APPLICATION OF LACTIC ACID IN PRESERVATION OF *Euthynnus lineatus* FISH

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Abstract: This research aims to study the effectiveness of lactic acid solution for inhibiting the growth of bacteria on fish and chicken meat samples. This work used an experimental method with observations on the sample's physical characteristics and identifying the presence of bacteria on tested samples. Three different preservation techniques were conducted with one negative control. The first technique is treatment with lactic acid, second is boiling and third is combination of boiling with addition of lactic acid. The result showed that the combination of boiling and lactic acid had given the best result in preserving the fish sample at room temperature until day 4. The fish samples did not show any sign of spoil and presence of bacteria. Thus, conclude that the combination of boiling technique and lactic acid can really reduce the number of bacteria and extend the life span of the samples.

Keywords: *Euthynnus lineatus, Lactic acid, Boiling and Gram staining*

INTRODUCTION

One of the most common bacteria associated with spoiled raw fish is *Pseudomonas spp*. *Pseudomonas spp.* bacteria are Gram-negative, rod-shaped bacteria that are widely distributed in the environment, including in water and soil [1]. They are known for their ability to grow at low temperatures, which makes them particularly relevant in the context of spoiled refrigerated or raw seafood [2]. To kill or reduce *Pseudomonas spp.* bacteria in raw fish, it's important to follow proper food handling and cooking practices. Cooking is the most effective method for eliminating harmful bacteria like *Pseudomonas* [3]. Even a number of prevention steps can be applied to reduce the risk of bacterial contamination, however no method can guarantee complete elimination of all bacteria [4].

Biopreservation, understood as a biological method for preserving foods with the use of microorganisms and their metabolites, has gained significant interest in recent years due to the increased awareness of consumers regarding chemical preservatives and their negative impact on health [5]. Lactic acid is commonly used in the preservation of meat and fish due to its antimicrobial properties. It is a natural organic acid produced by certain bacteria, and it plays a key role in the fermentation process of many food products [6]. When used in the preservation of meat and fish, lactic acid helps inhibit the growth of harmful bacteria and prolongs the shelf life of the products [5].

Lactic acid is typically used in combination with other preservation techniques such as refrigeration, packaging, and heat treatments. These methods work synergistically to ensure the safety and quality of preserved meat and fish products [7]. However, study on applying the lactic acid with heat on preservation of *Euthynnus lineatus* fish from bacteria making it last longer has not been established clearly. Therefore, the objectives of the study are to determine the most practical way in application of lactic acid as a preserving ingredient and identify any presence of bacteria.

METHODOLOGY

Sample preparation

Fish and chicken meat were bought from the fresh market at Tapah, Perak. Both samples were washed and cut into small pieces in cubic shape with 8 g each.

Preservation test

Three different preservation techniques were conducted with one negative control. The first technique is treatment with lactic acid, second is boiling and third is combination of boiling and addition of lactic acid. In lactic acid only treatment, samples were placed in 10 ml of lactic acid for 2 minutes. In the second preservation technique, samples were placed in boiling water for 10 minutes. While in the third technique, samples will first boil for 10 minutes and then placed in the lactic acid for 2 minutes. For negative control, the samples were not treated with any procedure. All samples from all four techniques were then left for four days inside a plastic bag at room temperature. The physical changes of the sample were observed and documented on each day.

Screening of bacteria

On day 4, screening on the presence of bacteria was conducted. A piece of treated sample was taken out and 2 ml of distilled water were placed in the middle of the slide. Next, the piece was mixed with water and slid through the flame at the Bunsen burner until the samples dried. Then, the slide was flooded with crystal violet for 60

seconds and washed with distilled water. Then, Lugol's iodine was flooded at the slide for 60 seconds and then rinsed with distilled water. The completely stained sample was then examined using a light compound microscope.

