DETECTION OF *Salmonella* sp. ON CANNED CRAB PRODUCT

Iis Rostini[1], Rusky I. Pratama[1]

[1] *Staff at Laboratory of Fisheries Processing Product, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Indonesia*

ABSTRACT

Crab (Portunus pelagicus) is one of Indonesian main export commodities to various countries in the world. One of the requirements for the export of fishery products is that they must be free from contamination by microorganisms, including being free from Salmonella. The purpose of this study was to test the content of Salmonella in canned crab products. The research method used is a survey with purposive sampling. The sample used in the identification is canned pasteurized crab meat. The Salmonella testing method used is in accordance with the applicable provisions, namely in accordance with the Indonesian National Standard (SNI) 01-2332.2-2006. There are 4 stages in the test to detect Salmonella in food, namely the pre-enrichment stage, the enrichment stage, Salmonella isolation, and the Salmonella test. Detection of Salmonella on canned crab meat samples showed negative results. This negative result indicates that the sample does not contain Salmonella bacteria. The test process is not carried out until the process of biochemical reactions because the initial process on Bismuth Sulfie Agar (BSA), Hektoen Enteric (HE), Xylose Lysine Deoxycholate (XLD) and Lysine Iron Agar (LIA) has shown a negative sign. Thus canned crab meat is suitable for export.

Keyword : - *canned crab, crab meat, microbiology contamination, Salmonella*

1. INTRODUCTION

Indonesia has a high potential for fishery products to be exported. The crab (*Portunus pelagicus*) has become the mainstay of Indonesian exports to various countries in the world. Indonesia has controlled the crab market share in the United States of 31%, more than 90% of the crabs exported are canned crabs. Of this amount, it turns out that 60% of both fishery products are exported to the United States and currently the country is increasingly tightening the requirements for importing or receiving products that enter the country. This is done so that its citizens are protected from various health threats caused by their food consumption.

Health threats from food can be caused by physical, chemical and microbiological contamination. Among these three contaminants, the most concerning is microbiological contamination. The presence of microbiological contamination in fishery products indicates a decrease in the quality of fishery products. Microbiological contamination can cause various potential illnesses because polluting bacteria are pathogenic bacteria or as contaminants where the origin of these bacteria is not from the fish product itself but from the environment. Factors that influence the presence of microbes are intrinsic factors and extrinsic factors. Intrinsic factors are factors that cannot be controlled by any effort, meaning factors that come from the fish themselves such as the presence of components of food substances needed by microbes, while extrinsic factors are factors that can be controlled by humans, such as preservation and environmental conditions [1].

One of the pathogenic bacteria in fishery products is Salmonella. Symptoms that arise if Salmonella contamination occurs generally are fever, diarrhea, nausea, vomiting, and abdominal pain within 8 to 72 hours after consuming food contaminated by Salmonella. This symptom is called Salmonellosis. In some cases, Salmonellosis can spread to the bloodstream resulting in more severe diseases such as arterial infections, endocarditis and arthritis [2]. The choice of Salmonella testing is because Salmonella is a very dangerous bacterium and a pathogen whose presence absolutely should not be present in the material. Food. Therefore, to be able to be aware of these microorganisms that can be healthy, it is necessary to identify Salmonella in canned crabs. The purpose of this study was to detection the content of Salmonella in canned crab products.

2. MATERIALS AND METHOD

2.1 Materials and Tools

The materials used in this study were canned pasteurized crab meat from the crab canning industry in Cirebon, West Java, Indonesia as many as 3 types of samples. The media used for Salmonella analysis were Lactose Broth (LB) as a pre-enrichment medium, Tetrathionate Broth (TTB) and Iodine as a selective enrichment medium. Rappaport-Vassiliadis (RV), Bismuth Sulfie Agar (BSA), Hektoen Enteric (HE), Xylose Lysine Deoxycholate (XLD), 70% alcohol, aquadest.

The tools used in this study were petri dishes, test tubes, test tube rack, pipette, stomacher, analytical balance, water bath, incubator, inoculation needle, vortex, Bunsen, autoclave, pH meter, spatula, filter apparatus, oven, hot plate and stirrer.

2.2 Research Method

The research method used in this research is the survey method. Sampling using purposive sampling method, namely the technique of determining the sample by considering certain criteria [3]. The parameter observed was Salmonella bacteria colony growth in each sample of canned pasteurized crab meat. The samples were tested at the Fishery Products Processing Laboratory, Universitas Padjadjaran.

2.3 Test Procedure

- Sample Preparation

Samples of canned crab meat were taken at random and cut into small pieces until the weight of each sample to be tested was in accordance with the provisions and the frozen sample was melted at the time of analysis. Melting is carried out for 18 hours at a temperature of 2-5^{o}C or below 45^{o}C and not more than 15 minutes.

