



ANALYSIS OF TOTAL MICROBIAL IN MARINATED SCALLOP *Gafrarium tumidum* DURING COLD STORAGE

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ABSTRACT

This study was conducted to analyze the number of microbes on marinated scallops *Gafrarium tumidum* during cold storage. The results showed that the number of microbes on the scallops marinated products *G. tumidum* ranged from 1.1×10^2 - 7.4×10^2 cfu/g, so that they meet the SNI 01-2725-1992, the maximum limit of microbial contamination is 5.0×10^5 cfu/g. Total plate count (TPC) value marinated scallops with storage time to 14 days is below the maximum limit is set, so that the marinated of shellfish products *G. tumidum* still meet microbiological standards and suitable for consumption.

Keywords: *Marinated; Scallops; Total microbes*

1. INTRODUCTION

Shellfish has been used for various purposes, both for ecologically and economically. Ecologically, shellfish have an important role in an ecosystem and become one of the inseparable elements of the food chain. In addition, shellfish can also be used as an indicator of an environmental condition. Economically, shellfish has been known as a delicious and nutritious food source. In addition, the shell can also be used for decoration or knick-knacks (Mikkelsen and Henne, 2011). In other countries like India and also in Southeast Asia, Bivalves are also a source of good and nutritious food, high protein content, and low prices (Jagadis and Rajagopal, 2007; Babu *et al.*, 2012). Reported by Babu *et al.*, (2012) amino acid composition of essential amino acids (EAA) and 22.2% non-essential amino acids (NEAA). Additionally also the composition of

marine bivalves is a nutritional guarantee for millions of people who suffer from malnutrition.

Research related to shellfish around Ambon Bay waters has been done, such as shellfish growth in Ambon Bay waters (Pattikawa, 2007), parameter of population of tropical shell *Anodontia edentula* (Natan, 2009), oyster shell growth in Ambon Bay waters out (Toja *et al.*, 2011), and “*kerang-kerek*” in Ambon Bay waters (Islami, 2014). Subsequently reported by (Srimariana *et al.*, 2015). About the mineral content of the *G. tumidum* taken from the waters of Laha State. One type of shell that is popular with the people of Maluku namely *G. tumidum* shells because it tastes delicious and also has a high nutritional content. In the coastal region of Maluku, *G. tumidum* shellfish is used by local people as an alternative food source besides fish, especially during the wave’s season where fish are hard to obtain. The shells are taken at low tide. Shrimp-taking activities during low tide are commonly referred to local term as *bameti*.

In general the characteristics of shell meat is rather hard or solid and chewy. For that one way to accelerate the process of meat baiting is by way of soaking the meat in marinated fluids (marinade). Marinade is a popular name of seasoned fluid that serves as a marinade of meat (including poultry and seafood), usually used to add flavors or enhance the tenderness of meat. Marinade is a fishery product that uses fish meat or fishery product section as raw material made by soaking fish meat on soaking medium consisting of acetic acid and salt solution so that it can cause a distinctive odor and serves as a preservative. Commonly used marinade raw materials are anchovy fish, herring (*Clupea harengus*), sardine (*Sardina pilchardus*), cod (*Gadus morhua*) and salmon (*Onchorynchus mykiss*). The most widely used types of anchovies are *Engraulis encrasicolus* (Duyar and Eke 2009; Cosansu *et al.*, 2010) and *Engraulis anchoita* (Capaccioni *et al.*, 2011; Yeannes and Casales, 2008). Marinade products can also be made using bonito or *Sardes sardes* (Duyar and Eke, 2009) and small *Atherina boyeri* (Bilgin *et al.*, 2011).

