSSION

1. Total plate count (TPC)

Based on the results of the TPC analysis of *Rebon* shrimp in the four samples with dilutions of 10^1 to 10^3 each, sample A1 had the highest number of colonies at 6.1 x 10^{2} .

while sample A4 was the lowest number of colonies at 1.9×10^2 . The results showed that *Rebon* from the 4 locations had the quality that met SNI 7388:2009 concerning the Maximum Limit of Microbial Contamination in Foodstuffs, namely TPC, a maximum of 1×10^5 CFU/g (Table 1).

No.	Samples Code	TPC (CFU/g)	
1.	A1	6.1 x 10 ²	
2.	A2	2.3×10^2	
3.	A3	$2.9 \ge 10^2$	
4.	A4	$1.9 \ge 10^2$	

Table 1. Total plate count (TPC) analysis results

Microbial contamination in food can be caused by the initial number of microbes affecting the number of subsequent microbes, increasing the amount of microbial contamination in fishery products (**Sukmawati, 2017**). The length of storage also influences it before being marketed or exposed to a too long marketing time.

The principle of preservation with the technique of using salt and drying aims to reduce water content to suppress or minimize the number of microbes (Marpaung, 2015). The results of TPC tests on four samples of dried *Rebon* shrimp at the Jargaria Market in Dobo City show a relationship between salt levels and TPC in dried *Rebon* shrimp where there is a growth of bacterial colonies, which shows that the bacteria that grow are halophilic. It is thought to be because the water content is high enough so halophilic bacteria can multiply adequately. According to Pelczar and Chan (1988), halophilic is one of the microorganisms that can grow at high salt levels. This result is supported by Madigan *et al.* (2000), who suggested that halophilic bacteria can be easily found in high-salt environments.

According to **Fardiaz** (**1992**), halophilic bacteria include *Halobacterium*, *Sarcina*, *Micrococcus*, *Pseudomonas*, *Vibrio*, *Pediococcus*, and *Alcaligenes*. The grouping of halophilic bacteria is divided into three groups, namely medium halophilic bacteria, with salt concentrations needed for optimum growth are 5-20%, 20-30% for extreme halophilic bacteria, and mild halophilic bacteria, namely halophilic bacteria that grow at salt concentrations of 2-5%.

These dried *Rebon* shrimp samples are stored in large clear plastic, and traders divide them into small crackle plastic bags for sale. A place to sell on the side of the road located inside the market. Another factor is microbial contamination caused by low sanitation and hygiene levels in the processing process and marketing sites (**Kadi** *et al.*, **2012**).

Processing also affects microbial contamination, and washing before drying uses unhygienic water or is polluted by microbes (**Indrawati & Fakhrudin, 2016**). The processing process of *Rebon* shrimp is washed with seawater to remove dirt after catching it. Arriving at the processing site, the shrimp are again washed with healthy (brackish) water to remove the remnants of existing soil. Microbial contamination in fishery products can also come from various sources, such as dust during the processing process, the respiratory tract of humans and animals, and storage areas (Ashok, 2008). An exemplary storage place is a storage area that is dry, cool, and has good ventilation. The dried *Rebon* shrimp were kept in a closed package so as not to be infested by flies, where flies are one of the sources of microbial contamination (Chouliara, 2004).

2. Mold analysis

Damage to processed products can be caused by physical, chemical, mechanical, and microbiological damage. Microbiological damage is very detrimental to fishery products and can cause disease to human health because it can produce toxins, and one of the causes is microorganisms, namely mold. The results of mold analysis can be seen in Table (2).

Table 2. Mold analysis results
 Samples Code Mold (CFU/g) No. [2.0] x 10² 1 A1 [2.5] x 10¹ 2 A2 [3.5] x 10¹ 3 A3 4 A4 [9.8] x 10¹

From the data obtained, the highest total mold colony was in sample A1 at 2.0 x 10^2 , and the lowest was in sample A2 at 2.5 x 10^1 . The analysis showed that all four samples were contaminated with mold but still within very reasonable limits. It is suspected that mold contamination has occurred since the processing place and the environment of traditional markets and selling sites beside the road where vehicles and pedestrians pass, and sellers generally sell directly on the floor (not using tables).

