Identification of Salmonella SP on Food Preparations (Seasoning and Rakik Chips) in Padang City

I Ketut Budaraga[1,*], Eddwina Aidila Fitria[1], Susilawati[1]

[1]*Agricultural Product Technology Study Program, Faculty of Agriculture Ekasakti Universit Padang, Indonesia*

[*]Correspondent author: iketutbudaraga@unespadang.ac.id

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Abstract. *The research aims to evaluate if the findings of microbiological testing on seasoning samples and rakik chips in Padang City comply with the provisions of SNI 01-4473-1998. Salmnolla sp. identification test in food utilizing the scratch method and a loop needle. The work procedure begins with (a) the sterilization of tools and materials. Glassware such as petri dishes, Erlenmeyer flasks, beakers, measuring cups, and test tubes are wrapped in paper before being sterilized in an oven at 180°C for 2 hours. Non-heat resistant instruments are sanitized with 70% alcohol, whereas metal tools, such as needles, are sterilized by burning them until they become red. (b) Media Creation includes RVS Media, MKTTn Media, BPLS Media, XLD Media, and BPW Creation. The next steps involve sample testing (preparation), sample homogenization, enrichment, isolation and inoculation, and confirmation. Confirmation tests are performed using traditional biochemical testing, as follows: (a) A microscopic examination. (b)Ui IMViC, serological test, and biochemical test using API 20E or RapID ONE kit or kit. Other relevant identification is carried out in accordance with the instructions in the kit. Microbiological testing on seasoning samples and rakik chips with Salmonella sp identification yielded negative results/25 g. The test results obtained using the test settings met the requirements outlined in SNI 01-4473-1998..*

Keywords*: identification, salmonella, seasoning, Rakik chips, food*.

1. INTRODUCTION

Food is defined components consumed on a daily basis to meet the needs of maintenance, growth, work, and the replacement of damaged body cells [1]. As a result, food is extremely important to humans as both a source of nutrition and an energy source. Food, on the other hand, has the potential to damage human health due to physical, chemical, or microbiological contamination [2].

Nowadays, public awareness of food focuses on the nutritional content and safety of the foods ingested. Food safety factors include whether or not food is contaminated with microorganisms, heavy metals, or chemicals that risk human health [3]. Food safety is critical in ensuring that food is safe and fit for consumption. Food safety is critical to ensuring that food is fit for eating. A healthy food supply not only preserves the health of Indonesian people, but also improves the quality of the younger generation. Indonesia already has food-product safety requirements, known as State requirements (SNI) [4]. This standard describes how to create the correct food, measures contamination, and specifies maximum acceptable limits of contamination. It is hoped that this standard can guarantee the safety of Indonesian food products [5].

Along with improving knowledge and understanding of the health of the food ingested, consuming safe food is something that must be paid attention to both producers and consumers [6]. Based on Food Law no. 7 of 1996, food safety is the conditions and activities needed to prevent food from

dangerous biological, chemical and other contamination that might disturb, impair and threaten human health. Safe food is food that does not contain biological or microbiological dangers, chemical hazards and physical hazards [7].

The correct use of food safety standards has been shown to promote food safety while reducing physical, chemical, or biological contamination in food ingredients. The Food and Drug Monitoring Agency is the government entity in charge of distributing food items throughout Indonesia. The Food and Drug Administration not only regulates food but also the distribution of therapeutic items, narcotics, psychotropics, other active drugs, traditional remedies, cosmetics, and hazardous chemicals [8].

In 2015, BPOM observed a food poisoning outbreak, with 61 cases reported from 34 Indonesian provinces. This food poisoning incident could be caused by either suspected or confirmed bacteria. Suspect microorganisms include E. coli, Staphylococcus, and Salmonella sp. Salmonella sp. is one of the bacteria that cause food poisoning [9].

