MICROBIAL CONTAMINATION TEST USING THE MOST PROBABLE NUMBER (MPN) METHOD ON SWEET SOY SAUCE AROUND UPN "VETERAN" JAKARTA PONDOK LABU CAMPUS

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ABSTRACT

Sweet soy sauce is a fermented soybean product widely used as a traditional Indonesian seasoning. Its production process involves specific microorganisms to develop unique taste, aroma, and texture characteristics. However, poor hygiene and sanitation can lead to microbial contamination, such as Coliform, which may degrade quality and pose health risks. This study aimed to evaluate the microbiological quality of sweet soy sauce using the Most Probable Number (MPN) method. Samples were collected from food vendors around the UPN "Veteran" Jakarta campus and tested using liquid media to detect the presence of Coliform. The results showed that sample 1 (street vendor) had an MPN value of 14 MPN/gram, exceeding the BPOM safety limit (3 MPN/gram), while sample 2 (packaged soy sauce from a minimarket) was within the safety limit at 3 MPN/gram. A confirmatory test detected the presence of Escherichia coli in sample 1 but not in sample 2. Proper product handling, implementation of Good Manufacturing Practices (GMP), and regular microbiological monitoring are necessary to ensure the safety of sweet soy sauce products.

Keywords: Coliform; Most Probable Number; Sweet Soy Sauce

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INTRODUCTION

Sweet soy sauce is a typical Indonesian product produced through fermentation of soybeans and used in various dishes. The manufacturing process involves soybeans mixed with flour, salt, water, and microbes such as *Aspergillus oryzae or Aspergillus zozae* (Meutia, 2015). Soy sauce has an important role as a seasoning that enhances the flavor and attractiveness of food (Kurniawati, 2017). Kecap manis is a typical Indonesian soy sauce in liquid form, produced from fermentation of soybeans (*Glycine max* L.) and sugar, including brown sugar, with or without caramelization process, and can be added with other ingredients. According to SNI 3543:2013 section 1, sweet soy sauce is made from fermented soybean liquid or soybean meal mixed with sugar and permitted food additives, with a total sugar content of not less than 40% (Meutia Y. R., 2015). In addition to fermentation, soy sauce can also be made through hydrolysis of vegetable proteins using acids. This hydrolysis process produces amino acids which are then mixed with sugar, coloring, and other flavor ingredients to mimic the taste of fermented soy sauce (Meutia Y. R., 2015).

The fermentation process of soy sauce consists of solid fermentation (koji) and liquid fermentation (moromi), each of which involves specific microorganisms to produce

bioactive compounds. In solid fermentation, *Aspergillus sp.* and *Rhizopus sp.* produce protease enzymes that hydrolyze soy protein. The moromy stage involves salt-resistant microbes such as *Hansenula sp.*, *Zygosaccharomyces sp.* and *Lactobacillus sp.*, which produce lactic acid and alcohol. These enzymatic and microbial interactions cause complex biochemical changes, creating the characteristic aroma, flavor and texture of soy sauce (Meutia Y. R., 2015).

Along with the times, public awareness of nutritional value and food safety is increasing, especially because of the many cases of poisoning due to contaminated food. Microbiological factors, such as microorganisms from soil, air, or water in the production process, can affect food safety (Humairoh, 2017). If the hygiene of the soy sauce consumed is poor, food can be contaminated by bacteria, fungi, and other microorganisms. Some factors that can cause food contamination include: the washing of tools and containers used, the water used for washing, the way food is stored, and the expiration date (Huda & Tuntun, 2015). If the population of microorganisms in food increases, various problems can occur, including a decrease in food quality, food spoilage, a means of transmission of several digestive system diseases, and food poisoning which can cause death.

Therefore, sweet soy sauce products that are marketed must meet food safety standards, including microbial count limits measured through Total Plate Numbers (TPC), Yeast Mold Numbers (YMN), and Most Probable Number (MPN). MPN (Most Probable Number) is a method used to calculate the number of microorganisms using data from the growth of microorganisms in test tubes using liquid media that is more specific so that the number of microorganisms is the closest approximation and refers to the MPN table (Harti, 2015). The MPN test is used to detect and determine *Coliform* bacteria contamination (Verawati *et al,* 2019). *Coliform* is a group of rod-shaped, gram-negative, non-spore-forming, facultative aerobic bacteria that ferment lactose in producing acid and gas within 48 hours at 37ºC (Jiwintarum, 2017).

The results of observations on food vendors around the UPN "Veteran" Jakarta neighborhood found that many food vendors carry out unhygienic practices towards the presentation of sweet soy sauce used for food additives. Based on the above background, the author wants to conduct research to find out how the microbiological quality of sweet soy sauce is based on the determination of the *Most Probable Number* (MPN) and determine the MPN value of sweet soy sauce.

MATERIALS AND METHODS

a. Materials

The tools and materials used are incubator, water bath, test tube rack, test tube, Durham tube, Petri dish, ose needle, autoclave, Laminar Air Flow (LAF), cotton swab, and spiritus.