The selection of marinade types depends on the ultimate goal to be achieved. Oil-based marinades can be used if the marination process is intended only to give a flavor to the meat. Conversely, if the main purpose is for the applying it should be used acid-based marinade. If the formation of flavor and tenderness becomes the goal of the marination

process, then the marine used is a combination of marinade based on oil and acid. Because the process of marinade absorption by meat during marination process is limited, marination mainly affects the flavor on the surface of the meat. Marination for longer periods can help penetrate the marinade to a deeper portion of the meat but this condition is not only ineffective but also at risk from the food safety aspect. The addition of acids that break the meat fibers will help the process of marinade absorption. It's just that the use of acid needs to be controlled so as not to cause inconsistencies of flavor and yield (Brooks, 2011). Meat processing by marination method initially serves as a spice, but on further development also serves to lower the content of bacteria in meat. Thus, marinated meat can be utilized to improve flavor, improve the physical properties of meat and is expected to also be used as a preservative to extend the shelf life. Various marine meat research results were also useful to improve food safety and added value. This is because marination materials are generally also antibacterial, so it is expected to meet the requirements according to SNI especially seen from the microbiological side.

One effort to maintain the quality of fishery products while increasing fish consumption in the community is the diversification of processed products into marinade. Sahabudin (2015), has made a marinade of skipjack tuna with the addition of papaya fruit. While marinade research using shells has not been done. Thus it is necessary to make marinade from other fishery products such as shellfish, so it can be used as an alternative of marinade raw materials, in addition to know the microbiological quality of marinade shell products.

2. RESEARCH METHOD

2.1. Material and Tool

The ingredients used for the manufacture of marinade in this study are: shellfish (*Gafrarium tumidum*) obtained from Laha Ambon village, and papaya flesh (*Carica papaya*), vinegar, white ginger, red onion, salt, sugar, water and analyzer Micro and chemical: PCA, NaCl 0.5%, Aquades. The equipment used in this research is glass bottles, scales, sample stickers, knives, plastics, cutting boards, blenders, incubators, analytical scales, autoclaves, petri dishes, stomachers and some other laboratory tools for analysis purposes.

2.2. Research Procedure

The fresh *G. tumidum* shell is separated from its shell and the entrails are discarded. Scallop *G. tumidum* was washed using clean water (fresh water), after which a raw papaya peeled skin, cut into 4 pieces, cut in the form of matches with the size of ½ x ½ cm. Soak the shellfish *G. tumidum* with papaya for 2 hours.

Boil shells *G. tumidum* with raw papaya for 1-2 hours with temperature 100°C, one ripe papaya fruit peeled, cut into 4 pieces, cut into a match with a size of ½ x ½ cm, so easy to input into a peppered cup containing seasoning. White ginger 100 g, 200 g onion and 1700 ml of white water in the input in a blender then in the blender into one filtered extract taken, and included as much as 150 ml extract into each glass bottle, add sugar 28 g, 4 g of salt included Into a spice solution and inserted 4 ml of vinegar into each glass bottle containing the spice solution, weight 30 g of papaya into the glass and 30 g (A1), 60 g (A2) and 90 g (A3) - inserted into a glass bottle that already contains a spice solution. Conducted storage using warm temperature (~50 °C) for 7 days and 14 days. Furthermore, TPC, pH, and moisture content were analyzed.

2.3. Treatment

In this research used 2 (two) treatment factor, that is factor A (Comparison of Shellfish and Papaya): Scallop *G. tumidum* without papaya 1: 0 (30 g : 0 g) A0; Scallop *G. tumidum* with 1: 1 papaya (30 g : 30 g) A1; Scallop *G. tumidum* with 1: 2 papaya (30 g : 60 g) A2; Scallop *G. tumidum* with 1: 3 papaya (30 g : 90 g) A3. Factor B (Storage Duration): storage time 0 day (B0); storage time 7 days (B1); and storage time 14 days (B2).

