

Microbiological Quality of Packaged Tea Beverages Using the 3M Petrifilm Rapid *E. coli*/Coliform Count Plate

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Abstract: Tea is a complementary drink which is currently being served in variety ways and can be found in many places. The form of packaging is also very diverse. Glass bottles, plastic bottles, metal cans, paper cups, and plastic cups are just few of the many different forms of packaging that are available. The Indonesian National Standards Agency has established several requirements that bottled drinking water must fulfill in order to be legally sold in the country; one of these requirements is that it cannot contain any bacteria that could potentially cause disease. However, the reality is that within the community there are still packaged drinks that are not safe for human consumption. This study tested the quality of bottled tea supplied in a university canteen using 10 brands in varying packaging. 3M Petrifilm Rapid *E. coli*/Coliform Count Plate is utilized. The test results showed that 10 brands of tea packaging that were sold negatively did not contain *E.coli* and Coliform, which could be seen by the lack of gas bubbles and discoloration on the petrifilm media. This means that all packaged beverage samples sold are safe to drink.

Keywords: Coliform, *E.coli*, Packaged Tea Drinks, 3M Petrifilm Rapid *E. coli*/Coliform Count Plate.

INTRODUCTION

Tea drinks are one of the complementary drinks that are becoming more and more varied in how they are served (Indrasti & Siliyya, 2022; Nuryanti & Rahman, 2008). As time and technology change, so does the way people think. Because people are always looking for ways to make their lives easier, almost all parts of society prefer to consume packaged drinks. People buy bottled beverage for a variety of reasons, including taste, simplicity, and lifestyle. But for most customers, safety and health benefits are important factors (Doria, 2006; Ward et al., 2009).

As a popular beverage option, tea is widely consumed across demographics and socioeconomic classes in Indonesia. According to studies conducted by (Valavanidis, 2019) tea is the second most commonly consumed beverage in the world, right behind water. There are a number of factors at play, but health is a major one. Alkaloids, flavonoids, amino acids, vitamins, and minerals are just some of the components found in tea that can combat free radicals and help to reduce the risk of lifestyle-related diseases.

There are numerous varieties of tea, but the most popular ones are green tea, black tea, oolong tea, dark tea, white tea, and yellow tea (Pan et al., 2022). Now, many new flavor variations have also been made. Also, the shapes of the packages are very different. At first, they came in glass bottles (Returnable Glass Bottle), but now they come in PET plastic bottles (*polyethelene terephthalate*), cans, paper, pouches, and plastic cups (Herdiana, 2014).

Bottled tea beverages are one of the beverages obtained by steeping tea, extract tea, instant tea, or its mixture in drinking water with or without other foodstuffs and sealed in an impermeable container. Conforming to the Indonesian National Standard, packaged tea beverages must meet health requirements (SNI). Similar to other foods and beverages, brewed tea beverages are susceptible to microbiological spoilage due to a number of factors. These factors include bacterial

contamination of the ingredients used to make brewed tea beverages, contamination of tools and environmental factors during processing. One of the causes of beverage poisoning can be the presence of pathogenic bacteria in the basic ingredients used to produce beverages (Buckle, 2009).

According to the Indonesian National Standards Agency (SNI) No. 01-3553-2006, bottled drinking water must not only contain pathogenic bacteria, namely *Salmonella* and *Pseudomonas aeruginosa*, but also cannot contain microbial contamination greater than 100 colonies/ml (Badan Standardisasi Nasional, 2009). Microbiological examination of drinking water is very important to be carried out as a measurement of the degree of microbial pollution, generally indicated in the presence of Coliform and *E. coli* bacteria. The microbiological quality of drinking water can be determined based on the results of the Petrifilm *E. coli* / Coliform Count Plate test. One way to test water quality that is easy, practical and fast is to use the petrifilm method. The Petrifilm test method was developed by the 3M company which is used to calculate the number of Coliform and *E. coli* bacteria. Petrifilm EC Count Plate already contains artificial medium, namely violet red bile (VRB) as an indicator of bacterial colony growth. Coliform bacteria are indicators of the presence of other pathogenic bacteria. More precisely, fecal Coliform bacteria are indicator bacteria of the presence of pathogenic bacterial pollution. The determination of fecal Coliform is an indicator of pollution because the number of colonies must be positively correlated with the presence of pathogenic bacteria.

