Wahana Matematika dan Sains: Jurnal Matematika, Sains, dan Pembelajarannya,
Vol. 14 No 1, April 2020
e-ISSN: 2549-6727 , p-ISSN: 1858-0629

Contamination Test Of Salmonella Sp. In Household Industry White Tofu Naimata Kupang

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Abstract

Industries in Naimata has been conducted. The test was conducted at the Microbiology Laboratory of the Food and Drug Supervisory Agency of the Province of East Nusa Tenggara in Kupang on August 3 - August 8, 2016. The research based on the level of consumption of tofu produced in home industries in Naimata is quite high by the community. The purpose of this study was to determine whether the white tofu produced in the household industry in Naimata was contaminated with Salmonella bacteria or not. The research process consists of three stages, namely, the Pre-enrichment stage, where the sample is weighed 25 grams aseptically and then mixed into 225 mL BPW media and incubated at 37 ° C for 24 hours, enrichment stage is the stage where 1 mL of culture in the previous stage was taken and 0.1 mL was then added to the media so that MKTTn and RVS were 10 mL which were then incubated at 37 ° C and 42.5 ° C for 24 hours, the Inoculation and Identification stage was culture from MKTTn media and RVS was taken then planted in the media XLD and BGA specific. The data analysis method used is only comparing the test results from the research sample with SNI: 01-3142-1998 regarding the Quality Requirements of Tofu as a reference. This test is also made a positive comparison or control, which is also made XLD and BGA media planted with culture of Salmonella bacteria. The results of the ceramics test of Salmonella bacteria in white tofu stated that the white tofu produced in the home industry in Naimata was not contaminated with Salmonella bacteria Sp. with a negative value of colonies / 25 grams of samples according to the Indonesian National Standard SNI: 01-3142-1998 regarding the quality requirements of tofu.

Keywords: *Bacterial Contamination ; Salmonella Sp ; White Tofu ; Home Industry*

INTRODUCTION

Food is a basic need that is very important for the life of every human being both physiologically and psychologically. Food development is carried out as a development effort across sectors that is related to meeting people's food needs evenly in terms of quantity and nutrition. The success of the food development of the Indonesian people will be influenced by the ability in the fields of production, processing, marketing and distribution of food. This can be realized if it is supported by the capacity of the processing industry sector that is adequate (Seto, 2001) [14].

Small industries have a very large role in the wheels of the economy of a country. According to Anoraga and Sudantoko (2002) [3], the role of small businesses can increase non-oil exports, employment, improve the quality of human resources and contribute to gross domestic product (GDP), this is a challenge for small entrepreneurs to improve their business. One small industry that has the potential to be developed is the tofu manufacturing industry.

Tofu is a food that is widely consumed by the public both as a side dish and as a snack. Tofu contains approximately 75% of water in addition to protein, carbohydrates and fat. This product is easily damaged so that it is a suitable medium for the growth of pathogenic microorganisms that cause the tofu to be stale and foul-smelling. Some diseases that are transmitted to humans through food include *Botulism*, *Salmonellosis*, and food poisoning by *Staphylococcus* (Limbong, 2003) [10].

The increase in tofu consumption by the people of Kupang city must also be supported by hygienic supervision both during the manufacturing process and during processing and storage to minimize contamination by microbes (Limbong, 2003) [10]. According to the results of research obtained by previous researchers, it was shown that tofu taken from the market of Kasih city Kupang positively containedbacteria *Salmonella Sp* and tofu taken from the home industry in Oebufu negatively containing *Salmonella Sp.* the researchers felt interested in researching whether or not contaminated white tofu was produced in the Home Industry in Naimata.

Bacteria *SalmonellaSp.* is a genus of *entro bacteriaceae* which can contaminate food and can multiply rapidly because the conditions of hot and humid environments stimulate its growth. Places that allow the spread of *Salmonella Sp.* for example: in homes, restaurants, dormitories, hotels, soybean factories and so on (Imam and Sukamto, 1999) [18].

Out whether there is contamination ofbacteria *SalmonellaSp.* in white tofu produced in the home industry at Naimata to be compared with SNI: 01-3142-1998 regarding the quality requirements of tofu.

RESEARCH METHODS

A. Types of Research

Using descriptive research types.

B. Location and Time of Research

study was conducted in the Microbiology Laboratory of Kupang POM Hall in August 2016.

C. Population

The population in this study was white tofu produced in the home industry in Naimata.

D. Samples and Sample Techniques Samples

Samples are taken from a population of 25 white tofu with a *random sampling techniquerandom sampling* becauseof members from the population regardless of the

strata in that population. This way is done if members of the population are considered homogeneous or have similarities. Thus, members of the selected population will be able to represent the condition of the population.