2.4. Analytical Procedures

In the process of TPC analysis (Fardiaz, 1993) first sterilization of equipment to be used, and every sampling should be done sterile. TPC analysis procedure is as follows: sample weighed as much as 25 g, add 225 mL 0.9 solution then blend for 1-3 minutes. The result of the blender is put back into the container and let stand so that it can be pipette. Blender samples resulted from blender is considered as the first dilution or 10⁻¹. Using a sterile pipette, take 1 ml of sample liquid and then inserted into 9 ml of 0.9% NaCl solution so that 10⁻² dilution can be obtained. Into 2 sterile petri dishes put 1 mL sample from each dilution, then pour as much as 10 -15 mL plate count agar (45). Then

the petri dish is shaken to form the number eight above the flat surface so that the colonies of bacteria that grow spreading. After the agar in the petri dish hardened, the cup is reversed and incubated for approximately 48 hours in an incubator with a temperature of 35 °C. To calculate the amount of bacteria content can be calculated based on the well known formula in biology.

2.5. Moisture Content Analysis

According to AOAC (2005), the usual oven method is one of the direct heating methods in determining the moisture content of a foodstuff. In this method the material is heated at a certain temperature so that all the water evaporates as shown by the constant weight of the material after a certain heating period. The weight loss of the material indicates the amount of moisture content. This method is mainly used for materials that are stable against rather high heating, as well as products that are not or low in the content of sucrose and glucose to such as starches and cereals.

The method is used by the method of oven (gravimetric). First weighing in oven bottle for 15 minutes, it aims to eliminate the moisture content in the bottle. Then it is 5 minutes long to keep moisture (RH). Then weigh the weigh as (a) gram to know the initial weight of the bottle. Then add 3 grams of material as sample for analysis. Weigh as (b) gram to determine the weight of the material and the weighing bottle. Then in oven done for 24 hours, it aims to know the changes in moisture content in the material. It is then subjected to 5 minutes for cooling and stabilizing moisture (RH). The last step is weighed with two weights to constant material weight. The formula of moisture content are:

$$\% \text{ Moisture content} = (B - (C - A))/B \times 100 \%$$

where: A = Weight of the cup; B = Material weight; C = Weight of plate + ingredients;
to find the total solids are: % total solids = (C - A) / B x 100% C - A = Dry matter.

2.6. Time and place of research

This research was conducted in November 2016 at Fishery Technology Laboratory, Faculty of Fisheries and Marine Sciences, Pattimura University Ambon.

3. RESULTS AND DISCUSSION

3.1. Microbiological Analysis for TPC

The analysis of the determination of microbial marinade number of shellfish was determined by microbial contamination method Total Plate Count (TPC) which is the calculation of microbial quantity contained in a product growing on the agar medium at the specified incubation time and temperature. Appropriate bacterial testing was performed using TPC method, which was calculated the amount of bacteria grown on a growth medium (media agar) and incubated for 48 hours. The growing bacterial colonies were calculated and figured out (Figure 1). The maximum limit of bacteria for ready-to-eat food is 1×10^4 cfu/g (Food and Drug Supervisory Agency, 2009). The principle of the TPC method is to grow living cells of microorganisms on agar media, so that microorganisms will multiply and form colonies that can be seen directly and counted with the eye without using a microscope.

