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Coliform Quality Test on Tofu Samples in Three Markets in Medan City

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ABSTRACT

Tofu is one of the products that are often consumed in the community because the price is cheap and easy to obtain. Tofu has high protein and water content and pH is close neutral. This makes tofu a suitable medium for growing pathogen microbial in food. The presence of pathogen microbial in tofu can cause health problems for those who consume them. **Objectives** This research was conducted to observe the microbial content in tofu snacks in three Medan city markets. **Method** This research was conducted by conducting laboratory tests and the results obtained in the form of a description of microbial content in the sample of tofu. The content of coliform microbes in tofu samples was tested using the Most Probable Number (MPN) method and biochemical identification. The tofu sample was selected using total sampling method from three markets in the city of Medan. **Results** A total of 15 tofu samples from three markets were analyzed in this study. From the MPN test, almost all samples had MPN levels exceeding the limits set in the Regulation of the Badan Pengawas Obat dan Makanan No. 13 of 2019, which is below 3 MPN / g. Five samples of tofu (33.3%) of E.coli bacteria, 9 samples (60%) of Klebsiella sp and 1 sample (6.7%) contained Enterobacter sp. **Conclusions** Bacteria found in this study were Escherichia coli, Klebsiella sp and Enterobacter sp. The presence of microbial content in the tofu sample that exceeds the threshold must be a concern of various parties in the supervision of snacks being sold.

Keywords: coliform, identification, most probable number, tofu

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INTRODUCTION

Everyday, humans need to consume proteins to assist the body in carrying out its physiology process. Protein intake can be obtained through dairy and plant-based products. One of the alternative products that is rich in protein is Tofu. Based on the National Socio-Economic Survey (SUSENAS), in March 2018, Tofu consumption in Indonesia reached 0.158 kg per capita in a week. Tofu consumption continues to increase throughout the years due to its relatively low price and the fact that it has been widely known by the community.[1]

Bean curd or otherwise known as tofu is one of the oldest snacks in the world, which is believed to have originated from during Han Dynasty about 2000 years ago.[2] The basic principle of making tofu is to utilize the presence of protein in soybeans that will coagulate when mixed with vinegar. The water from the soybean essence is separated by pressing or squeezing the soybeans, which will leave out a lump of protein. These particular lump of protein is what referred as Tofu.[3]

These procedures are the reason why Tofu is rich in protein. The composition of Tofu contains about 6.0% - 8.4% protein, 79% -87% water and has a pH close to neutral (5.2-6.2).[4] These composition makes tofu a suitable medium for microbial growth, owing to the fact that it is rich in protein, water and has a suitable pH for microbes.[5] Hence why tofu should be consumed immediately, and furthermore, it can only be stored for 2-3 days at 4°C.[6] Microbes that often cause damage to foods with high concentration of water and an almost neutral pH, are Bacteria, such as, *Bacillus sp, Klebsiella sp, Coliform, Pseudomonas sp, Staphylococcus sp* and *Leuconostoc sp.*[7] The existence of these microbes will not only cause the quality of tofu to decrease, but also able to bring diseases to individuals who consume them.

The existence of pathogenic microbes in tofu can cause food-borne diseases. The symptoms of food borne diseases usually share similarities to gastrointestinal symptoms, which includes nausea, vomiting, abdominal pain and diarrhea.[8] These microbial contaminations can originate from both the production process and environmental contamination such as water, soil and air.[9] In addition, these contaminations can also originate from food-handler, a person who plays a direct role in food management. A study by Nenni et al (2019) discovers that equipments and hands used by food handlers were contaminated with *E.coli, Klebsiella pneumonia,* and *S.aureus.*[10]

In testing the quality of microbial content in food, it is not possible to detect any pathogenic microbes in food samples. To detect the content of microbial contamination in a sample such as water, food, or agricultural products, indicator microbes are needed. Indicator microbes that are widely used to measure the content of microbial contaminants are coliform group bacteria. Coliform group bacteria is a group of gram-negative bacteria, which are in the form of non-spherical rods, facultative aerobics, and can ferment lactose characterized by the formation of CO2 gas within ± 48 hours at 35°C.[11,12,13]

About 10% of microbes in the digestive tract of humans and animals are coliform bacteria.[13] So if this coliform bacteria pollutes a water source, it indicates that the water source has been contaminated with faeces.[11] Tofu that is contaminated with coliform group bacteria indicates that the tofu has been contaminated feces. This can happen if the water used in making tofu is not clean or self-hygiene and equipment hygiene are ignored during the process of making tofu. The presence of coliform bacteria in a sample of water or food makes the water or food not suitable for consumption.