E. Research Variables Research

variables in the form of a single variable that is knowing whether or not there is contamination ofbacteria *SalmonellaSp*. in white tofu produced in the home industry in Naimata.

F. Operational Definitions

1. Tofu is a product made from soy protein obtained from home industries in Naimata.
2. Test for contamination, namely the test phase in the form of pre-enrichment test, enrichment and identification tests conducted to determine whether or not a substance is contaminated by *Salmonella Sp.* on tofu obtained from home industries in Naimata contamination
3. *SalmonellaSp.* is *Salmonella Sp*. originating from soil, water and air also come from equipment and facilities used during the process of making tofu in home industries in Naimata
4. Bacteria *SalmonellaSp.* is a bacterium that will be tested on white tofu produced in the home industry of Naimata Stomacher

G. Tools and Materials

1.tools

bag, test tubes *(Iwaki Pyrex),* 1.0 mL pipette volume *(Iwaki Pyrex),* 10 pipette volume, 0 mL *(Iwaki Pyrex),* Erlenmeyer *(Iwaki Pyrex),* Autoclave *(Hirayama),* Bunsen lamp, *Laminar air flow (Esco),* Needle osse,*Memmert*, *Incubator* (*Memmert*), Petri dish *(Normax)*

2. Materials

White tofu samples from home industries in Naimata, *Buffered Peptone Water* (BPW), *Muller Kaufmann Tetrathionate Novobiocin Broth* (MKTTn) , *Rappaport Vassiliadis Medium + Soya* (RVS), *Brilliant Green Agar* (BGA), *Xylose Lysine Deoxycholate* (XLD)

H. Research procedures

1. Field Observation

Covers the environment where sampling, seller hygiene and environmental sanitation.

2. Sampling Procedure The

tofu sample was taken aseptically by ± 25 g, then put into a *glass beaker* sterile.

3. Preparation of Tools and Materials

Before conducting the experiment, all laboratory equipment to be used in the research are sterilized and the materials are ready for use.

a. Sterilization of equipment that includes petri dishes, pipette volumes, test tubes, erlenmeyer, beaker glass, is carried out in the following way

1) Equipment that is ready to be sterilized, put into the oven and sterilized at 180 ° C for 2 hours.

2) If it has reached 2 hours. The oven lid is opened and left to cool

3) The tools are ready for use.

b. Preparation and sterilization ofmedia *Buffered Peptone Water* (BPW), *Muller Kaufmann Tetrathionate Novobiocin Broth* (MKTTn), *Rappaport Vassiliadis Medium + Soya* (RVS), *Brilliat Green Agar* (BGA), *Brilliat Green Agar* (BGA), *Xylose Lysine Deoxycholate* (XLD)

4 Bacteriological examination (Anonymous, 2002)

a. Aseptically weighed 25 grams of cuttings were cut into a suitable sterile container, added 225 mL *BPW*, homogenized using a *stomacher*, then incubated at 37 ° C for 18-24 hours.

b. Enrichment

By aseptically piped pre-enriched cultures of 1 mL each into 10 mLmedia *MKTTn* incubated at 37 ° C for 24 hours and 0.1 mL into 10 mLmedia *RVS* incubated at 41.5 ° C for 24 hour. So that the maximum incubation temperature is not more than 42.5 ° C.

c. Inoculation and Identification

Ofcultures *MKTTTn* and *RVS* were inoculated 1 each at the surface of *BGA* and *XLD*, then incubated at 37 ° C for 24 hours, the growing colonies were observed.

Culture suspected of *Salmonella Sp.* positive, if:

At the *BGA*	:	colonies are colorless, pink to red, and transtulent to cloudy with pink to red circles.
In *XLD*	:	transparent colonies with black spots in the middle, and surrounded by a reddish transparent zone.

RESULTS AND DISCUSSION

analysis of the results is done by comparing the test results with the standard limits for contamination ofbacteria *SalmonellaSp*. according to the Indonesian National Standard SNI: 01-3142-1998 namely Negative / 25 g.

This study was conducted on white tofu samples obtained from home industries in Naimata to obtain data on the presence or absence of contamination ofbacteria *SalmonellaSp.* in knowing what is produced by the industry. The sampling process is done by aseptic method, ie the sample is taken randomly from the upper right corner to the lower left corner (diagonally), inserted in aOffice in *glass beaker* sterileand then taken to the Microbiology laboratory of the Food and Drug SupervisoryKupang to be crushed before weighing as many samples 25 grams for testing to the next stage.

