MICROBIAL CONTAMINATION NUMBERS IN THE PROCESSING OF "OTAK-OTAK IKAN" FISH JELLY PRODUCT

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ABSTRACT

Fishery products are products that have a high protein content and physical conditions that are very suitable for the growth of spoilage microbes. "Otak-otak", which categorize as fish jelly product, is one of the diversified fishery products which was the analyzed sample in this study. The purpose of this study was to determine the number of microbial contamination identified in the sample during the processing steps. The research method used is a survey method. The sample consisted of raw materials, dough, and the final product of "otak-otak". The total plate count method was used to analyze the number of microbial contamination in the samples. The Total Plate Number analysis consists of 3 stages, namely the analysis stage, the calculation stage, and the interpretation of the results. The results of the analysis were that there are differences in the number of bacteria before and after the process of making "otak-otak" due to the boiling process. The "otak-otak" produced are suitable for consumption and based on the results, has the bacterial number of 2.5 x 10^4 colonies/gram. Microbiological quality requirements according to national standard (SNI.77557.2013) regarding "otak-otak" are 5.0 x 10^4 colonies/gram. It is recognized that the composition of raw materials affects the number of bacteria in the product of "otak-otak".

Keyword: *bacterial count, fishery products, raw material, "otak-otak", total plate number*

1. INTRODUCTION

Fish is considered as a perishable food, this is due to several things, such as high protein content and environmental conditions that are very suitable for the growth of spoilage microbes. The water content in fish is the main factor causing food spoilage. The higher the water content of a food, the greater the possibility of deterioration, both as a result of internal biological activity (metabolism) and the appearance of harmful microbes [1].

Quality or microbiological standards are parameters that are not visible to the eye but greatly determine the safety and durability of food ingredients [2]. Microbiological quality variations and changes would cause food products to be unsuitable to market and consume. Numerous studies show that the consumption of food whose microbiological values deviate or exceed the standard can cause diarrhea, dizziness, vomiting, nausea and fever. Even certain bacteria can cause fainting, nerve cell damage and even loss of life [3]. The government through the local Food and Drug Monitoring Agency and the Indonesian National Standard (SNI) have prerequisite microbiological criteria for most food ingredients and products. In general, the criteria for analyzing food products are the total microbial value or total plate number, total mold and yeast, and coliform bacteria. Certain products also require an analysis of the pathogenic bacteria presence. Food products that require microbiological criteria include fresh products, processed products ready for consumption, semi-finished products such as flours and food additives [4].

"Otak-otak" fish jelly are processed fishery products that use minced fish meat or surimi of at least 30% mixed with flour and other ingredients, with or without vegetables and coconut milk, that have been formed, with or

without wrapped in leaves, and cooked [5]. This product is one of the diversified fishery products that have long been known by the public. "Otak-otak" is a product that is susceptible to microbial growth due to raw materials derived from fish and flour, and contamination during the processing. Therefore, it is necessary to perform a study on analyzing the microbial contamination in "otak-otak" fish jelly. The "otak-otak" sample testing includes raw materials, dough, and the final product of "otak-otak" to determine the feasibility of consuming the "otak-otak" which refers to the number of bacteria using the Total Plate Number analysis of bacteria in the samples. The purpose of this study was to determine the number of microbial contamination contained in the "otak-otak" during the processing steps, thus it can be considered whether the product is suitable for consumption by the public or not.

2. MATERIALS AND METHOD

The materials used in the research are as follow: "otak-otak" fish jelly made from mackerel as raw material, dough and the final product; Plate Count Agar (PCA), used as a medium for bacterial growth; Butterfiel's phosphate buffered solution, used as a diluent and aquadest, used to dissolve agar media. The equipment used in the study were stomacher, test tube, test tube rack, petri dish, micropipette, Erlenmeyer, incubator, analytical balance with an accuracy of 0.1 g, autoclave, Bunsen burner, spatula, hotplate and stirrer, colony counter and hand counter. This study used survey method. The samples were fresh fish as raw material, "otak-otak" dough mixture, and the resulting "otak-otak" products. Samples were taken from fisheries processing industry in Cirebon, West Java, Indonesia.

The Total Plate Count (TPC) testing procedure includes the analysis and calculation or interpretation stages of the results. The procedure for testing the Total Plate Number of [6] (SNI 01-2332.3-2006) is as follows:

1) Analysis Stage
    a. Samples are prepared and weighed as much as 25 grams.
    b. 25 grams of sample and 225 mL of sterile Butterfiel's Phosphate Buffered solution were put into plastic aseptically, then homogenize by mashing it using a stomacher.
    c. 1 mL of the suspension was pipetted into the Butterfiel's phosphate buffered solution to obtain a 10^{-2} dilution. Later, the dilution (10^{-3}) was prepared by taking 1 mL of the sample from the 10^{-2} dilution using a sterile pipette and put into 9 ml of the Butterfiel's phosphate buffered solution.
    d. 1 mL of each dilution above was pipetted and put into a sterile petri dish which was carried out in duplicate for each dilution.
    e. In each dish/plate that already contains the sample solution, 12-15 mL of PCA is added and then shaken to distribute evenly. It was then incubated in an incubator at 35ºC for 48 ± 2 hours.
    f. Afterwards, perform the calculations on the plates that have the number of colonies 25-250 with the colony count.
2) Calculation

    To report a microbiological analysis result, a standard is used which explains how to count colonies on a plate and how to select available data to count colonies in a sample. Instructions for calculating and reporting the Total Plate Number are as follow:

    a. Plate less than 25 colonies

        If duplicate plates from low dilutions yielded fewer than 25 colonies, count the number in the plates from each dilution. Average the number of colonies per dish and multiply by the dilution factor to determine the estimated Total Plate Number.
    b. Plate more than 250 colonies

        If the number of colonies per plate is greater than 250 in all dilutions then report the result as too many to count (TMTC), but if any of the dilutions has a colony count close to 250 report it as an estimate of total plate number.
    c. Spreaders

        Colony spread is usually divided into three forms:
        i. Colony chain, the colonies are connected to each other because the bacteria are grouped together.
        ii. The spreader comes from the water layer between the agar and the bottom of the dish.
        iii. Spreader comes from a layer of water on the side or edge of the dish or on the surface of the agar. If the plate is overgrown with a spreader greater than 25% then report it as a spreader.
            - Type 1 spreader, if there is only one chain then declare it as one colony.