The materials used were soy sauce test sample 1, soy sauce test sample 2, Buffered Peptone Water media, Lactose Broth media, Brilliant Green Lactose Bile Broth media, Endo agar, and Fuschin 25%.

b. Method

1. **Tool Sterilization**

   The glass tools used were prepared, washed, dried, wrapped in parchment paper, and placed in an oven at 170°C for 1 hour.

2. **Preparation of Buffet Peptone Water (BPW)**

   Weighed 20 grams of BPW media, put into an erlenmeyer, added

1 liter of distilled water, dissolved until homogeneous, then put into an autoclave, sterilized at 121°C for 15 minutes.

3. Preparation of Lactose Broth (LB) Media

Weighed 13 grams of anhydrous media (single) and 26 grams of anhydrous media (double), put into an erlenmeyer, added 1 liter of distilled water, dissolved until homogeneous, put into a test tube that has contained a durham tube as much as 9 ml, covered with cotton, then put into an autoclave, sterilized at 121 ° C for 15 minutes.

4. Preparation of Brilliant Green Lactose Bile 2% Broth (BGLB) Media

Weighed 40 grams of BGLB media, put into an erlenmeyer, added 1 liter of distilled water, dissolved until homogeneous, put into a test tube that has contained 9 ml of Durham tube, covered with cotton, then put into an autoclave, sterilized at 121 ° C for 15 minutes.

5. Most Probable Number (MPN) Method

a. Presumptive Test

1) Sample preparation: the packaging of the test sample was opened, and the sample was taken aseptically. Weighed 5 g of sample into a bottle containing 45 ml of diluent solution (BPW) to obtain a dilution of10^1 and then homogenized.
2) Inoculated 1 mL each of the solution from each dilution level (10^{-1}, $10^{-2}, 10^{-3}$) into three tubes containing lactose broth in which there is an inverted Durham tube.
3) Put the tube into an incubator at 35°C for 48 hours.
4) Observe the tube at 24 hours. If any tube contains gas, then the tube is positive.
5) The tubes that did not contain gas were continued to incubate for up to 48 hours.
6) Note the formation of any amount of gas after 48 hours incubation.

b. Confirmative Test

1) Transfer one Ose from each positive lactose broth tube into a different BGLB Broth tube.
2) Incubate the BGLB broth tubes in an incubator at 35ºC for 24 hours, tubes that form gas are positive.
3) If negative, incubate and check again at 48 hours. If gas forms then the tube is positive.
4) Calculate the coliform MPN using the MPN table.

c. Complementary Test

1) Tubes that are positive in the confirmation test are continued by taking the culture using an ose needle.
2) Inoculated on the surface of Endo agar media by streak plate method in duplicate.
3) Incubate at 37℃ for 24-48 hours until bacterial colonies form.

6. Gram *Staining* Method

a. Bacterial samples are taken, then applied to a glass slide and fixed by heating.

b. Crystal violet stain was dripped onto the entire smear and left for 1 minute. After that, the glass slide was rinsed with running water.
c. An iodine solution is added to the smear to form a stable dye complex. This dye is left for 1 minute, then washed off with water.
d. Alcohol is slowly dripped to remove the main dye on Gram-negative bacteria. This process is stopped by immediately rinsing the glass slide with water.
e. Safranin secondary dye is applied to the smear and left for 30-60 seconds to color Gram negative bacteria. After that, the glass slide is again rinsed with water.
f. After the smear was dried, the sample was examined using a microscope with an immersion lens. Gram-positive bacteria were observed to be purple in color, while Gram-negative were pink in color.

Test	Repli-cation	10^{-1}	10^{-2}	10^{-3}
	1	+	-	-
Presumptive	**2**	+	-	+
	3	+	-	-
	1	+	ND	ND
Confirmative	**2**	+	ND	+
	3	-	ND	ND
	1	-	ND	ND
Complement	**2**	-	ND	+
	3	ND	ND	ND

RESULT

Table 1. Test results of sample 1

Table 2. Test results of sample 2

Test	Repli-cation	10^{-1}	10^{-2}	10^{-3}
	1	-	-	-
Presumptive	**2**	-	-	-
	3	+	-	-
	1	ND	ND	ND
Confirmative	**2**	ND	ND	ND
	3	+	ND	ND
	1	ND	ND	ND
Complement	**2**	ND	ND	ND
	3	+	ND	ND

Description; ND = Not Done

DISCUSSION

In this test, two types of samples were used. Sample 1 is soy sauce that has been removed from the primary packaging and used by traders around the UPN "Veteran" Jakarta Campus to sell daily. Meanwhile, sample 2 is soy sauce obtained from the convenience store in new condition, still sealed in the primary packaging, and serves as a negative control.