Almost all dietary items are contaminated by microbes from the surrounding environment. Salmonella sp., Staphylococcus aureus, Escherichia coli, mold, yeast, and other dangerous bacteria can all be present in foods. Microbial contamination of food occurs as a result of direct or indirect contact with microbial pollution sources such as soil, air, water, dust, and the digestive and respiratory systems of humans and animals [10]. Only a few of the numerous types of pollution serve as initial sources of bacteria, which then grow in food to a certain extent. Within certain limits, the microbial composition of food has no effect on its durability; nevertheless, if environmental conditions allow microorganisms to thrive and develop more rapidly, the food will suffer as a result [11].

Salmonella sp. is a bacteria that causes salmonelosis. This bacteria dwells in the digestive tracts of people and animals and can be transmitted through food. Salmonellasis instances have been documented in numerous countries. These bacteria can cause infections and pollution practically anywhere in the world [12].

The findings of a study conducted by a research team at the National Institutes of Health in the United States can help explain how Salmonella sp spreads rapidly in humans. This research team uncovered a reservoir in which these germs reproduce fast in epithelial cells, infecting additional cells. A mechanism removes salmonella germs from the epithelial layer, allowing them to infect other cells or multiply in the colon [13].

Salmonella sp. is known to cause digestive organ disorders. Typhoid fever can be caused by Salmonella sp. contamination in food products, and symptoms include high fever, constipation, abdominal pain, disorientation, itchy skin and reddish blotches, and even loss of consciousness [14].The problem is defined as whether there is Salmonella sp bacterial contamination in the food preparations that will be examined, specifically seasoning samples and rakik chips. The goal is to determine the results of microbiological testing on seasoning samples and rakik chips that match the requirements outlined in SNI 01-4473-1998, as well as the method used to test for Salmonella sp in seasoning samples and rakik chips and identify the food samples

2. METHODS

A. Material

This study was conducted at the Padang Food and Drug Administration's (BPOM) Microbiology Testing Laboratory, Jln. Gajah Mada No. 172 (Mount Pangilun), 25137, Padang, West Sumatra. From October 24, 2022 to November 25, 2022. Stomacher bag, Analytical Scale, Tweezers, Scissors, Spatula, Ose needle / sling, 100 ml measuring cup, 1000 ml measuring cup, Spirit lamp, Spray bottle, Laf (Laminar Air Flow/BSC Bio Safety Cabinet), Funnel, Petri dish/petridish, Measuring pipette, Electrical pipette, Test Tube, Test tube/sample rack, Oven, Autoclave, Incubator, Hot plate, Stirring rod, refrigerator. Next, the items used were: spice samples and rakik chips, 25 grams each, obtained at random from a market in Padang City. Buffered Peptone Water (BPW) 225 ml; Aquadest pH 7 450 ml; Muller-Kaufmann Tetrathionate Novobiocin Broth (MKTTn) 10 ml.

B. Research Procedures

1) *Sterilization of Tools and Materials*

Glassware such as petri dishes, Erlenmeyer flasks, beakers, measuring cups, and test tubes are wrapped in paper before being sterilized in an oven at 180°C for 2 hours. instruments that are not heat resistant are sterilized with 70% alcohol, whereas metal instruments, such as needles, are sterilized by burning them in a flame until they become red. The scratch test with a loop needle is used to identify Salmnolla sp. in food.

2) *Media Creation*

a. RVS Media: Medi RVS powder was weighed at 41.8 g and transferred to a 1000 mL Erlenmeyer flask. Then 1000 mL of distilled water (pH 7) was added. The mixture is then cooked on a sticker hot plate until it is homogenous and

boiling. The boiling media was placed in 10 mL test tubes apiece. The Erlenmeyer is closed and placed in cans before being sterilized in an autoclave at 115°C and 1 atm pressure for 15 minutes.

b. MKTTn Media: MKTTn media must be sterile because they cannot be sterilized in an autoclave. MKTTn media was weighed at 89.5 g and transferred to a 1000 mL Erlenmeyer flask. Then 1000 mL of distilled water at pH 7 was added. The media is heated with a hot plate stirrer until it becomes homogenous and boils. Cool to 45-50°C, then add 20 mL of Iodine-Potassium Iodide Solution to the Erlenmeyer along with the rehydration contents of one vial of MKTTn supplement (FD203), stirring until uniformly combined. How to Make Iodine-Potassium Iodide Solution: Weigh 5 grams of potassium iodide, then add 4 grams of iodine and water, shaking until homogenous.