An unhygienic market environment is one of the most significant sources of contamination. According to **Rahayu** *et al.* (2014), mold spore contamination generally comes from the background around processing and containers/equipment used in processing. In addition, mold grows optimally in a warm environment (25-30°C). The growth of microorganisms such as mold can be prevented by applying good sanitation and hygiene from the processing stage to marketing or at the storage stage.

High relative humidity for the tropics and warm temperatures contribute to high levels of fungal contamination in food. Fungi have an optimal growth temperature between 20 - 35°C, and the group of psychrophilic fungi has a minimum growth between 0-7°C, namely for *Fusarium, Clasdosporium, Penicillium,* and *Thamnidium* species (**Pitt & Hocking, 1985**).

3. Mold identification

Mold isolates incubated for seven days after being macroscopically identified produced several mold species from four samples, including *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Mucor* sp.

Fusarium sp. species were found in samples A1 and A3 with macroscopic characteristics that had circular colonies and spread in all directions. At the beginning of growth in the PDA medium, the colony is white like cotton, then changes to slightly yellowish white or cream, as shown in Fig. (1).



Fig. 1. Mold Fusarium sp.

Fusarium can grow at a 22-24% moisture content and soil temperature between 10-24°C, although this also depends on the fungal isolate (**Soesanto, 2002**). The fungus is suitable for growing on acidic soils with a pH range of 4.5-6.0 (**Sastrahidayat, 1989**). Some types of fungi can cause disease and even death in organisms, such as *Fusarium* sp. and *Phycomycetes* sp. fungi that can kill shrimp for 24 hours (**Hastuti, 2013**). *Fusarium* sp. is a type of fungus that often infests shrimp gills. *Fusarium* sp. causes black gill disease. *Fusarium* sp. can produce toxins in the form of fumonisin B1 (FB1), which is destructive to feedstuffs.

Fumonisin contamination in animals and humans has not received attention in Indonesia, but its existence needs to be watched out for, considering that these mycotoxins are found together with aflatoxin, which can increase the toxicity of the two mycotoxins (Maryam, 2000).

Mucor colonies initially appear white will turn gray to brown as they age. The fungus produces growth spores, and features hyphae that are not septate, erect, and often enlarge, occasionally forming branches. According to **Gandjar** (1999), several species of the genus *Mucor* can sporulate at temperatures of 5°C to 20-30°C. The research results show that the genus *Mucor* sporulates rapidly when grown on PDA media at room temperature. These *Mucor* colonies were present in each sample with little growth but were found more in sample A2 (Fig. 2).



Fig. 2. Mold Mucor sp.

According to **Purwati and Hamidah** (2018), the genus *Mucor* can act as a decomposer that helps fertilize the soil. The genus *Mucor* can also produce proteases, which are enzymes that play a role in the nitrogen cycle in the soil (Saraswati, 2007). *Rebon* shrimps are caught in muddy water, allowing the existence of *Mucor*-type molds that live in the habitat preferred by *Rebon* shrimp, which consists of mud and sand.

Penicillium mold colonies were present in sample A4 (Fig. 3). According to **Fardiaz (1992)**, molds can grow at low water content because molds are microorganisms that require water content for growth. The water content in food affects the resistance of these foods to microbial growth. Microbial growth never occurs in the absence of water. According to **Benwart (1989)**, *Penicillium* sp. can grow at a moisture content greater than 22%. According to **Beuchat (1978)**, the optimum value for the growth of *Penicillium* sp. is 0.22, temperature at 25-35°C, pH 6-7, moisture content at 12-16%, and salt content at 30%.

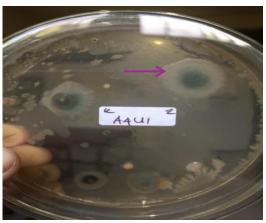


Fig. 3. Mold Penicillium sp.