Based on (Handayani et al., 2017) the results of the estimator test showed the presence of Coliform bacteria in home industrial packaged tea samples, complementary tests were carried out to determine the presence of *E. coli* bacteria. From the results of the complementary test, it was found that the sample containing Coliform bacteria in the positive estimator test contained *E. coli*. This proves that the presence of Coliform bacteria in the sample is an indication that there is a possible presence of *E. coli* bacteria. Based on some of the descriptions above, it is necessary to conduct research to determine whether there is contamination of *E. coli* and Coliform bacteria in packaged tea drinks circulating in university canteen using the Petrifilm *E. coli* / Coliform Count Plates test method.

MATERIALS AND METHODS

Qualitative descriptive research methods were used for this study. Ten different packaged tea brands were chosen for the sample. They came in bottles, mugs, and cardboard boxes from three different cafeterias at one of Malang's public colleges. The 3M company's *E. coli*/Coliform Count Plate petrifilm is used in the testing procedure. This petrifilm contains a medium developed for the purpose of detecting *E. coli* and coliform bacteria. It is not necessary to sterilize a Petrifilm AC Plate before using it for testing because the plate is impervious to contamination even in its unprocessed state. 3M Microbiology is already officially ISO 9001 certified.

The test procedure using petrifilm is as follows;

- 1) Put petrifilm on a flat surface.
- 2) Use a micropipette to take a 1 ml sample.
- 3) Open the upper film and drop the sample on the center of the lower film and carefully close it without any gas bubbles
- 4) If the sample has not been flattened, use a spreader to do so until a gel formed.
- 5) Stack petrifilm to a maximum of 20 layers and incubate for 48 hours at 35°C.

- 6) Count the number of colonies using the colony counter. The indicator of a red colony with gas is coliform and a blue colony associated with gas is *E. coli* (Bird et al., 2021; Laily et al., 2022). The total amount of Coliform and *E. coli* colonies on each 3M Petrifilm plate is next recorded in a notebook.

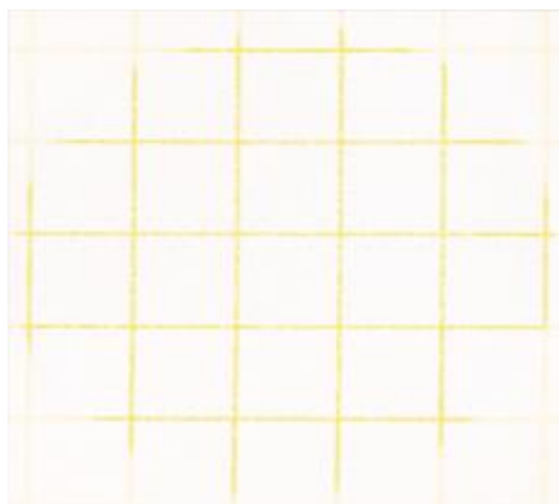


Figure 1. Example Petrifilm Aerobic Count plate without Colonies

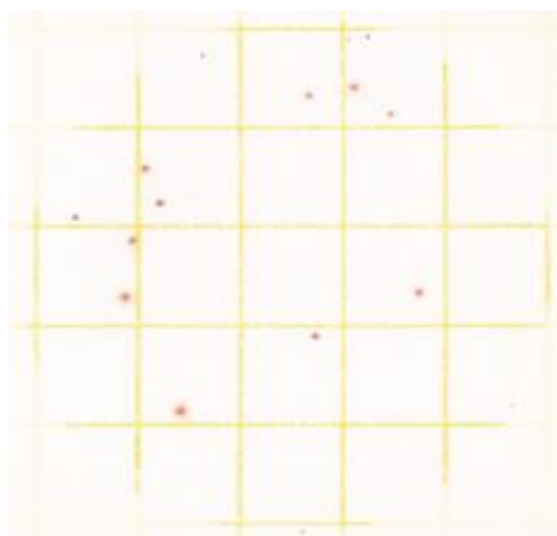


Figure 2. Example Petrifilm Aerobic Count plate with a few bacterial colonies (16).