c. BPLS Media: A 1000 ml Erlenmeyer flask was filled with powdered BPLS medium weighing up to 51.0 g. Add distilled water pH 7 until the volume reaches 1000 mL. The mixture is then cooked with a hot plate stirrer until it is homogenous and boiling. In addition, sterilization was performed using an autoclave at 121°C for 15 minutes.

d. XLD Media: Making XLD media requires sterility because it cannot be sanitized in an autoclave. XLD medium was weighed up to 55 g and placed in a 1000 mL Erlenmeyer flask. Add distilled water pH 7 until the volume reaches 1000 mL. The mixture is then cooked with a hot plate stirrer until it is homogenous and boiling.

e. Making BPW: BPW is manufactured by combining 25 g of BPW powder with 1000 cc of water. Using a hot plate stirrer, heat until completely homogenous. The Erlenmeyer is closed with cotton and paper, then tied. It was then sterilized for 15 minutes in an autoclave set to 121°C and 1 atm pressure.

3) *Implementation of Sample Testing*

a. Sample preparation: Before the sample is weighed, the scales, glassware, other tools, and the area around the scales are disinfected with 70% alcohol to maintain them sanitary and clean. The sample component to be opened is first disinfected using 70% alcohol-infused cotton and then opened aseptically with sterile scissors.

b. Sample Homogenization: Samples weighing up to 25 g were placed in a sterile plastic stomacher, mixed with 225 ml of BPW, and then incubated at 37°C for 18 hours. All actions take place in the LAF or BSC (Bio Safety Cabinet).

c. Enrichment: By aseptic approach, 1 mL of incubated BPW culture is taken and inoculated into 10 mL of MKTTn, which is then incubated at 37 °C for 24 hours, and 0.1 mL of BPW culture is inoculated into 10 mL of RVS, which is then incubated at 41.5 °C for 24 hours. The maximum incubation temperature does not exceed 42.5 °C.

d. Isolation and inoculation: The inoculation technique involves taking a selective enrichment culture and employing one of its loops. It is then etched onto the surfaces of the BPLS and XLD media. Next, the plate was incubated upside down at 37°C for 24 hours. Typical and non-typical colonies suspected of being Salmonella sp grew and were observed. On BPLS, colonies are red or light pink with a crimson border around them. On XLD: Red colonies with or without black specks in the center. Notes: 1. Salmonella sp, which does not release H_2S (S Paratyphi A), appears pink with a dark patch in the center. 2.Salmonella sp. that ferment lactose appear yellow with or without black patches.

4) *Confirmation*

The confirmation test is designed to ensure that the bacteria infecting the sample are actually Salmonella sp bacteria. Previously, the BPLS/XLD plate results were inoculated into TSA/NA media and incubated at 37°C for 24 hours. Confirmation tests can be carried out using traditional biochemical testing as follows:

a. Microscopic examination: Gram staining of the TSA/NA culture was performed and viewed using a microscope. Salmonella spIt is a Gram-negative rod-shaped bacteria.

b. IMViC UI: IMViC UI consists of TSIA, serology test, and biochemical test. In TSIA, one cycle of NA/TSA culture was inoculated on TSIA media utilizing a loop of scratching and pricking. The samples were then incubated at 35-37 °C for 24-48 hours. Observe the change in culture color, Salmonella sp develops a red color on the surface (slant) and a yellow color without gas on the bottom (butt) with or without H_2S. In Serology test. A suspension was prepared from the tilted NA/TSA culture using 1 drop of 0.85% NaCl on a glass slide. Polyvalent O Salmonella antisera was introduced into the culture and homogenized by shaking the slide. Observations are conducted for one minute; if agglutination occurs, Salmonella sp is positive. In Biochemical test with API 20E or RapID ONE kit or kit. Other

relevant identification is carried out in accordance with the instructions in the kit.

c. Interpretation of Results: The identification of Salmonella sp in seasoned vegetables and peanut butter yielded no Salmonella sp germs that contaminated the recipe.

d. Condition: Food sample seasoning mixes and rakik chips must be free of Salmonella sp.