To measure the levels of coliform bacteria in a sample, presumptive, confirmatory and complementary tests are performed. The whole process of presumptive,

confirmatory and complementary tests requires at least 4 days since incubation. The number of tubes in the presumptive test that gives positive results can indicates the number of microbes in the sample.[13]

Very few studies regarding the microbial content of Tofu in Indonesia can be found at the moment. However due to the high consumption of tofu in Indonesia and the possibility of microbial contamination, leads to the aim of study which to identify the microbial content of the tofu sold in various market in Medan.

Methods

This descriptive study with a microbial content test is conducted at the Microbiology Laboratory of the Faculty of Medicine, University of North Sumatra. The sample size was chosen by using total sampling, the sample came from all tofu that was entirely exposed to environment sold in three different markets. The type of Tofu used in this study is white tofu. Samples that have been purchased are then put into sterile containers and sent to the Microbiology Laboratory in a span of less than 2 hours. Before being tested, 25g of tofu was weighed, then diluted by using 225mL of Buffered Peptone Water (BPW) (10^{-1}dillution). Then, as much as 10 ml of tofu sample is transferred into a sterile tube.

The quality test of microbial content was carried out using the Most Probable Number (MPN) technique. This method is generally used to calculate the estimated number of microorganisms in food, water and agricultural products.[11] The first MPN test (presumptive test) divided into 3 groups of tubes, each group contained 5 tubes housing a medium of 10 mL lactose broth and within each tube, a Durham cylinder positioned upside down to trap the gas formed is placed. A total of 1 ml of tofu sample was diluted into a second tube containing 9 mL of sterile water (10^{-2} dillution) and from the second tube, another 1 ml was taken and diluted back to the third tube containing 9 mL of sterile water (10^{-3} dillution). Every 1 ml from the 1st dilution tube is put into group 1 tube, the 2nd dilution is put into group 2 tube and so on. Then the lactose broth tube was incubated for 24-48 hours at 35°C ± 2°C.

The lactose broth tube with a positive result(marked by the presence of gas formed), using a loop, move the positive culture into the Brilliant Green Bile Lactose Broth (BGBLB) tube(confirmed test) that contained an inverted Durham cylinder. Then the tube was incubated for 24-48 hours at a temperature of 35°C ± 2°C.[11,14] The positive results were marked by the formation of gas in the Durham cylinder. For bacterial identification, culture from BGBLB media was planted into Eosin Methylene Blue (EMB) media .

Besides being cultured on EMB media, the test bacteria will also be put on the following biochemical test:[15]

- Indole Test

Indole test aims to detect the ability of bacteria to produce the tryptophanase enzyme (to break down the amino acid tryptophan into indole). Indole test results are said to be positive if red rings are found on the surface of the media and negative if yellow rings form on the surface of the media. Positive test results were found in *E. coli, Proteus vulgaris, Vibrio cholerae bacteria* and negative test results were found in *Klebsiella, Shigella sp, Salmonella sp, Pseudomonas sp bacteria.*

- Methyl-red

On glucose phosphate media, some bacteria ferment glucose to produce acids (lactate, acetate) which will make the pH below 4.4. The results are said to be positive if the media turns reddish on the test of *Escherichia coli* and *Proteus* bacteria. The results are said to be negative if no media discoloration (yellow) was found in the test of *Klebsiella* and *Enterobacter* bacteria

