Content analysis of Escherichia coli in Sliced Yellowfin Tuna (Thunnus albacores)

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Abstract

The purpose of this study is to determine Escherichia coli bacterial content in chunks of yellowfin tuna (Thunnus albacores) sold in Central Market Gorontalo. This study uses descriptive analysis method which gives an idea as carefully as possible about an individual, state, symptom, or a particular group. Based on observations of the total MPN Coliform samples A, B and C have MPN coliform exceed ISO standards for quality and food safety requirements of fresh fish, ie<3 MPN / g.MPN value low of sample A1 = 2.40 and the highest score> 24.00 MPN / g. Test EMBA (Eosin Methylene Blue Agar (EMBA) indicates that the Tuna Yellowfin positive for the bacterium Escherichia coli with colors that are characterized by green metalic. Pieces of tuna sold in the central market town Gorontalo safe to eat at 08:00 am, while at 10:00 am and 12:00 am bacterial content becomes a lot more. The longer the fish are displayed at table without any effort to maintain the quality of the fish, the more the bacteria contained in the fish.

Keywords: E. coli; yellowfin tuna; Thunnus albacores; pieces; content analysis.

Introduction

Fishery products should be consumed in a safe state by observing sanitation and hygiene and is expected to be free of bacteria. Peltzer and Chan (1988) states that the bacteria can cause food poisoning, most polluted through processing by human. Generally, foods become a source of infection and poisoning by bacteria is low acidity foods such as meat, eggs, fish and dairy foods. The bacteria that can cause infections one of which is Escherichia coli. These bacteria are easily spread by contaminated water and contaminating materials that come in contact with it (Faith, 1999, in Ashari, 2007). The fisheries products which are consumed by the people of Gorontalo is a type of yellow fin tuna (Thunnus albacares) is usually marketed in the form of pieces - pieces. Gorontalo City Central Market is one of the places visited by many people to buy tuna that have been in slices.

Fish that have been cut are usually placed on the table not cover material or without packaged. It is feared that the pieces - pieces of fish are contaminated with bacteria due to come into direct contact with the air and the surrounding environment. Based on the Indonesian National Standard (SNI 2729-2013) that the requirements of quality and safety of fresh fish to the test parameters of microbial contamination is <3 APM / g.

Escherichia coliisa gram-negative rod-shaped bacteria are not encapsulated. These bacteria produce more acid in the medium of glucose, which can be seen from the red indicator metal, producing indole, but does not produce acetoin and can not use citrate as a carbon source. Escherichia coli can cause diarrhea in humans called Entro pathogenic Escherichia coli (EEG). Infection of EEG can cause diseases such as cholera and dysentery in children - children and adults. The incubation period is 8-44 hours (Nuraeniet al, 2000, in Azhari, 2007).

Research Methodology

This study uses descriptive analysis method which gives an idea as carefully as possible about an individual, state, symptom, or a particular group (Koentjoroningrat, 1985).

This research was conducted at the Laboratory of Microbiology, Department of Biology, Faculty of Science, State University of Gorontalo to isolate Escherichia coli of yellowfin tuna (Thunnus albacores) in June - July 2014, followed by the preparation of the thesis to the test.

The tools used in this study are: the Petri dish, Colony Cauter, measuring cup, Micro pipettes,

Erlenmayer, Stirer Magnetic Hot Plate, Autoclave, Incubator, Laminar flow iar, Analytical Scales, Needles Ose, Test Tube, Tube Huss.

The materials used in this study are: Pieces tuna (Thunnus albacores),Plate Count Agar (PCA),Butterfield Phosphate (BFP), Lauryl Triptose broth (LTB), Emba Agar`.

Samples for microbiological testing isyellowfin tuna chunks of meat taken from the Central market Gorontalo, which is placed in a sterile plastic that has been coded. Total sample taken 9 samples from three fishmonger. Seller first coded A, both the seller and the seller B coded three coded C. Each code is done three times retrieval.

Sampling was performed three times, with each taking a sample comprising A, B and C. The first decision at 08.00 which samples A1, B1 and C1.Further testing in the laboratory for observation.Decision to two at 10.00 which is the sample code A2, B2 and C2 further testing in the laboratory.Then sampling all three at 12.00 with code samples A3, B3 and C3.