For technology and engineering studies, methods should specify the time and place when this research was conducted. All materials such as chemicals and methods, treatment and experimental design should be stated clearly and concisely. References of original methods/procedures must be stated and all modifications of procedures (if any) should be explained.

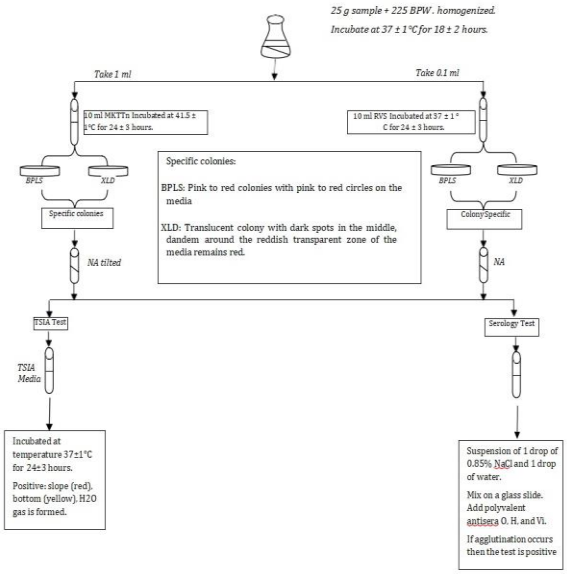

Figure 1. Flow chart for testing Salmonella sp.

C. RESULTS AND DISCUSSION

The Salmonella sp. on food findings reveal that the sample tested meets the Salmonella sp standards, indicating that the food sample tested does not contain Salmonella sp bacteria. Table 1 shows the findings of observations made on seasoning samples and rakik chips cultivated with MKTTn and RVS inoculated on XLD.

According to the table above, these two samples, the seasoning sample and the rakik chips, both yielded negative results/25 gr, indicating that the results of these two samples met the requirements.

Table 1. Observation results of seasoning samples and rakik chips

Sample no.	Enrichment media	Condition	Results	Information
		Negative/25 g	Negative/25 g	
550	MKTTn	Negative/25 g	Negative/25 g	M.S
550	RVS	Negative/25 g	Negative/25 g	M.S
299	MKTTn	Negative/25 g	Negative/25 g	M.S
299	MKTTn	Negative/25 g	Negative/25 g	M.S

[a] 550 = Seasonings.

[b] 299 = Rakik Chips.

[c] M.S = Meets the Requirement.

a b c

Figure 2. Observation results of Salmonella sp bacteria: a and b positive test results (XLD Agar), c negative test results (XLD Agar)

Salmonella sp was tested on food samples that had been seasoned and served with rakik chips. This test is designed to identify the quality of a product based on its packaging or microbiological qualities. Microbiological testing of food samples will always relate to recognized food standards [15]. The Salmonella sp bacteria identification method is based on qualitative examination. This qualitative analytical approach has multiple stages that aim to determine whether or not a microbe is present in food, including pre-enrichment, enrichment, inoculation, identification, and confirmation [16].

The isolation technique for Salmonella sp is based on how the Kit operates, which includes pre-enrichment, selective enrichment, and inoculation on selective media. On the first day, 25 grams were mashed with a stomacher, then placed in Buffered Peptone Water and incubated; on the second day, 0.1 mL of Buffered Peptone Water was incubated in RVS medium and 1 mL in MKTTn media and incubated again. On the third day of culture, RVS and MKTTn were streaked onto various XLD Agar media and incubated. Salmonella sp infection was not detected, as evidenced by the absence of red colonies with black cores on Xylose Lysine Deoxycholate Agar.

The first stage is non-selective pre-enrichment, which attempts to proliferate and repair Salmonella sp bacterial cells caused by sample

handling conditions such as heating, cooling, drying, and pressure. At this point, the media employed is Buffered Peptone Water (BPW), which is a pre-enrichment media designed to assist restore damaged Salmonella sp before being transferred to selective media [17]. BPW medium contains peptone protease, which provides carbon, nitrogen, vitamins, and minerals. In addition, sodium chloride can maintain osmotic equilibrium, and a phoslate buffer mechanism avoids bacterial harm caused by fluctuations in media pH [18].

