

Relationship Long Time Storage of Trunk Fish (*Euthynnus Affinis*) Smoke With The Presence Of *Staphylococcus Aureus* Bacteria At Bobotsari Traditional Market

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ABSTRACT

Fresh fish can experience spoilage due to enzyme activity and microorganisms contained in the body of the fish itself due to the oxidation process in body fat by air. Preservation method is done to reduce the decay. Preservation alternatives can be done, among others, by salting, cooling, drying, and smoking. Excess fumigation contains phenol which can function as an anti-microbial so that microbes will die and not develop. The purpose of this study was to determine the relationship between long storage of smoked tuna (*Euthynnus affinis*) and the presence of *Staphylococcus aureus* bacteria in Bobotsari traditional markets. The samples in this study were 12 samples of smoked cob fish which were sold in Bobotsari traditional markets. The sampling technique used is random sampling. This type of research was experimentally analyzed using the Kruskal Wallis test. The results of isolation of 12 samples of smoked tuna obtained positive results (dilution 10-1, 10-2 and 10-3). The results of gram staining on 12 samples of smoked tuna were round or oval in shape. From the results obtained, it can be concluded that there is a relationship between the length of storage of smoked tuna (*Euthynnus affinis*) and the presence of *Staphylococcus aureus* bacteria in Bobotsari traditional markets.

INTRODUCTION

Mackarel tuna (*Euthynnus affinis*) is a type of small tuna fish characterized by an elongated body without scales and a hard dorsal fin texture. Fish belonging to the family *Scombridae* genus *Euthynnus*. It has a fairly large body size, gray skin and thick, dark red flesh. This fish has nutritional content including 69.40% water, 1.50% fat, 25.00% protein, 0.03% carbohydrates (Basu, 2013)

Fresh fish can rot due to the activity of enzymes and microorganisms contained in the fish's body due to the oxidation process of body fat by air. The fish's body contains quite a lot of water, namely 60-80% and has a pH of 7.2 so it can be a good medium for the growth of putrefactive bacteria. Factors that can accelerate spoilage in fish include the composition of chemical compounds, the quality of very high protein content and fat (Ndahawali, 2016)

The preservation method is used to reduce decay. Alternative preservation methods include salting, cooling, drying and smoking. The advantages of fumigation are due to the

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fumigation process going through several stages, namely salting, heating, drying, and chemical reactions resulting in the most commonly used preservation method being fumigation. Fumigation contains phenol which can function as an antimicrobial so that microbes will die and not grow.

Wahyudi's research (2021), said that there are bacteria *Staphylococcus aureus* on smoked fish sold at the traditional market in Kedungadem District. Jeujan's research (2022), stated that the smoked tuna fish that was marketed did not find any type of bacteria *Staphylococcus aureus* so it can be stated that the smoked fish has met the quality standards in accordance with the microbiological quality requirements issued in the Indonesian National Standard (SNI) 2725:2013 that the quality and safety requirements for smoked fish with hot smoking are namely *Salmonella* negative sp and *Staphylococcus aureus* max 1.0×10^3 cfu/gram.

METHOD

This research uses an experimental method with the research design used is *quasi experimental*. This research was conducted at the Integrated Microbiology Laboratory, Muhammadiyah University, Purwokerto. This research variable consists of two independent and dependent variables. The independent variable in this research is the storage time for tuna (*Euthynnus affinis*) smoke and the dependent variable is the number of bacteria *Staphylococcus aureus*.

The population in this study was tuna fish (*Euthynnus affinis*) smoke sold at the Bobotsari Purbalingga traditional market. The samples used were 12 smoked mackerel fish taken using the technique *total sampling*. The tools used in this research are *autoclave*, petri dishes, erlenmeyer tubes, bunsens, matches, test tubes, micropipettes, *hot plate*, newspapers, blue tips, tube racks, gloves, masks, incubators, stir sticks, analytical scales, measuring pipettes, dropper pipettes, *filter pump*, measuring cup, tube clamp, spatula, durham tube, tweezers, vortex, microscope, *object glass*, *cover glass*. The data analysis used in this research data normality test in table 4.5. It is known that the data is not normally distributed at $p < 0.05$, so a data transformation test is carried out. After the data transformation test is carried out, the data is still not normally distributed so the Kruskal Wallis and Fisher non-parametric test is carried out.