Based on identification by **Anggraeni and Usman (2015)**, *Penicillium* sp. at first, is white. Then it changes to greenish-blue, greenish-gray, or olive-gray. These characteristics can be seen in the identification results above. The *Aspergillus* has septal

hyphae and hyalines (Fig. 4). It is supported by the research of **Noerfitryani and Hamzah (2018)**, who reported that the macroscopic characteristics of *Aspergillus* fungi on PDA media are that the surface is bright green to dark green and black and has a flour-like texture. According to **Mahfoeld (1993)**, *Aspergillus* can grow at more than 18% moisture content.



Fig. 4. Types of molds Aspergillus sp.

Aspergillus sp. has insulated and branched hyphae, at the end of the hyphae, especially in the erect enlarged part of the conidiophore. The conidiophore at the end is rounded into a phenolic. The vesicle has short rods called sterigmata or filadia, usually colored or colorless. In sterigmata, conidia grow and form chains that are green, brown, or black (Fardiaz, 1992). One of the fungi that pollute food is Aspergillus sp. mushrooms. Aspergillus sp. is one of the fungi that produce aflatoxin, a toxin that can kill humans because it can cause liver cancer if it enters the body through food (Djarir, 1993).

Aspergillus sp. fungi can cause aspergillosis disease, especially Aspergillus flavus and Aspergillus furnigatus, which can cause granulomatosis inflammation of the bronchus, ears, mucous membranes of the eyes, sometimes on the skin and subcutaneously in the bones, lungs, and meninges. Food sanitation needs to be considered, especially environmental factors, including avoiding dust and pollution so that food remains clean because Aspergillus sp. contaminates food through the air.

Based on the physiological properties that support the growth of molds in this study, among others, the incubation temperature is the optimum temperature for the growth of molds such as *Aspergillus* sp. and *Penicillium* sp., which grow in the temperature range of 35-37°C, allowing molds to grow because the optimal incubation temperature for growth is 37°C. Humidity is one of the microbial growth factors, the higher the temperature, the lower the humidity, the lower the temperature, the higher the humidity. In addition, the high-water content from this study is a mold growth factor. The water content obtained in this study was 19.05%. Mushrooms can grow in various places, from simple to complex content. Most fungi produce hydrolytic enzymes, e.g., amylase,

pectinase, proteinase, and lipase; therefore, they can increase foods containing starch, proteins, and lipids.

PDA media is a good medium suitable for mold growth because it contains nutrients needed for mold growth. PDA media is made from potato extract with carbohydrate sources (starch). PDA media also has a low pH of 4.5 - 5.6, which is preferred by molds, so the four types of molds produced in this study can grow well (**Cappucino & Sherman, 2014**). There are also inhibitory components produced by *Aspergillus* and *Penicillium* that can eradicate other organisms so that their growth runs well. This component is called an antibiotic that is mycostatic, an inhibitor of fungal growth or fungicide, which kills fungi.

4. Water content

Based on the analysis above, the highest water content of dried *Rebon* shrimp was found in sample A2 at 19.05%, while the lowest was found in sample A4 at 12.35% (Fig. 5).

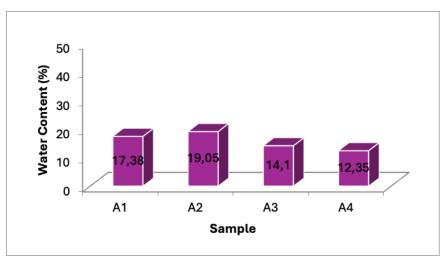


Fig. 5. Histogram of water content

From the results of this study, it is much lower than the water content of dried *Rebon* shrimp based on **TKPI** (2019), which is 21.6%, so it can be said that the dried *Rebon* shrimp from this study has good and decent quality.

The water content in food determines the durability, freshness, and shelf life of the foodstuff. The average fishery product has a very high-water content, between 60-80%; high water content in food can cause bacteria, mold, and yeast to multiply quickly because the water content is a suitable medium for microbial growth so that spoilage becomes faster and reduces the quality of food (**Siahaya**, **2020**).