Next is the enrichment stage, at this stage Rappaport Vassiliadis Soya (RVS) media is used. In RVS media, there is magnesium chloride which causes the medium's osmotic pressure is high so that Salmonella sp bacteria which are able to survive at relatively high osmotic pressure can develop well [19]. Apart from that, RVS media also contains malachite green which can suppress the growth of gram-negative bacteria other than Salmonella sp. So at this enrichment stage the growth of Salmonella sp is optimized and the growth of other bacteria which can interfere with the growth of Salmonella sp is optimized, thereby minimizing false negative results.

Aside from RVS media, this stage also employs MKTTn medium, which is designed to enhance the development of several Salmonella sp species that may be present in the sample. Some Salmonella strains can grow well on RVS media but not on MKTTn, and vice versa. The enrichment stage findings can be noticed after planting in selective media because they do not directly demonstrate the growth of a certain type of microbe [20]. The next stage is inoculation and identification, which involves the use of BPLS and XLD medium.

XLD media, which contains sodium deoxycholate, lactose, phenol red, sucrose, and agar, inhibits gram-positive bacteria and non-enteric gram-negative bacteria, resulting in the growth of only Salmonella sp bacteria on this medium. The presence of translucent colonies with a black spot in the middle and surrounded by a reddish transparent zone indicates that the media is positive.

Salmonella sp bacterial colonies are black because they can produce hydrogen sulfide (H2S). If it does not produce H2S or thiosulfate reductase, the colonies that form will be white or not clear. Lactose, peptone, bile salts, iron (III) citrate, and retusal red indicator are the most important components of XLD media that contribute to its selectivity [21]. The metabolic capacities of these microorganisms serve as the primary basis for differentiation. Bacteria from the Salmonella genus can create H2S and thiosulfate reductase, resulting in dark black colonies and an unpleasant odor [22].

Salmonella sp colonies on Brilliant-green Phenol-red Lactose Sucrose Agar (BPLS agar) media grow pink with a red zone surrounding them. This is because the lactose in this culture media is destroyed and becomes alkaline, as demonstrated by the phenol red pH indicator, which changes color to dark red [23]. The brilliant-green color significantly inhibits the growth of the accompanying Gram-positive microbial flora, Salmonella sp. [24]. A more nutrient-rich environment, on the other hand, aided Salmonella sp growth. The resulting rise in microbe growth is significantly reduced by raising the Brilliant-greent concentration. Salmonella sp. cannot ferment lactose or sucrose. In contrast to BPLS agar, the sucrose in this medium enables for the identification of lactose-positive or lactose-negative microorganisms that are sucrose-positive [25].

The colonies that grew on BPLS and XLD media did not display specific colony growth for Salmonella sp, implying that the samples tested were negative. Therefore, there is no need to conduct more tests. Thus, the sample is deemed to meet the Salmonella sp test parameters. According to SNI 01-4473-1998 [26], the sample examined was deemed negative because no typical signs of Salmonella sp bacteria were detected. Characteristics that indicate the presence of Salmonella bacterium. If the sample is incubated for 24 hours, it will smell terrible and then be isolated on XLD media, resulting in a red colony with or without a black patch in the center.

The presence of seasoning samples and rakik chips that are declared negative after a microscopic examination indicates that the sample has undergone microbiological testing up to the point of being declared negative. The seasoning samples and rakik chips were physically free of bacteria and fungi, as evidenced by their shape, texture, color, flavor, and packaging. As a result, a microbiological test is required to see what the naked eye cannot see, as well as additional sample testing to confirm that the sample includes salmonella sp bacteria.

D. CONCLUSION

Microbiological testing on seasoning samples and rakik chips with Salmonella sp identification yielded negative results/25 g. The test results obtained using the test settings met the requirements outlined in SNI 01-4473-1998.

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