- Voges-Proskauer Test

This test is carried out on media that uses glucose phosphate, as in the methyl-red test. Some types of bacteria can produce acetoin as the final product of glucose fermentation. Acetoin will be oxidized to diacetyl (when 40% KOH is added) and this acetyl can react with α-naphthol which will give a red color to the media. Positive results were found in the *Klebsiella pneumonia and Enterobacter* bacteria, while negative results were found in the *E.coli, Shigella,* and *Salmonella* bacteria

- Simmon's Citrate

This test detects the ability of some bacteria to use citrate as a carbon source for their growth by producing alkaline metabolite products. This test is said to be positive if the media changes from green to blue in the *Klebsiella, Citrobacter,* and *Enterobacter* bacteria test

- Urease test

This test is to observe the bacteria that produce urease to break down urea into ammonia. The test results are said to be positive if the media color becomes pink in the *Helicobacter pylori, Proteus,* and *Klebsiella pneumonia* bacteria test and negative in the *E. coli, Shigella* and *Salmonella* bacteria test.

- Triple Sugar Iron (TSI) Test

This media contains 3 types of sugars, namely glucose, sucrose, and lactose with a ratio of 1:10:10. The media also contains phenol red as an indicator of acid production and ferric salt as an indicator of H_2S production.

- Sugar Fermentation Test

This media is used to test bacteria that can ferment carbohydrates (sugars). This sugar fermentation test generally uses glucose, lactose, sucrose, mannitol and maltose.

Result and Discussion

From three different markets, there were 15 tofu samples to be analyzed in this study. The presumptive test is the initial stage in the microbial content test in this study. That uses the properties of coliform bacteria which can ferment lactose as a carbon source to form acids and gases hence the results are deemed to be positive and meant that the samples may contain coliform bacteria.[16] The presumptive test in this study found that all samples were positive (gas formation occurs in the Durham cylinder, table 1).

Table 1. The presumptive test results

Sample	Total of Positive Tubes			MPN	Microbe Quality
	10^{-1} mL	10^{-2} mL	10^{-3} mL		
T1	1	0	0	2	Safe
T2	5	1	0	33	Not Safe
T3	5	1	1	46	Not Safe
T4	4	1	1	21	Not Safe
T5	3	1	1	14	Not Safe
T6	2	0	0	4,5	Not Safe

T7	1	1	1	6,1	Not Safe
T8	1	1	0	4	Not Safe
T9	1	1	1	6,1	Not Safe
T10	4	1	1	21	Not Safe
T11	5	1	1	46	Not Safe
T12	3	1	1	14	Not Safe
T13	5	1	1	46	Not Safe
T14	5	1	1	46	Not Safe
T15	5	1	1	46	Not Safe

The MPN method uses the principle of gradual dilution to measure the approximate number of microbes in a sample. The MPN method is useful for measuring microorganism concentrations below 100 CFU / g in water, food and agricultural product samples.[17] The MPN value of a sample is perceived by using a combination of five tubes that give positive results. Then the combination is matched using the MPN / 100 mL index table. A total of 14 tofu samples in this study has MPN values that exceeds the microbial contamination threshold (4-46 MPN / g). Based on the regulations from the Food and Drug Supervisory Agency No. 13 of 2019, the microbial content in the tofu sample must be under 3 MPN / g.[18] This illustrated that the tofu sample is not fit to be consumed raw.

From the positive presumptive test results, the confirmation test was continued using BGBLB media. This confirmation test aims to verify the presence of coliform bacteria from the positive presumptive results, this is done because other bacteria besides coliform can ferment lactose, therefore, it can give the impression of a positive result in the presumptive test.[16] Cultures from lactose broth media are planted into BGBLB media that contain gallbladder, served to inhibit Gram positive bacteria, whereas Brilliant green, used to inhibit certain types of Gram negative bacilli bacteria so that it helps fertilize coliform bacteria.[19]

The positive culture results of BGBLB is then planted into EMB media. This media contains lactose and sucrose as a source of growth of Gram negative bacteria. Other than that, the content of methylene blue in the media is used to inhibit Gram-positive bacteria and the content of the eosin Y dye can change from colorless to blackish purple due to acidic atmosphere. *E.coli* bacteria that are cultured on EMB media will give the characteristic of a metallic green colony (figure 1).[20]