RESEARCH RESULTS AND DISCUSSION

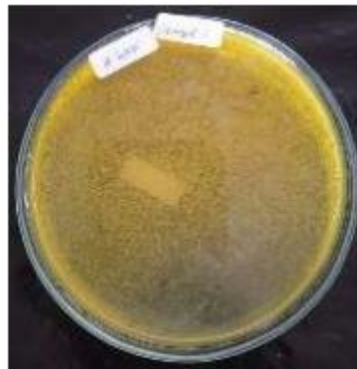
Bacterial isolation process *Staphylococcus aureus* starting by taking samples of smoked mackerel fish sold in the market. Next, fish samples are prepared and planted on appropriate selective media, such as Mannitol Salt Agar (MSA), which contains salt and mannitol as a selector for bacteria. After incubation, colonies that grow on MSA media will be examined to ensure the presence of typical bacterial morphology, such as Colonies are yellow, resistant to salt, and have a characteristic round or ovoid shape. Next, the colony will be isolated and purified to obtain pure bacteria. The next process is a biochemical test, which involves a series of tests to identify the metabolic characteristics of bacteria, such as catalase test, coagulase test, to accurately confirm the presence and species of bacteria. *Staphylococcus aureus* on samples of smoked tuna.

Tabel 4.1 Hasil Pertumbuhan Pada MSA

Sampel (kode)	Sampel	2 Hari	4 Hari	6 Hari	Deskripsi koloni
A	Ikan tongkol A	+	+	+	Berbentuk bulat, diameter 1-2 mm, warna kuning atau kuning keemasan
B	Ikan tongkol B	+	+	+	Berbentuk bulat, diameter 1-2 mm, warna kuning atau kuning keemasan
C	Ikan tongkol C	+	+	+	Berbentuk bulat, diameter 1-2 mm, warna kuning atau kuning keemasan
D	Ikan tongkol D	+	+	+	Berbentuk bulat, diameter 1-2 mm, warna kuning atau kuning keemasan
E	Ikan tongkol E	+	+	+	Berbentuk bulat, diameter 1-2 mm, warna kuning atau kuning keemasan
F	Ikan tongkol F	+	+	+	Berbentuk bulat, diameter 1-2 mm, warna kuning atau kuning keemasan

Keterangan: + ada pertumbuhan bakteri, - tidak ada pertumbuhan bakteri

Based on table 4.1. It is known that the results of bacterial isolation on Mannitol Salt Agar media showed positive bacterial growth leading to colonies *Staphylococcus aureus* because it has the ability to decompose (glycolize) mannitol. If grown on MSA media, these bacteria will produce acid due to mannitol fermentation, and change the color of the red media to yellow.

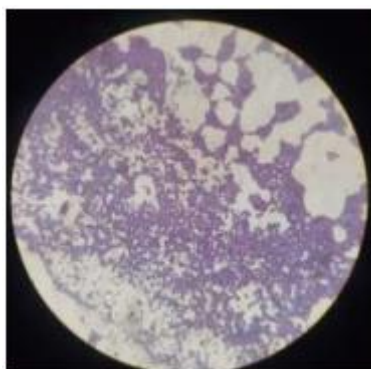


Gambar 4.1
Pertumbuhan koloni bakteri pada medium MSA

In table 4.1, the results of bacterial growth in the media followed by pure culture in the MSA medium tilt tube show bacterial growth and the media changes color to yellow, this indicates that the bacteria growing are probably *Staphylococcus aureus*, these results only provide a preliminary indication, and for confirmation, gram staining and biochemical testing are required.