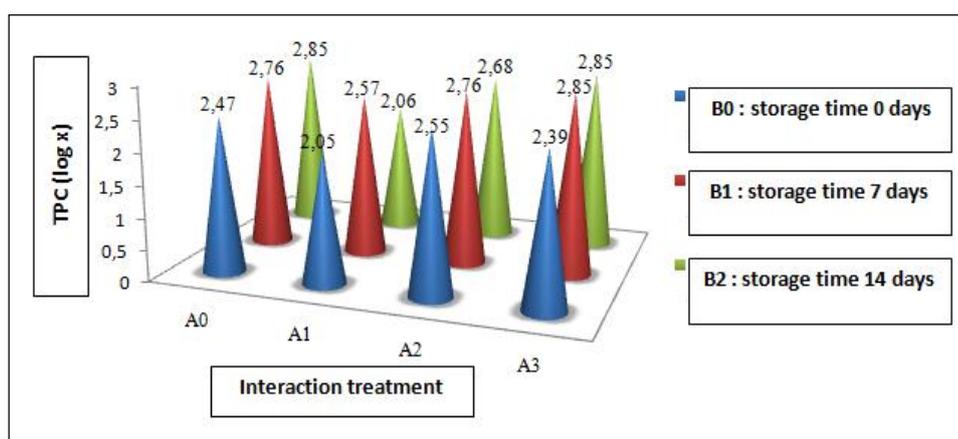


Figure 1. Histogram Analysis of TPC Marinade.

TPC test result on marine product of *G. tumidum* shell in 0th day storage (B0) showed different change of microorganisms. At treatment (A0) with the number of microorganisms 2.47 log-x or 3.0×10^2 cfu/g, treatment (A1) with the number of microorganisms 2.05 log-x or 1.1×10^2 cfu/g, treatment (A2) with number of microorganisms 2.55 log-x or 3.5×10^2 cfu/g, and treatment (A3) with the number of microorganisms 2.39 log-x or 2.5×10^2 cfu/g, These results prove that at the beginning of storage of marinade products *G. tumidum* shells have been contaminated by microorganisms, this is caused by environmental factors of shellfish *G. tumidum*, and the

unfavorable processing factor so that at the beginning of storage of marine products of *G. tumidum* shells have been contaminated.

In the 7th day storage on treatment (A0) with the number of microorganisms 2.76 log-x or 5.8×10^2 cfu/g, the treatment (A1) with the number of microorganisms 2.57 log-x or 3.8×10^2 cfu/g, Treatment (A2) with the number of microorganisms 2.76 log-x or 5.8×10^2 cfu/g, and the treatment (A3) with the number of microorganisms 2.85 log-x or 7.4×10^2 cfu/g. These results indicate that at day 7 storage the TPC value continues to increase during storage. This is caused by the lack of storage process, the water content is so high that it becomes the growth medium of microorganisms.

As for the 14th day storage by treatment (A0) with the number of microorganisms 2.85 log-x or 7.4×10^2 cfu/g, treatment (A1) with the number of microorganisms 2.06 log-x or 1.1×10^2 cfu/g, treatment (A2) with the number of microorganisms 2.68 log-x or 4.8×10^2 cfu/g, and treatment (A3) with the number of microorganisms 2.85 log-x or 7.4×10^2 cfu/g. The result of TPC test of marinade product of *G. tumidum* shell in 14th day storage experienced different result, some increase, some decreased. This is caused by the storage process, the longer the storage process the higher the number of microorganisms. Storage using cold temperatures can inhibit the growth of microorganisms and some even die so that some of the microorganisms are decreasing and some are increasing.

Figure 1 shows that at the beginning of cold storage the shellfish *G. tumidum* marinade product has been contaminated by bacteria (anaerobic or facultative aerobic). By demonstrating the extrinsic factor that the nutritional value contained in clam meat is high enough, the marinade of *G. tumidum* shells is a good medium for bacterial growth. However, the influence of intrinsic factor that is condition in chilling storage and processing method that is chemical compound attached to marinade surface of shell, hence bacterial growth will be inhibited even some bacteria will die. Cold temperatures can cause bacterial metabolism run slowly, so bacterial growth goes slow too (Moelyanto, 1992).

The value of TPC marinade shellfish *G. tumidum* increased during storage is closely related to factors affecting the growth of microorganisms as found by Mossel (1971) in Buckle *et al.*, (1987), that is intrinsic (properties of the material food), processing

(changes from early microbes as a result of processing), extrinsic (environmental conditions of handling and storage) and implicit (the nature of the organism itself).

Based on the results obtained the average value of TPC marinade shells *G. tumidum* is the lowest of 2.5 log-x or 1.1×10^2 cfu/g, while for the highest average value of 2.85 log-x or 7.4×10^2 cfu/g.