Figure 1. *E.coli* metallic green colony on EMB media

To find out the type of coliform microbes in the tofu sample, an identification test consisting of indole, urease, Simmon's citrate, TSI, methyl red, motility, Voges-Proskauer tests, and confectionery tests (figure 2) was conducted. From the biochemical identification test, 5 samples of tofu (33.3%) contain *E.coli* bacteria, 9 samples (60%) of *Klebsiella sp* and 1 sample (6.7%) of *Enterobacter sp*. The three types of bacteria found in this study were coliform microbial groups. Coliform microbes refer to a group of Gram-negative, rod-shaped, facultative aerobics, non-spore, lactose fermenters and can produce gas within 48 hours at 35°C.[11,12,14]

Figure 2. Bacterial identification test (from left to right, sugar fermentation test +, methyl red -, indole -, *Voges-Proskauer +, citrate* +, urease +, motility -, TSI {A/A, gas +, H_2S -})

A study conducted by Verawati et al (2019) discovers two tofu samples from two tofu industries were found to have positive samples containing coliform bacteria and Salmonella sp.[21] Another study by Ananchaipattana et al (2012) analyzed 133 tofu samples, found that there were 10 types bacteria present. Out of the 10 types of bacteria the 3 most commonly found were *Enterococcus sp,* lactic acid bacteria and *Pseudomonas sp.*[22] Tofu samples in Brazil were found to contain *E.coli* and *Staphylococcus sp.* and most of the samples contain bacteria that exceeds the specified safety limit, as reported by Ribeiro et at (2017).[23]

To evaluate fecal contamination in water and food products whose sanitary conditions are unknown, a test is needed to determine the levels of coliform group

bacteria which are indicator microorganisms. This indicator microorganism functions as a marker of the presence of microbes in a food or the environment.12 Ideally, the bacteria used as indicator microorganisms must be able to meet the criteria, which are: in large numbers in the intestines and human feces; not pathogenic; can be detected easily and cheaply; do not multiply in the environment outside the intestine; and greater in number than the pathogenic bacteria.[24]

Detection of pathogenic microbes in a test sample is not possible. This is due to the large variety of pathogenic microbes and the high cost required to detect it. For this reason, coliform bacteria from the same source (human feces) are used, the same as pathogenic microbes. Simply put, the presence of these coliform bacteria indicates the possibility that a test sample also contains pathogenic microbes.[25] Most coliform bacteria do not cause disease. Only a few types of bacteria such as E.coli O157: H7 can cause disease from mild diarrhea to bloody diarrhea. Several outbreaks due to E.coli O157: H7 have occurred and are associated with food consumption from animals.[26]

The degree of contaminating bacteria that exceed this threshold when consumed without being cooked properly can cause diseases, especially gastrointestinal infections for those who consume them. The pathogenic microbes in tofu were found to come from various sources. Food contamination can occur in each step, starting from production until consumption ("from the field to the fork"). In addition, the materials used during the manufacturing process can also affect the microbial content of food.

The microbial content that contaminates tofu can come from water used for tofu production, various equipment used during tofu production to distribution, and hygiene of tofu producers and sellers. These things are the main factors in the presence of microbes in tofu. In addition, tofu that is rich in water, protein and neutral pH can become good growth medium for various pollutant microbes.

CONCLUSION

From the results of this study it was found that almost all samples contaminated by microbes exceeded the specified threshold of 3 MPN / g. Bacteria found in this study were *Escherichia coli*, *Klebsiella sp* and *Enterobacter sp*. The fact that the coliform bacteria found in the tofu samples exceeded this threshold indicates that, the tofu sample is not suitable for raw consumption, therefore, the public is encouraged to thoroughly cook tofu products before consumption in order to kill the germs. Therefore, supervision is needed in conducting surveillance on the food sold in an open market. It is necessary to hold counseling for tofu merchants to maintain the hygiene and sanitation of the food they sold.

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