Tabel 4.2 Hasil Pewarnaan Gram

Sampel (kode)	Hasil Pewarnaan Gram	Deskripsi Sel Bakteri
A	+	Sel berbentuk bulat, berkelompok tidak teratur seperti buah anggur
B	+	Sel berbentuk bulat, berkelompok tidak teratur seperti buah anggur
C	+	Sel berbentuk bulat, berkelompok tidak teratur seperti buah anggur
D	+	Sel berbentuk bulat, berkelompok tidak teratur seperti buah anggur
E	+	Sel berbentuk bulat, berkelompok tidak teratur seperti buah anggur
F	+	Sel berbentuk bulat, berkelompok tidak teratur seperti buah anggur
G	+	Sel berbentuk bulat, berkelompok tidak teratur seperti buah anggur
H	+	Sel berbentuk bulat, berkelompok tidak teratur seperti buah anggur
I	+	Sel berbentuk bulat, berkelompok

Gambar 4.3
Hasil Pewarnaan Gram

Microscopic examination was carried out using the Gram staining technique to determine the character and morphology of the cells. The results of gram staining show that the morphology of the bacteria is roundclustered like the arrangement of grapes, the colonies are gray to dark yellow, which indicates that the bacteria are gram positive (figure 4.3).

Catalase and coagulase tests are laboratory tests used to identify bacteria *Staphylococcus aureus* specifically. Positive results on both tests indicate the presence of bacteria *Staphylococcus aureus* in smoked tuna samples.

Based on the data normality test, it was found that the data was not normally distributed at $p < 0.05$, so a data transformation test was carried out. After the data transformation test was carried out, the data was still not normally distributed so a Kruskal Wallis non-parametric test was carried out.

Tabel 4.5 Hasil Analisis Uji Kruskal Wallis

Lama Penyimpanan	Jumlah Sampel	Hasil	Rata-rata (Min-Max)	P value
2 HARI	12	+	1203.83 (761-1801)	0,000
4 HARI	12	+	31532.25 (28338-35885)	
6 HARI	12	+	471991.00 (211420-977864)	

Based on Table 4.5, the average growth of bacteria is known *Staphylococcus aureus* storage for 2 days 1203.83cfu/ml, at 4 days storage it was 31532.25 cfu/ml and at 6 days storage it was 471991.00 cfu/ml. There are significant differences between growth *Staphylococcus aureus* at 2 days, 4 days and 6 days storage $P=0.000$ ($P<0.05$)

Tabel 4.6 Hasil Analisis Uji Fisher

lama penyimpanan * HASIL Crosstabulation

			HASIL	
			POSITIF	Total
lama penyimpanan	2 hari	Count	12	12
		% within lama penyimpanan	100.0%	100.0%
	4 hari	Count	12	12
		% within lama penyimpanan	100.0%	100.0%
	6 hari	Count	12	12
		% within lama penyimpanan	100.0%	100.0%
Total		Count	36	36
		% within lama penyimpanan	100.0%	100.0%

Based on Table 4.6, it is known that the results for 2 days of storage are: 12 samples, at 4 days storage there were 12 samples and at 6 days storage there were 12 samples. If there are positive results, it can be concluded that there are relationship between storage time for tuna (*Euthynnus affinis*) smoke with the presence of bacteria *Staphylococcus aureus* in storage for 2 days, 4 days and 6 days

CONCLUSION

1. There are bacteria *Staphylococcus aureus* in mackerel (*Euthynnus affinis*) Smoke is stored for 2, 4.6 days.
2. There are differences in bacterial growth *Staphylococcus aureus* in mackerel (*Euthynnus affinis*) smoke stored for 2 days 1203.83 cfu/ml, 4 days 31532.25 cfu/ml and 6 days 471991.00 cfu/ml.
3. There is a relationship between the storage time of tuna fish (*Euthynnus affinis*) smoke with the presence of bacteria *Staphylococcus aureus*.

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