The results of microbiological analysis in the observation of Hecer (2011) showed that there was no growth of pathogenic bacteria during the storage period of marine products. The length of storage of seafood marinade salad products is five months. It shows the ratio between the mixture of salt and acetic acid enough to protect the product and the production process is declared hygienic. The results are reinforced by research by Bilgin *et al.*, (2011) that the use of 3% acetic acid is an ideal concentration because it can decrease the pH and create environmental conditions suitable for the growth of acid-resistant bacteria, not pathogenic bacteria. Study of Capaccioni *et al.*, (2011) also proves that the higher the concentration ratio of the solution: the raw material then the penetration speed of acid and salt in the higher the raw material. Solution ratio: 3: 1 raw materials are considered ideal because they can shorten the marinating time without causing sensory damage and marinade product stability.

The maximum limit of microbial contamination in marinade according to SNI 01-2725-1992 is 5×10^5 cfu/g. Thus, the value of TPC marine shellfish with a long storage period of up to 14 days is still below the maximum limit set by SNI 01-2725-1992. This shows that the shellfish marinade *G. tumidum* still meet the quality standards and still feasible for consumption.

3.2. Moisture Content

Moisture content is the percentage of a moisture content of a substance which can be expressed on a wet basis or on a dry basis. Wet weight moisture content has a theoretical maximum limit of 100 percent, whereas moisture content based on dry weight can be more than 100 percent.

The result of the analysis showed that the moisture content in the interaction of treatment A3B2 was significantly different from other treatment interactions. The average value of marine moisture content of shellfish *G. tumidum* with the addition of the lowest

papaya flesh is reached at 0 day storage (B0) equal to 59.18 and the mean value of marine moisture content of the highest *G. tumidum* shellfish is reached in storage day 14th (B2) of 70.74 (Figure 2)

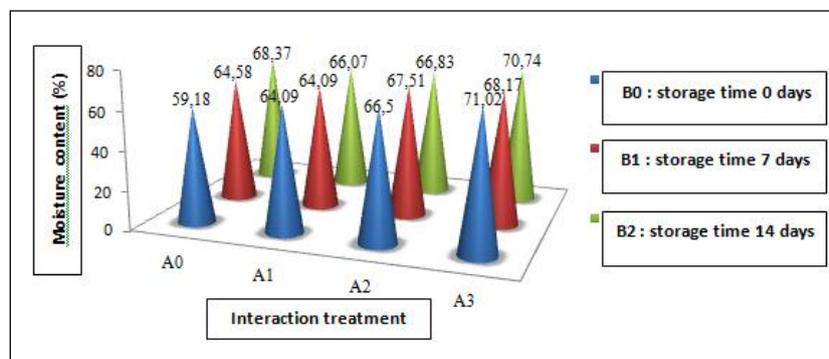


Figure 2. Histogram Value of Moisture Content of Marinade.

The results in (Figure 2) show that the difference in the composition of the shellfish and the papaya flesh on the marinade product of *G. tumidum* shells led to a very significant increase in moisture content. The data identify that a 1: 3 (A3) ratio for all storage times at cold temperatures is very high in moisture content. This is caused by the addition of papaya flesh which resulted in the moisture content contained in the flesh of papaya into one with the moisture content of the shellfish and the storage process so long that the value of moisture content of marine products *G. tumidum* shells the higher. So the longer the process of storage of marine products *G. tumidum* shells, the higher the value of moisture content of the marine product of *G. tumidum* shellfish.

4. CONCLUSION

There are two conclusions in this research. First, the value of TPC marine shellfish with a storage period up to 14 days is still below the maximum limit set by SNI 01-2725-1992, so the marinade of *G. tumidum* shells still meet the quality standard and still feasible for consumption. Second, differences in the comparison of shellfish and papaya flesh on the marinade product of *G. tumidum* shells cause the increase of moisture content during chilling storage.

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