Testing the presence of bacteria by using Total Plate Count conducted by SNI 01-2332.3.2006. All the equipment used during the analysis sterilized by autoclave at a pressure of 15 psi with a temperature of 121 ^{0} C for 15 minutes. The calculation of the number of bacterial colonies in accordance with SNI 01-2332.3.2006.

Principles of microbiological testing is to grow the bacteria Escherichia coli in a medium and calculations are based on the number of positive tubes coliform after incubated at a temperature and time.

Stage - the stage of the tests performed in this study as follows; ie prediction coliform test or presumptive coliform test and confirmation of coliform / E.coli estimation (Source: SNI testing of E. coli 01-2332.1-2006).

Gram stainingTest according Ijong (2003), aims to determine the characteristics of the bacteria, either the reaction of bacteria to the Gram stain, cell shape and size. Stages in Gram staining bacteria were performed with steps - steps as follows:

a. Prepared sterile glass slide, free from dirt, especially oil. Dib • • stronger sign of a circle with a marker with a diameter of about - about 1 cm on the bottom side of glass slide.
b. Bacteria cultured on agar slant taken loopful, were transferred to a glass slide was then fixed over a Bunsen burner. Furthermore, preparations have been applied a few drops of crystal violet bacteria and allowed to stand for 1 minute.
c. Excess crystal violet substances discharged by tilting the glass slide and then rinsed with distilled water. Furthermore drip with Lugoland left for 45 seconds and then rinse with distilled water. Then the glass slide with a few drops of alcohol to bleaching, and then rinsed again with distilled water. After that drip with safranin solution and left to stand for 30 seconds, then rinsed again with distilled water.
d. Rest - the rest are located around the glass preparations are absorbed by tissue.
e. Observations were made using a microscope. In this test Escherichia coli will look red and or rod-shaped bacilli (G ram negative).

Results and Discussion

Test of Total Plate Count (TPC)

Calculation of Total Plate Count and the detection of the presence of Escherichia coli bacteria in fish yellowfin tuna (Thunnus albacores)was conducted to see the number of microbial contamination of the tuna sold in pieces - pieces in the Central market Gorontalo.

In this observation the number of bacterial contamination via test Total Plate Count, from 3 samples taken at regular intervals where each taking 3 samples are samples A, B and C. making 08.00 coded samples A1, B1, C1. Sampling 10:00 coded samples A2, B2, C2. While sampling 12.00 coded samples A3, B3 and C3. The total number of samples in this study were 9 samples.

After incubation, the colonies growing on a petri dish is calculated by number of colonies acceptable 25-250 colonies per plate. The calculation is performed using a colony counter.

Sampling at 08.00 at the A1 sample was found the amount of bacteria that can be calculated that the column 10 $^{-1}$ and 10 $^{-2}$ then showed the number of bacteria 480.95 Log namely 2,682 results (A1 = 4.8 x 10 ^{2} colonies / gram). In sample A2 column can be calculated that the column 10 $^{-1}$ and 10 $^{-2}$ found the number of bacteria Log 1436.4 3,157 results (A2 = 1.4 x 10 ^{3} colony / salt). Samples A3 columns that can be calculated are kolom10 10 $^{-2-1}$ and column number of the bacteria found are the result of 1513.63 Log 3.1800 average yield of the sample A = 1143.66 and an average of 3.058 log (A3 = 1.5 x 10 ^{3} colonies / gram). Log average - average A1, A2 and A3 is 3,058.

Samples B1 column can be calculated that the column 10^{-1} and 10^{-2} 695.2 Log number of bacteria found 1,487 results (B1 = 6.9 x 10^{1} colony / gram). Samples B2 can be calculated 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} found the number of bacteria Log 4318.2 3,635 results (B2 = 4.3 x 10^{3} colonies / gram). In sample B3 column can be calculated that the column 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} discovered bacterial count 3,705 5,075 Log results average yield of 3141.3 and Log on average 3,497 (B3 = 5 x 10^{3} colony / gram). Log Average - Average B1, B2 and B3 is 3,497.