Water content determines the freshness and durability of food, and very high-water content will result in easy entry of bacteria, yeasts, and molds to multiply so that changes in nutrition can accelerate spoilage (**Pratama, 2014**). Water content is affected by salt content during the processing process. The higher the salt content, the lower the water

content in the material because salt has hygroscopic properties that allow it to absorb water.

The moisture content in food is closely related to the product's durability. Water reduction, either in drying or adding other materials, aims to preserve food ingredients to withstand chemical and microbiological damage.

Water activity is an essential factor that affects the stability of dry food during storage (Gita & Danuji, 2018). The water content in a food ingredient dramatically affects the quality and shelf life of the feed ingredients. The moisture content of feed ingredients that do not meet the requirements will experience physical and chemical changes characterized by the growth of microorganisms in the feed, so the spread is unsuitable for consumption. Fitriani (2008) stated that the ability of materials to release water from their surface would be more significant with the increasing temperature of the drying air used and the longer drying process so that the resulting water content is lower.

Oviantari and Purwata (2007) stated that uneven drying and fluctuating temperature changes affect water content. The longer the drying time, the lower the moisture content contained in a material.

At the beginning of drying, the moisture content and drying rate are high, then the moisture content drops rapidly, slopes, and is very slow when heading toward the equilibrium water content. The drying time increases as the drying temperature decreases.

Makapedua *et al.* (2014) stated that the longer the drying time, the lower the moisture content value. The water content in foodstuffs affects the resistance of food to microbial attacks expressed Aw, which is the amount of free water that microorganisms can use for growth.

Various microorganisms have a minimum Aw to grow correctly; for example, bacteria Aw: 0.90; yeast Aw: 0.80-0.90; mold Aw: 0.60-0.70. To extend the durability of a material, some of the water in the material must be removed in several ways depending on the type of material. Generally, drying is done either by drying or with an artificial dryer (**Winarno, 1992**). Water content is also a significant characteristic of feed ingredients because water can affect the appearance and texture of the feed ingredients and determine their freshness and durability. Water content causes bacteria, mold, and yeast to multiply, changing feed ingredients. Dry materials are cooled before weighing; the material is placed in a dry, closed room, e.g., in an excitatory or desiccator. Determining the moisture content of a food depends on the nature of the food itself; from materials tested for water content, materials with low water content have better shelf life (**Andarwulan, 2011**).

5. Salt content

The varying salt content is caused by the erratic salting process and the use of salt by fishermen during the processing of dried *Rebon* shrimp (Fig. 6). The amount of salt contained in dried *Rebon* shrimp influences the water content and number of bacteria.

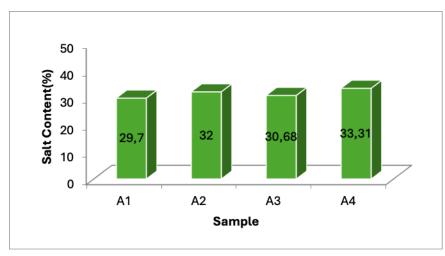


Fig. 6. Histogram of salt content

In theory, high salt content is associated with low microbial activity because the higher the salt content, the lower the Aw in the material. The high salt content in the sample causes the selection of bacteria so that only halophilic bacteria can grow on dried *Rebon* shrimp.

According to **Wijnker** *et al.* (2006), salt is a chemical component that is bacteriostatic and bactericidal. In addition, salt has hygroscopic properties, so it can absorb water contained in materials and bacterial cells, disrupting bacterial metabolism due to lack of fluid, which causes bacteria to die.

CONCLUSION

The analysis of dried *Rebon* shrimp from Jargaria Market in Dobo City confirmed the presence of mold microorganisms, including *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Mucor* sp. The study also revealed variations in microbial contamination, moisture content, and salt concentration among the samples. The findings highlight the importance of proper processing, storage, and hygiene practices to minimize mold and bacterial contamination. Ensuring better sanitation during processing and distribution can help maintain product quality and reduce potential health risks associated with mycotoxin contamination.

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