- Sample Examination for Salmonella sp. According to [4]

1. Pre-Enrichment

A sample of 25 grams was weighed and put into a sterile plastic bag then added 225 ml of Lactose Broth. Samples were homogenized for 2 minutes for analysis. Aseptically, the sample solution was transferred into a suitable sterile container and left at room temperature for 60 minutes with the container closed. Shake gently and determine the pH to 6.8±0.2. Then shaken well and the lid of the container is loosened sufficiently. Incubated for 24 ± 2 hours at a temperature of 36 ± 1^{o}C.

2. Enrichment

The test tube was tightly closed and shaken slowly, left for pre-enrichment. 0.1 ml of the sample solution was transferred into 10 ml of Rapport Vassiliadis (RV) media and 1 ml of each was taken, then transferred into 10 ml of Tetrathionate Broth (TTB) and 10 ml of Selenite Cystine Broth (SCB). RV media was incubated for 24±2 hours at 42±0.2^{o}C (water bath); TTB was incubated for 24±2 hours at a temperature of 43±0.2^{o}C (water bath).

3. Salmonella isolation

The test tube was shaken using a vortex and a needle loop. After incubation the TTB was streaked onto HE, XLD and BSA media. BSA was prepared one day before use and stored in a dark place at room temperature. RV Broth or SCB etched into the same substrate. HE, XLD and BSA plates were incubated at 35±1^{o}C for 24 hours.

- Observation of typical Salmonella colony morphology

The removal of two or more Salmonella colonies from each agar medium was selective after 24 hours of incubation. Typical Salmonella colonies are as follows:

1. HE Agar, bluish green to blue colonies with or without black core. Generally, Salmonella cultures form large colonies, shiny black nuclei or almost all colonies are black

2. XLD Agar, pink colonies with or without black core. Generally, Salmonella cultures form large colonies, shiny black nuclei or almost all colonies are black
3. BSA Agar, colonies brown, gray or black, sometimes metallic. Usually the media around the colony is initially brown and then turns black with increasing incubation time.

2.2 Data Analysis

Data is presented in tabular form. The results of the Salmonella test were analyzed descriptively with reference to the Indonesian National Standard 01-2332.2-2006.

3. RESULT AND DISCUSSION

Microbiological testing includes quantitative tests to determine the quality and durability of a food, and qualitative tests of pathogenic bacteria to determine the level of safety, as well as indicator bacteria tests to determine the level of sanitation of the food [5]. The analytical method used to identify the presence of Salmonella bacteria, the method used is a qualitative analysis method, which aims to determine the presence or absence of Salmonella bacteria in a food. Identification of Salmonella in canned crab fish products needs to be done so that it is not contaminated with the danger of Salmonella when consuming it. Identification is in accordance with the procedures of the Indonesian National Standard 01-2332.2-2006 so that the results are maximum and in line with expectations. The results of Salmonella bacteria examination on 3 samples of canned pasteurized crab meat are presented in Table 1.

Table -1: Results of Detection of Salmonella Bacteria in canned pasteurized crab meat

Number	Sample	Code	Salmonella Test Result
1	Pasteurized crab meat	A1	Negative
		A2	Negative
2	Pasteurized crab meat	B1	Negative
		B2	Negative
3	Pasteurized crab meat	C1	Negative
		C2	Negative

Based on Table 1, it can be seen that the results of the examination of 3 samples of canned pasteurized crab meat showed that all samples did not contain Salmonella bacteria, so that the three canned crab products met the requirements set by SNI 01-2332.2-2006 which states that food products are not allowed to contain Salmonella. This was caused by unsuitable conditions for the growth of Salmonella in canned pasteurized crab meat.

Canned pasteurized crab meat is a processed product of fishery products with raw materials of crab that undergoes a process of steaming, exfoliating, sorting the type of meat, weighing, packaging in hermetic containers, pasteurization, cooling, labeling and packing in cold conditions [6]. Pasteurization process is a relatively low heating process (generally carried out at a temperature of 60-80^{o}C). The purpose of pasteurization is to kill all pathogenic bacteria, inactivate enzymes and extend shelf life. Pasteurized products must be packaged or stored at low temperatures to control microbial growth [7].

Salmonella testing requires a fairly long stage with complete testing so that it can find out and conclude the presence of Salmonella. There are 4 stages in detecting the presence of Salmonella in food. The first stage is the pre-enrichment stage. Salmonella bacteria present in food may be in small quantities. In addition, the processing process can cause bacterial cells to become damaged. Therefore, pre-enrichment was carried out on selective on media to stimulate growth and restore damaged bacterial cells to a stable condition. The media most often used for pre-enrichment is Lactose Broth. This is because the fermentation of Lactose Broth in the media can lower the pH, so that Salmonella can grow well while other bacteria are stunted.

The second stage is enrichment aimed at increasing the number of Salmonella bacteria by inhibiting the growth of competing bacteria such as Coliform, Proteus and Pseudomonas. Selective media that contains inhibitors and is widely used at this stage is Tetrathionate Broth (TTB). An important factor in the enrichment stage is

agitation during incubation to promote the growth of Salmonella. The addition of oxygen can be done by lowering the height of the sample suspension by using a larger tube, by loosening the tube cap. At this stage all procedures are carried out correctly so that they can show the appropriate results.

