THE EFFECTS OF CHITOSAN IN MACKEREL TUNA (Euthynnus affinis) BRINE SHREDDED AGAINTS HALOPHILIC BACTERIA

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ABSTRACT

*Mackerel tuna (*Euthynnus affinis*) brine salting has short shelf life because consist of high protein and water. Shredded fish is food technology to prolong shelf life of mackerel tuna brine. Addition of chitosan in mackerel tuna brine shredded has functioned as antibacterial. Halophilic bacteria, is bacterial resource from salinity environment and can lived in high temperature, likes seafood. The aim of this research is to investigate if chitosan can against the growth of halophilic bacteria. The samples were mackerel tuna brine salting, mackerel tuna brine shredded non-chitosan and mackerel tuna brine shredded adding with 50 mg chitosan, were enriched in Triptyc Soy Broth (TSB) media and streaked on Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar plates, incubate at 37°C for 24 hours. Mackerel tuna brine shredded's chitosan agar plate was negative. Total two TCBS strains were isolated from TCBS of mackerel brine salting and mackerel brine shredded non-chitosan agar plates. All strains were indentifying using gram staining and 20E API kit. The result shows, two strains were classified as Proteus vulgaris and Pseudomonas luteola. The conclusion is to process mackerel tuna brine into shredded fish by adding 50 mg chitosan makes result in inhibit the growth of halophilic bacteria.*

***Keywords**: chitosan, halophilic bacterial, mackerel tuna (*Euthynnus affinis*), brine salting, mackerel tuna (*Euthynnus affinis*), brine shredded*

INTRODUCTION

Indonesia surrounded by the ocean makes Indonesia has lots of fish potential as protein resource beside meat. Mackerel tuna or "tongkol" (*Euthynnus affinis*) is usually processed as mackerel tuna brine-salting [1]. This seafood for Balinese people is very popular and becoming daily consumption for family [2]. Because of mackerel tuna brine salting high in protein and water, bacteria can growth easily [3]. Shredded fish is one of food technology to make mackerel tuna has long shelf life. Pan frying method of processing shredded fish, showed can prolong the shelf life until 40 days [5].

The contamination of bacteria could give impact on reducing of nutrition value and the quality of the shredded fish. Seafood with high contamination of bacteria also makes diseases to the consumer. Some of bacteria which classify as halophilic and thermophilic bacteria have characteristic can survive in salinity environment and high temperature, common found in seafood [6]. Bali as one of tourist visit, has to improve the service of culinary tour and ensure the seafood is safety to eat [7].

Chitosan known as antibacterial made from natural product crustacea shell. It can inhibit the growth of gram positive and negative bacteria including fungus [8]. In this research we have added 50 mg chitosan inside the mackerel tuna shredded. The aim on this research by adding chitosan can inhibit the growth of bacterial and will prolong the shelf life of shredded fish, in case chitosan can use as alternative antimicrobial without side effect in seafood.

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METHODS

The mackerel tuna (*Euthynnus affinis*) brine salting bought from Badung market. The mackerel tuna process to shredded fish by adding 50 mg of chitosan in 100 gr shred mackerel tuna and left for 15 minutes before cooked. The seasoning such as shallot, garlic, kencur, lengkuas, tumeric, cumin, and coriander blended and fried. Palm sugar, bay leaves and lime leaves adding into the fried seasoning until the color became brownish. The shred mackerel tuna was entered into the seasoning fried and added coconut milk powder. Then continued fried the shredded mackerel tuna in 1 liter coconut oil until brownish. Other shredded mackerel tuna cooked without chitosan.

Three samples consist of mackerel tuna brine salting, mackerel tuna shredded chitosan and mackerel tuna shredded non-chitosan, respectively was taken 10 gr and enriched in 10 ml Tryptic Soy Broth (TSB) media, incubated in 37°C for 24 hours. All samples were streaked on selective media Thiosulfate Citrate Bile Salts Sucrose (TCBS), incubated in 37°C for 24 hours. One colony was taken and subculture in TCBS, incubated in 37°C for 24 hours. Taken only one colony bacteria and indentify by gram stain. The gram-negative bacteria will continue identified by API 20E kit.