A. Pre-enrichment

test This test generally uses non-selective search media that contains enough nutrients to strengthen very weak or diseased bacterial cells caused by food processing (BPOM, 2009). In this test, the results obtained were tofu samples that had been suspended withmedia *BPW* as much as 225 mL then incubated at 37 ° C for 24 hours. The results obtained were that the suspension had undergone a color change from the previous cream to brownish color. These results show that in the sample there are microbes but it is not certain that *Salmonella* but the bacterial growth activity in the sample may be due to factors - factors such as:

1. The cleanliness of the environment and air where the food products are made.
2. Cleanliness of workers when carrying out food production processes
3. Cleanliness and sterility of tools and materials used during the production process, and
4. During the food storage process.

B. Test enrichment

This test is carried out using selective liquid media (*MKTTn* and *RVS*) enriched with sufficient nutrients so that bacterial colonies can be isolated (BPOM, 2009) [4]. In this test, themedia *MKTTn* and *RVS* used were 10 mL sacks, each of which was prepared in a test tube. Bacterial culture onmedia was *BPW* taken as much as 1 mL each to be mixed intomedia *MKTTn* and 0.1 mL to be mixed intomedia *RVS* then incubated at 41.5 ° C ± 1 ° C for 24

hours. The results obtained after incubation were opaque blue color onmedia to *MKTTn* be somewhat cloudy and onmedia *RVS* which was previously clear translucent blue became rather dark because of the microbial life of the media. Based on the results of this test, it can be seen that the test sample contains microbes but it is not certain that it is a type of *Salmonella*.

C. Inoculation and identification tests for *Salmonella.*

Each microbial colony to be identified must be completely pure and to obtain pure culture selective media are used which allow for the isolation of suspect microbial colonies based on the biochemical characteristics of microbes which will affect the bacterial growth properties of a specific medium, namelymedia *BGA* and *XLD* (BPOM, 2009) [4].

Tests at this stage will be taken for each bacterial culture loop onmedia *MKTTn* and *RVS* suspected of *Salmonella* to be scratched onmedia *BGA* and *XLD* by the quadrant stroke method which will then be incubated at ± 37 ° C for 24 hours. Based on the results of observations made at this stage, negative results were obtained because the BGA and XLD media did not show a collection of bacterial colonies that showed specific characteristics as listed in the literature.

In this test positive controls were also made on BGA and XLD selective media planted with cultures of Salmonella Thypimuruium bacteria. Based on observations obtained when plantingculture *Salmonella* on specificmedia *BGA* and *XLD* , it was seen that there wasbacterial colony *Salmonella* a growing, the testing would be carried out with a confirmation test.

Table 1.Bacteriology Test Results *Salmonella Sp.*

No.	Samples	Pre - enrich ment	Enrichment		Inoculation and identification	
			MKTTn	RVS	BGA	XLD
1.	whiteKnow	Cloudy	Cloudy	Cloudy	(-) *Salmonella*	(-) *Salmonella*

2.	positive control *SalmonellaThypimurium*	Cloudy	Cloudy	Cloudy	(+) *Salmonella*	(+) *Salmonella*

(source: primary data research , 2016)

In the confirmation test, the results of positive control cultures were taken 1 cone and then planted on Nutrient Agar media and then incubated at 37 ° C for 24 hours. *Salmonella is* positive if the biochemical test and serological test results are as follows:

1. *TSIA* : butt (+), slant (-), positive or negative gas and H_2S positive or negative.

2. urea hydrolysis: negative

3.test *β-galactosidase* : negative

4.production: negative

Indole5. Proskauer voges reaction: negative

6. Serological test: agglutination occurs in addition of polyvalent antisera O, H, and Vi.

D. The confirmation test stages are as follows:

1.Test *TSIA*

In thetest the *TSIA* color of the media slant turns red because these alkaline bacteria indicate that this bacterium does not ferment lactose and sucrose. In the medium the yellow butt area of the media turns yellow indicates the bacteria ferment glucose. Positive gas formation is the result of fermentation of H_2 and CO_2 can be seen from the outbreak and the lifting of the order.Hformation$_2$PositiveSis indicated by the presence of black deposits. TSIA to contain lactose and sucrose in a concentration of 1%, 0.1% glucose and phenol red as an indicator that causes changes in color from orange to yellow in an acidic atmosphere. TSIA also contains sodium trisulfate, which is a substrate for producing H_2S, ferro sulfate produces FeS (precipitat), which is black to distinguish H_2S bacteria from other bacteria.