Sampling with different conditions was intended to compare the presence of bacteria in sample 1 that had contact with the environment and sample 2 that had not been in contact with the environment, so that the effect of environmental exposure on microbial contamination could be known.

Positive results of the presumptive test are characterized by the formation of gas

bubbles in the Durham tube and changes in media turbidity, these characteristics are the result of the metabolism of *Coliform* bacteria. (Aji and Fiani, 2021). Based on the results of the presumptive test, all tubes did not show bubbles, but some tubes were said to be positive with sediment or turbidity. Sample 1 had a total of 4 positive tubes, consisting of 3 tubes at dilution 10^{-1} and 1 tube at dilution 10^{-3}. On the other hand, sample 2 only had one positive tube, which was at dilution 10^{-3}.

The booster test aims to confirm the presence of *Coliform* bacteria in the test sample. In the booster test, the positive tube results of the presumptive test are then inoculated into a tube containing BGLB (Brilliant Green Lactose Bile Broth) media containing selective agents in the form of brilliant green and bile salts. (Aji and Fiani, 2021). The component in BGLB, peptone, is useful as a source of essential nutrients for the metabolism of contaminated bacteria, unhygienic equipment, water used in the production process, and handling practices that are not up to standard (Onyeaka *et* al, 2022). *Coliform* bacteria itself is a sanitary indicator because its presence indicates the possibility of pathogenic microorganisms that can endanger health (Ayer *et* al, 2023).

Positive samples in the booster test then proceed to the supplementary test stage to determine the presence of *Escherichia coli* bacteria. In the complementary test, tubes that showed positive results were taken using an ose and scratched on the surface of endo agar media that had been solidified in a petri dish. The inoculated media was then incubated at 37^{o}C for 2 x 24 hours. A positive result in the complementary test is indicated by the formation of a metallic red color on the agar medium which indicates the presence of *E. coli* bacteria. In the complementary test, a positive result was shown by sample 1 at the 10^{-3}dilution level. The results of this test were then reconfirmed with a gram staining procedure which also showed the presence of *E. coli* bacteria.

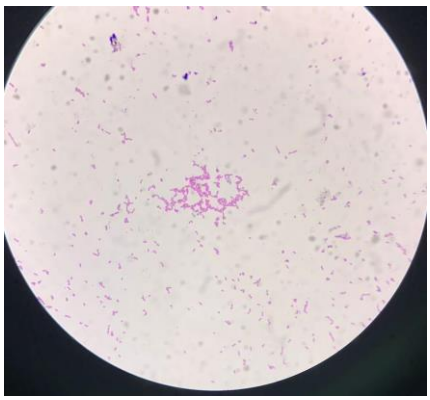

Figure 1. Confirmation of *E.Coli* by *gram stain* procedure

Based on the test results, the MPN value obtained for sample 1 was 14 MPN/gram and in sample 2 a value of 3.6 MPN/gram was obtained. It can be seen in the MPN table of sweet soy sauce that both test samples are not within the safe limit of use because the value does not match the existing standards. The presence of *coliform* bacteria contamination still needs to be watched out for because it can indicate poor sanitation and hygiene conditions in product handling. Bacterial contamination of soy sauce can occur through several pathways such as contaminated raw materials, unhygienic equipment, water used in the production process, and handling practices that are not up to standard (Onyeaka *et* al, 2022). *Coliform* bacteria itself is a sanitary indicator because its presence indicates the possibility of pathogenic microorganisms that can endanger health (Ayer *et* al, 2023).

Preventing microbial contamination in soy sauce can be done by implementing *Good Manufacturing Practice* (GMP) and good

sanitation starting from the selection of raw materials, production process, packaging, to storage. The use of tightly closed containers, storage at appropriate temperatures, and routine cleaning of equipment can minimize the risk of microbial contamination. Consumption of soy sauce containing *coliform* bacteria can lead to the risk of gastrointestinal illness, symptoms of food poisoning, and further contamination if not stored properly. Although soy sauce has characteristics that can inhibit microbial growth such as high salt and sugar content, contamination is still possible especially in products that are poorly handled. Therefore, regular monitoring of microbiological quality is still carried out to ensure the safety of soy sauce products on the market.

CONCLUSIONS

Based on the test results, it can be concluded that the *Most Probable Number* (MPN) method is effective for detecting and identifying *Coliform* bacteria contamination. This is important to do because excessive accumulation of *Coliform* bacteria in the body can cause health problems such as diarrhea, infection, and colon bleeding. Based on the National Standardization Agency (BSN) standard, the maximum acceptable limit of *Coliform* bacteria in food is 3 MPN/g. Test results on two soy sauce samples, namely sample 1 obtained from around the UPN "Veteran" Jakarta campus and sample 2 from a nearby minimarket, showed that both samples did not meet safety standards. Sample 1 contained *Coliform* bacteria of 14 MPN/g, while sample 2 had *Coliform* bacteria of 3.6 MPN/g, which far exceeds the limit set by BPOM of 3 MPN/g.

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