The methods section describes detailed actions, instruments, techniques of analysis utilized in the study in order to investigate the research question(s), and explains the rationale for the application of specific procedures or techniques used to identify, process, and analyze information applied to understanding the problem. The writing should be direct and precise; it should be written in the past tense.

FINDINGS AND DISCUSSIONS

Bacteria Culture

Samples are mackerel tuna brine salting (P), mackerel tuna shredded brine non-chitosan (A) and mackerel tuna shredded brine chitosan (AK). The samples were weight 10 gram respectively, and enriched into TSB media. Halophilic bacteria, has to be enrich in media capable for anaerobes, aerobes, micro-aerophilic, and fastidious organisms. Tryptic Soy Broth (TSB) has content with NaCl between 0-30percent which tolerances to halophilic bacteria growth [9]. All samples were incubating for 24 hour in 37°C. The next day, each samples cultured on TCBS media by streak method and continued incubate in 37°C for 24 hour. The halophilic bacteria can grow in TCBS media by alkalinity resource in the media [10].

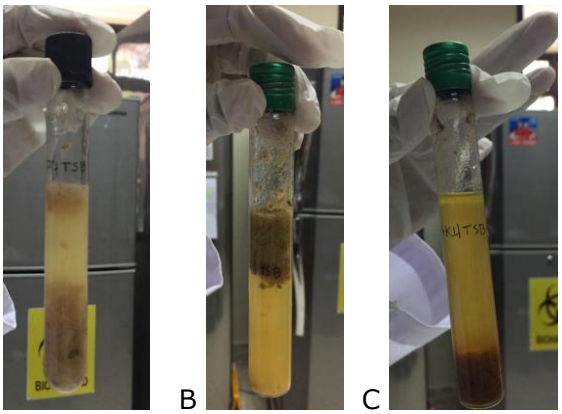


Figure 1, A: sample mackerel tuna brine salting (P). B: sample mackerel tuna brine shredded non-chitosan (A), C: mackerel tuna brine shredded chitosan (AK), enriched in TSB media.

All the bacteria colonies which had grew on TCBS media taken only one colony and subculture on new TCBS media and incubate. The colonies have been growing identified by gram staining. If the bacteria were gram-negative, it will continue to API E20 kit to classify the species. It is showed on Figure 1.

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After incubating for 24 hour, each sample were culture on TCBS media selective for halophilic bacteria and incubated in 37°C for 24 hour. Some bacteria colonies were growing on TCBS media were from mackerel tuna brine-salting and mackerel tuna brine shredded non-chitosan. It wasn't found bacteria grew on TCBS media from mackerel tuna shredded chitosan (Figure 2). The yellow zone formed of bacteria able to metabolize sucrose and reduce the pH. Other halophilic can grow but cannot metabolize sucrose, it makes the color's bacteria's colonies won't be yellow. Proteus strains can grow to form yellow, vibrid-likes colonies [10].

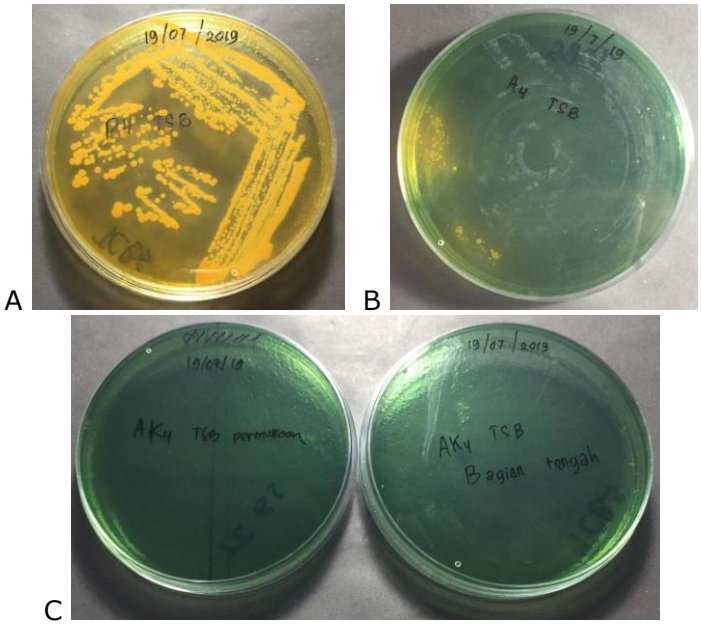


Figure 2. Culturing samples A: mackerel tuna brine-salting (P). B: mackerel tuna shredded non-chitosan (A). C: mackerel tuna shredded chitosan (AK) on TCBS media.