Sample C1 column can be calculated that the column 10^{-1}, 10^{-2}, 10^{-3} Log 1227.3 amount of bacteria found 3,088 results (C1 = 1.2 x 10^{3} colonies / gram). Samples C2 columns can be calculated that the column 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} discovered bacterial counts 3.69 4916.7 Log results (C2 = 4.9 x 10^{3} colonies / gram). Samples C3 columns can be calculated that the column 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} 168 500 Log number of bacteria found 5226 results (C3 = 1.6 x 10^{5} colonies / gram). Log on average C1, C2 and C3 is 4,765. More details ALT test results are presented in Table 1.

Table 1. Total Plate Count (ALT)

Sample	Collecting time (wita)	Value ALT(cfu/gr)
A1	08.00	5.0 X 10^{2}
A2	10.00	1.4 X 10^{3}
A3	12.00	1.5 X 10^{3}
B1	08.00	7.0 X 10^{1}
B2	10.00	4.3 X 10^{3}
B3	12.00	5.0 X 10^{3}
C1	08.00	1.2 X 10^{3}
C2	10.00	5.0 X 10^{3}
C3	12.00	1.6 X 10^{5}

Coliform test with Most Probable Number (MPN/gram)

Based on the total MPN Coliform test which showed that all the samples had total coliform MPN exceeded standards ISO 7530.1: 2009 for quality and food safety requirements of fresh fish, ie<3 MPN / g.Coliform test results can be seen in the table below.

Based on observations of total Coliform MPN obtained, the sample tested had exceeded the value set by SNI's requirements for quality and food safety of fresh fish, ie<3 MPN / g.Values obtained from MPN test is its low point of the sample A1 = 2.40 and the highest score> 24.00 MPN / g.

Test of the bacteria Escherichia coli

Based on the test results an affirmation using selective media E. coli Eosin Methylene Blue Agar (EMBA) positive reaction seen in the sample C2 and C3 samples.This suggests that one of the points of sale Yellowfin Tuna cut in the central market town of Gorontalo positive contaminated with bacteria Escherichia coli to sampling at 10.00 and 12.00.For more details EMBA test can be seen in Table 2.

Table 2. Test Results bacterium Escherichia coli with media Eosin Methylene Blue Agar (EMBA)

Sample	Collecting time (wita)	E. coli
A1	08.00	-
A2	10.00	-
A3	12.00	-
B1	18.00	-
B2	10.00	-
B3	12.00	-
C1	18.00	-
C2	10.00	+
C3	12.00	+

(+) Positive (Escherchia coli found)
(-) Negatif (Escherchia coli NOT found

Growth of E. coli is characterized by a distinctive color: green methalik seen in the picture C2 and C3. EMBA test results shown in Figures 1 and 2.

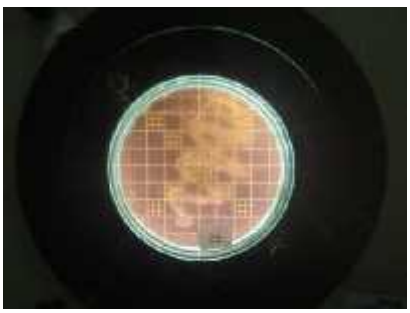

Figure 1. .Sample C2

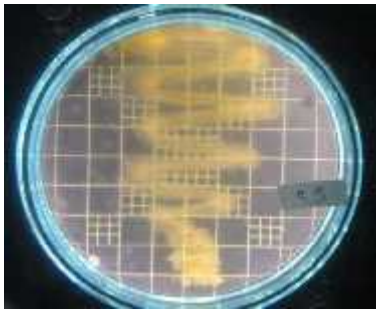

Figure 2. Sample C3

Conclusion

Based on these results, the total MPN Coliform analysis showed that in all samples except the samples A1 and B1 have exceeded the standard ISO 2729-2013 (<3 MPN / g) for the requirements of quality and safety of fresh fish.Test EMBA (Eosin Methylene Blue Agar) showed that pieces of yellowfin tuna were positive for the bacteria Escherichia coli only on the sample C2 (at 10:00 am) and the samples C3 (12.00 am).

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