Based on the results of themedia test *TSIA*, it was seen that there was a black precipitate at the bottom of the tube which indicated the presence of bacterial activity suspected of *Salmonella.*

2. Test *Urease*

Urease test is used to determine the ability of microbes to hydrolyze urea to ammonia. The urease enzyme will break down urea into ammonia. The urease test shows a positive result if there is a change in color from yellow to purplish red. The urease test results are negative if there is no change in color from yellow to purplish red.

Based on the results of this test the results obtained were negative because there was no change in color from yellow to purplish red.

3. Test of *β-galactosidase,*

tests *β-galactosidase* used weeks to identify several types of bacteria such as *Salmonella.* The *beta-galactosidase* enzyme is an enzyme that can convert lactose to glucose and galactose. Some microorganisms such as E. coli, can use lactose as a carbon source. Apart from lactose, the natural substrate of this enzyme is a very important ingredient and also ONPG discs (o-nitro-phenyl-β-D-galactopyranoside). Gal-galactosidase can catalyze ONPG to galactose and o-nitrophenol. ONPG is colorless but after hydrolysis to o-nitrophenol, yellow will appear in an alkaline solution. several types of bacteria that are capable of fermentingcarbohydrates *Streptococcus, Lactobacillus, Zygomonas, Saccaromycetes, Escherichia, Enterobacter, Salmonella.*

Based on the above test, the results obtained after addition ofdiscs *ONPG* and incubated for 24 hours at 37 ° C were negative because the solution did not change to yellow color.

4.Test *IndolIndol*

Test aims to determine the ability of bacteria to break down the amino acid tryptophan. This media is usually used in rapid identification. The indole test results obtained are negative because there is no pink (ring) layer formed on the culture surface, meaning that these bacteria do not form the indole of tryptopan as a carbon source, which can be identified by adding the kovacs solution. The amino acid tryptophan is a component of amino acids that is commonly found in proteins, so that these amino acids can easily be used by microorganisms due to protein decomposition.

5. Proskauer Voges test Voges Proskauer

Test aims to identify the type of bacteria to distinguish *Escherichia coli bacteria, Enterobacter aerogenes* and *Salmonella*. The result of this test is negative, because there is no red color on the medium after adding alpha - napthol and KOH, meaning that the end result of this bacterial fermentation is not acetyl methyl carbinol (acetoline).

6. Serological Test

In this test after adding polyvalent O antisera then drops of a few drops of aquades and observed with a dark background it turns out that the results of agglutination have occurred.

Because the results of testing from bacteriological tests on different samples with positive control results, the colonies that grew from BGA and XLD cultures in the sample were declared notbekteri *Salmonella,* so the results of this test could be expressed as negative colonies / 25 grams. These results have met the requirements as in SNI 01-4473-1998 which requirescontamination *Salmonella* to know is a colony / 25 gram negative.

CONCLUSIONS AND RECOMMENDATIONS

A. Conclusions

Based on microbiological testing conducted at the Laboratory of Drug and Food Control in Kupang on August 3 - 8, 2016 against white tofu samples obtained from home industries in Naimata, the results obtained were non-polluted samplesbacteria *SalmonellaSp* (negative colony / 25 g sample). In other words, the samples tested have met the quality requirements of the Indonesian National Standard SNI 01-01142-1998 regarding the quality requirements to know the parameters tested werecontamination *Salmonella*

Table 2. Quality requirements to know according to SNI: 01-3142-1998

No	Test Type	Unit	Persyratan
1	Odor	-	Normal
2	Flavors	-	Normal
3	colors	-	Normal white or normal yellow

No	Test Type	Unit	Persyratan
4	Sightings	-	Normal, not slimy and not moldy
5	Abu	% (b / b)	Maximum 1.0
6	Protein	% (b / b)	Minimum 9.0
7	Fat	% (b / b)	Minimum 0.5
8	Crude fiber	% (b / b)	Maximum 0.1
9	Lead Containers	Mg / kg	Maximum 2.0
10	Copper	Mg / kg	Masksimal 30.0
11	ContaminationsZeng	Mg / kg	Maximum 40.0
12	ContaminationsTin Contaminants	Mg / kg	Maximum 40.0
13	Contaminants Arsenic	Mg / kg	Maximum 1.0
14	Contents of *Eschericia Coli*		Maximum 10 APM
15	Contamination Salmonela		Negative/25 g

(source: SNI 01-3142-1998)

B. Recomendations

1. For tofu producers, it is expected to always maintain hygiene during the production process so that the quality produced is maintained so as not to endanger the consumers.
2. For the community to be able to pay attention to good food processing methods.
3. For students, hopefully this paper can be useful as additional knowledge and also as a reference for conducting further research related to bacterial contamination of food products.

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