Bacteria Subculture and Gram Staining

Subculture needed to make sure the purity of the colonies bacteria. One colony was taken and streak on new TCBS media (Figure 3). The colonies were growing on the next day after incubated. First step to identify the morphology from the gram class of bacteria is by gram staining (Figure 4).

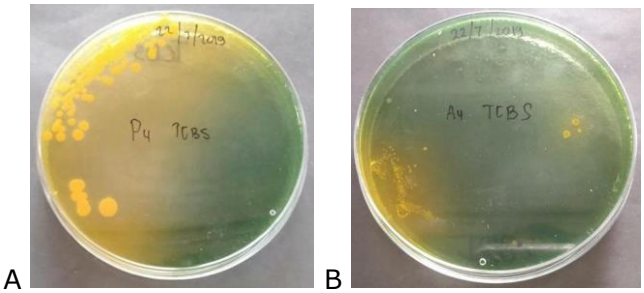


Figure 3. Subculture colonies grew on TCBS media: A. Mackerel tuna brine-salting (P); B. Mackerel tuna shredded non-chitosan (A)

The result based on gram staining from mackerel tuna brine-salting and mackerel tuna brine shredded were found that both bacteria were gram-negative. All the bacteria can classify the species using API 20E kit. One colony from mackerel tuna brine-salting shows %ID 99.2% is *Proteus vulgaris* and mackerel tuna shredded non-chitosan %ID 98.1% is *Pseudomonas luteola*.

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Both are halophilic bacteria which can grow in salted environment. These bacteria have produced specific protein that can active in salted condition. It because in the cytoplasm, it has Na+, K+ and Cl+ to reduce osmotic movement, it can avoid the damage of cell walls [9]. In the present of salt, the halophilic bacteria can grow. *Pseudomonas luteola* is micro flora on fish but if the growth is not in control can cause strawberry disease on fish's skin [11]. In people, known can cause pneumonia. The bacteria as mesophilic can found frequently in soil, water or damp environment [12]. The presence of *P. luteola* in mackerel tuna brine shredded it can be growth during cooling process.

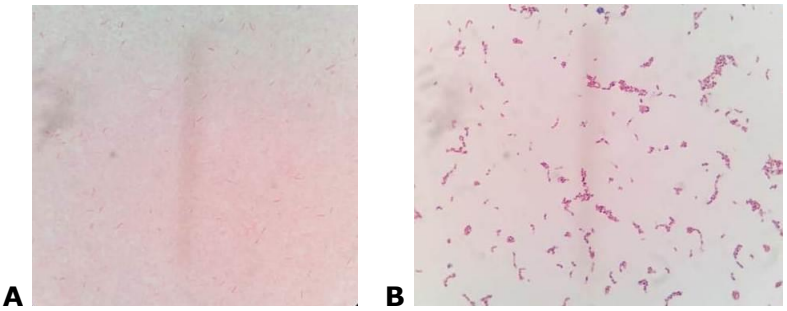


Figure 4. Bacteria gram-negative. Sample A: mackerel tuna brine-salting (P) and B: mackerel tuna shredded brine non-chitosan (A).

Proteus vulgaris is bacteria present in water and soil. In this case show as indicator of fecal pollution in marine water. Water animals such as fish absorbs the bacteria because of marine chain food and transferred to human after consumed animals water contaminated with *P. vulgaris*. In spoiled fish product like sardines, *P. vulgaris* can produce histamine [13]. These two bacteria can produce biogenic amines such as histamine and tyramine [14]. These biogenic amines can cause allergic reactions in humans [15].

CONCLUSION

The conclusion on this research the halophilic bacteria still can grow in mackerel tuna brine-salting and mackerel tuna brine shredded non-chitosan. This contamination can arise due to salted environment during selling in market and the capability of thermophilic bacteria survives in extreme temperature. Addition of 50 mg of chitosan in mackerel tuna brine shredded have been capable to inhibit the growth of halophilic bacteria such as *Proteus vulgaris* and *Pseudomonas luteola* in mackerel tuna *(Euthynnus affinis*) brine shredded.

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