- If one or more chains appear to come from different sources, report each source as a colony.
- Type 2 and 3 spreaders generally come from different colonies and report each as one colony.

d. Plates with 25 to 250 colonies and spreader free
Record the dilution used and count the total number of colonies. Calculation step is following Total Plate Number formula below:

$$N = \frac{\sum C}{[(1xn_1) + (0{,}1xn_2)]x\,(d)}$$

Information:
N = number of product colonies, expressed in colonies per mL or colonies per g
$\sum C$ = number of colonies in all counted plates
n_1 = number of plates in the first calculated dilution
n_2 = number of plates in the second calculated dilution
d = first calculated dilution

3) Interpretation of Results
   a. Round the number to 2 appropriate numbers if the third number is 6 or above, then the third number becomes 0 and the second number increases by 1, for example 456 becomes 460.
   b. If the third number is 4 or below, then the third number becomes 0 and the second number is even, for example 445 becomes 440.
   c. If the third number is 5, then the third number becomes 0 the second digit increases by 1 digit, for example 456 becomes 460

3. RESULTS AND DISCUSSION

The total plate number analysis was carried out on fishery diversification products that were well known to the Indonesian public, namely "otak-otak" fish jelly. Otak-otak product samples comes from a company in Cirebon. There are 3 products that will be tested using the Total Plate Number analysis method, namely (A) "otak-otak" raw material, (B) "otak-otak" dough mixture, and (C) "otak-otak" final products. After going through the incubation stage for 48 hours at a temperature of 35ºC, the number of bacteria in the petri dish were observed and calculated. The interpretation and calculation results of the Total Plate Number on the sample products are presented in Table 1.

Table 1. Total bacterial count on "otak-otak" fish jelly samples

Samples Code	Product	Bacterial Count
A	Raw material	$6{,}2 \times 10^3$
B	Dough mixture	$4{,}3 \times 10^4$
C	Final product	$2{,}5 \times 10^4$

The Total Plate Number analysis used in this study refers to SNI 01-2332.3-2006 [6]. Based on Table 1, there is a change observed in the number of bacteria from raw materials and dough to the final finished product of "otak-otak" samples. The number of bacteria is reduced after being a final product of "otak-otak", this is due to one of the processes of making "otak-otak", namely boiling process. The number of bacteria is reduced because they perish due to hot temperatures during boiling process. According to [7], at least the bacteria will perish or stop their activities at a temperature that is lowered to 0°C or increased above 100°C.

Based on Table 1 as well, the highest number of bacteria in the "otak-otak" dough mixture was 4.3 x 10^4 cfu/g, while the lowest number of bacterial count was found in the raw material of "otak-otak" 6.2 x 10^3 cfu/gram. The number of bacteria in all samples is nevertheless supposed to not exceed the normal threshold for the number that must be present in accordance with [5] (SNI.77557-2013) regarding "otak-otak" fish jelly, which is 5.0 x 10^4 colonies/gram. In addition, regarding to the referred standard of bacterial count in the "otak-otak", the products produced are suitable for consumption. If the number of bacteria in the product exceeds the standard that has been established, the individual consuming it will experience diarrhea, nausea, fever, and so on. [8] stated that changes in microbiological quality resulted in food products being unqualified for marketing and consumption. Many studies show that the consumption of food whose microbiological values deviate or exceed the standard can cause diarrhea, faintness, vomiting, nausea and fever. Even certain bacteria can cause fainting, nerve cell damage and even loss of life [3].

The raw material used in the sample is mackerel fish which is still fresh with suitable handling, consequently that the number of bacteria nevertheless meets the requirements stated in Indonesian National Standard. The high and low number of bacteria in "otak-otak" products is caused by the raw materials used. The analysis results of the raw materials obtained by Total Plate Number method showed that it does not exceed the standard threshold for the number of bacteria that exist according to [9], which is 5.0 x 10^5 colonies/gram.

The quality of the raw materials used is still fresh due to proper handling of the fish from the time the fish is caught until it reaches the consumers. According to [10], mishandled or inappropriate fishery products handling are several reasons which could cause low quality of most fresh and/or processed fishery products. The quality of fresh or processed fishery products is often below the specified requirements due to improper handling of the fish since the fish is caught, during distribution and or when it reaches the consumer.

4. CONCLUSIONS

The Total Plate Number a method is used to determine the number of bacteria in foodstuffs including fishery products. The results showed that there were differences in the number of bacteria before and after the process of making "otak-otak" fish jelly. This is due to the boiling process, hence the number of bacteria is reduced. The "otak-otak" produced in this study are suitable for consumption, namely the number of bacteria is 2.5 x 10^4 colonies/gram. It is nevertheless in accordance with microbiological quality requirements according to Indonesian National Standard (SNI.77557:2013) which standardize "otak-otak" fish jelly (5.0 x 10^4 colonies/gram).

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