RESULTS AND DISCUSSION

For food spoilage observation, the raw fish preserved with lactic acid lasts longer than the one with no lactic acid. It takes 3 days for raw fish to be spoiled while 4 days for the raw fish preserved with lactic acid. It is reported that *Pseudomonas* spp. was the most important spoilage microorganisms at low storage temperatures [8], which may lead to the decomposition of nitrogenous substances and the production of ammonia, trimethylamine and hydrogen sulfide in salmon under cold chain logistics [9]. This may lead to an increase of enzymatic activity, denaturation of the muscle proteins and structural damage of membranes, which can result in increased weight loss, reduction of water-holding capacity, textural changes, and off-odor [2].

On studying the physical changes of the sample, observed that lactic acid on the raw fish caused changes in color (darker) and a little shrink. The acid helps to break down the muscle fibers and can result in a more tender and juicier final product. While boiled preserved fish with lactic acid, the heat of boiling had increased the preservation of fish. There is no sign of contamination within the testing period. No unusual odor, off color mold growth or abnormal texture observed on sample in combination of boiling technique and lactic acid sample. This explained that the application of heat and lactic acid together works for fish preservation. Lactic acid, when applied, creates an acidic environment that inhibits the growth of pathogenic microorganisms [5]. With heat treatment on the sample this then kills or inactivates pathogenic bacteria, parasites, and viruses that might be left on the surface or within the flesh of the fish [10]. This study finding agrees [7] that a combination of approaches is rationalized by the belief that synergism may occur by exposing undesired microorganisms to a series of obstacles to their growth and survival. Furthermore, if synergism occurs, preservative doses or technological treatment intensities may be reduced.

Table 1. Physical characteristics changes on fish sample from Day 1 to 4

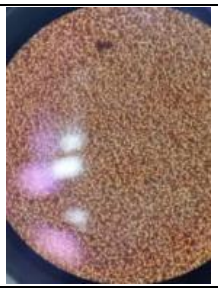
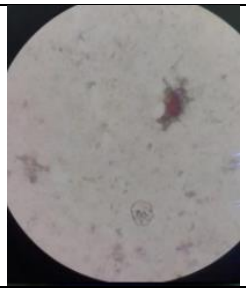
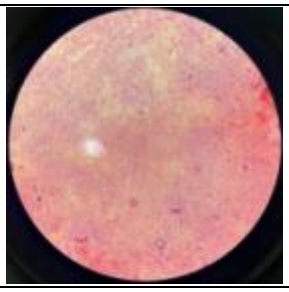
Day	Control	Boiled	Raw+lactic acid	Boiled + lactic acid
1				
2				
3				
4				

For bacteria screening, the results found that there are several bacteria that can be found in the spoiled fish products. Apparently it is the Gram-negative bacteria that is most likely the *Pseudomonas spp*. The control treatment of raw fish gives the highest presence of bacteria. This is explained as *Pseudomonas spp*. are known for their ability to grow at low temperatures, which makes them particularly relevant in the context of spoiled refrigerated or raw seafood [2]. This is then followed with raw lactic acid and a boiled sample. The boiled lactic acid sample showed the least sign of bacteria presence.

The lactic works best with heat in reducing the number of bacteria and extending the lifespan of the sample. The combination of heat treatment (boiling) and the addition of lactic acid helps to kill or inhibit the growth of microorganisms, reducing the chances of bacteria presence. However the techniques still cause growth of the gram negative bacteria at minimal amounts. It is suggested that the *Pseudomonas spp*. could form spores that are highly resistant to heat thus boiling alone may not be sufficient to kill these spores [11]. The contamination also could

come from improper handling post-boiling as the sample is not handled and stored properly, thus contaminated with bacteria from the environment [12].

Table 2. Observation on bacteria presence in preserved fish sample at 100x magnification

Preservation technique	Control	Boiled	Raw+lactic acid	Boiled + lactic acid
Presence of bacteria				

CONCLUSIONS

In conclusion, it was successfully determined that lactic acid exposure in combination with heat technique had increased the sample raw fish preservation. The Pseudomonas spp bacteria contamination had been decreased using a combination of lactic acid and heat, but it's important to note that the effectiveness of this method may vary depending on factors like concentration, exposure time, and temperature. Thus it is recommended to check the environmental factors that could fasten up food spoilage.

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