The third stage is the agar selective test, namely growing bacteria on selective media and separating the bacteria to be tested from other bacteria. Various selective media have been recommended for growing Salmonella from the enrichment stage. These media contain alkaline nutrients with the addition of dyes, inhibitors or indicators to inhibit the growth of bacteria other than Salmonella and give the characteristics of Salmonella colonies. The media commonly used are BSA, HE and XLD.

At the stage of observing the morphology of a typical Salmonella colony, the samples observed using HE, BSA and XLD media did not show positive or suspected Salmonella colonies in these various media. Thus the next stage is not carried out because if it is continued, the results will remain negative.

Regulations issued by the [8] that fish food and fishery products must not contain salmonella bacteria, and based on SNI 01-2332.2-2006 the quality and food safety requirements for canned crab meat microbial contamination of the type of Salmonella must be negative. If the results of the biochemical test are positive on TSI and LIA Agar, with the results on TSI, Agar is red, and Agar is upright, it is yellow, while on LIA, Agar is tilted and Agar is purple, it is purple in all tubes, further tests must be carried out, namely further biochemistry and serological tests.

Prevention of the dangers of Salmonella contamination in food can be done by controlling sanitation hygiene in food processing. By decreasing the frozen storage temperature and adding a few minutes of cooking time, it can reduce the risk of Salmonella contamination in meat [9]. Reduction of Salmonella contamination in food can be done biologically, physically (sterilization with heat, radiation and filters) and chemically [10]. Biological reduction of Salmonella contamination in food is by using bacteriophages. Changes in temperature from 37^{o}C to 50 and 60^{o}C can kill salmonella [11].

4. CONCLUSIONS

Detection for Salmonella bacteria has been carried out in accordance with the Indonesian National Standard 01-2332.2-2006. Salmonella bacteria test on canned pasteurized crab meat samples showed negative results in all samples. Based on these negative results, it can be concluded that all samples of canned pasteurized crab meat tested did not contain Salmonella.

5. REFERENCES

[1]. Irianto, H.E. dan Gayatmi, S. (2009). “Teknologi Pengolahan Hasil Perikanan”. Universitas Terbuka, Jakarta
[2]. Sartika, D. (2012). “Efektivitas dan Keamanan in vivo fage litik FR38 dari Limbah Domestik dalam Menurunkan Cemaran Salmonella P38 Indigenous pada Sosis, Susu dan Air”. Disertasi. Sekolah Pascasarjana Institut Pertanian Bogor. Bogor.
[3]. Sugiono. (2015). “Statistika Nonparametris Untuk Penelitian”. Alfabeta. Bandung.
[4]. [BSN] Badan Standardisasi Nasional. (2006). “Cara Uji Mikrobiologi-Bagian 2: Penentuan Salmonella pada Produk Perikanan: SNI 01-2332-2-2006”. Badan Standardisasi Nasional. Jakarta.
[5]. Fardiaz, S. (1993). “Analisis Mikrobiologi Pangan”. PT. Raja Grafindo Persada. Jakarta.
[6]. [BSN] Badan Standardisasi Nasional. (2016). “Daging Rajungan (Portunnus pelagicus) Pasteurisasi Dalam Kaleng: SNI 6929:2016”. Badan Standardisasi Nasional. Jakarta.
[7]. Khurniyati, MI., Teti, E. (2015). “Pengaruh Konsentrasi Natrium Benzoat dan Kondisi Pasteurisasi (Suhu dan Waktu) terhadap Karakteristik Minuman Sari Apel Berbagai Varietas”. *Jurnal Pangan dan Agroindustri* Vol. 3, No. 2, pp. 8-12.
[8]. Badan Pengawas Obat dan Makanan Republik Indonesia (BPOM RI). (2009). “Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia Nomor HK.00.06.1.52.4011 tentang Penetapan Batas Maksimum Cemaran Mikroba dan Kimia dalam Makanan”. Badan Pengawas Obat dan Makanan Republik Indonesia. Jakarta.
[9]. Gonzales-Barron, UA., Redmon, G., Butler, F. (2012). “A Risk Characterization Model of Salmonella typimurium in Irish Fresh Pork Sausages”. *Food Research International* Vol. 42, Issue 20, pp. 1184-1193.
[10]. Madigan MT., Martinko, JM., Stahl, DA., Clark, DP. (2012). “Biology of Microorganism”. 13th ed. Pearson Education, Inc. San Fransisco.

[11]. Migeemanathan, S., Bhat, R., Min-Tse, L., Wan-Abdullah, W. (2011). "Effect of Temperature Abuse on The Survival, Growth, and Inactivation of *Salmonella typimurium* in Goat Milk". *Foodborne Pathogens and Disease* Vol. 8, Issue 11, pp. 1235-1240.