RESULT AND DISCUSSION

According to petrifilm tests conducted on 10 brands of package tea served in numerous cafeterias at one of Malang's public colleges, all samples were negative. This indicates that neither *E. coli* nor coliform bacteria have been identified in packaged tea. A blue colony with gas indicates the presence or absence of *E. coli*, whereas a red colony with gas indicates coliform contamination. If the colony concentration on Petrifilm is high, the entire growing area will become red or pink.

Occasionally, if the overcrowded film is covered with colonies, the colony's center may be less visible, but numerous minor colonies can be seen around the colony's periphery. When this occurs, the colony is classified as Too Numerous To Count (TBUD). When a precise amount is required, a higher dilution should be considered.

The following are the findings of a petrifilm test conducted on ten brands of packaged tea.

Table 1. Test Result of Packaged Tea Drink with 3M Petrifilm Rapid *E. coli*/Coliform Count Plate

Code for Packaged Tea Drink	Packaging Type	Petrifilm Test Results		Information
		U1	U2	
A	Plastic Bottle	(-)	(-)	No bubbles and discoloration
B	Plastic Bottle	(-)	(-)	No bubbles and discoloration
C	Plastic Bottle	(-)	(-)	No bubbles and discoloration
D	Plastic Bottle	(-)	(-)	No bubbles and discoloration
E	Plastic Bottle	(-)	(-)	No bubbles and discoloration
F	Plastic Bottle	(-)	(-)	No bubbles and discoloration
G	Plastic Bottle	(-)	(-)	No bubbles and discoloration
H	Carton	(-)	(-)	No bubbles and discoloration
I	Carton	(-)	(-)	No bubbles and discoloration
J	Plastic Cups	(-)	(-)	No bubbles and discoloration

The absence of colony growth in petrifilm that is characterized by no discoloration and the association of bacteria with gases stands out among all of the indicators that have already been described. This indicates that manufacturers of packaged tea have complied with the health requirements specified in the Indonesian National Standard (SNI).

Food-borne or water-borne microbial infections are the most common cause of disease in some countries (Mengistu et al., 2022). Numerous harmful bacterial species found in foods and beverages, including *Salmonella*, *Shigella*, *B. cereus*, *E. coli*, *Staphylococcus aureus*, and others (Barreira et al., 2024). Beverages are a popular choice on campus due to their availability, variety of tastes, and high nutritional content. It makes sense that these products are devoid of dangerous microbes (Biva et al., 2019).

In packaged beverages, water, sugar, tea flavorings, and food preservatives are utilized as ingredients (sodium benzoate, potassium sorbate). To inhibit the development of bacteria in food products, numerous types of preservatives are employed. As preservatives in food, sodium benzoate, potassium sorbate, and sodium nitrate are frequently employed. Sodium benzoate or sodium benzoate is a common food preservative (Stanojevic et al., 2009).

The results of observations on Petrifilm revealed that no bacteria were detected on the film. This is because the factory-made packaged tea contains preservatives, including sodium benzoate, which inhibits the growth of beneficial bacteria *E. coli* and Coliform bacteria. These preservatives are used to inhibit the growth of various microorganisms, including mold, yeast, and bacteria, and to kill them. Benzoates suppress microorganisms by disrupting the permeability of cell membranes, the structure of the microbial genetic system, and the activity of intracellular enzymes (Hesti, et al., 2016). The way that benzoates and their salts work is based on the fact that non-dissociated acid molecules can pass through the membranes of microbial cells. The pH of everything inside a microbial cell is always 7. If microbial cells become acidic or basic, there will be changes in the organelles of the cells, which will slow down metabolism and cause some cells to die. Mold cell

membranes can only let through acid molecules that haven't broken apart. To be most effective, they should be used in an acidic environment (Khurniyati et al., 2015).

In this test, plastic bottles and cartons were used to package the tea. (Nisa et al., 2012) says that food packaging is an important way to prevent pollution. Microorganisms, the environment, and chemistry can all be sources of pollution. Cleanliness and hygiene are taken into account when packaging tea. The procedure is a step to make sure that the process is free of bacteria, fungi, and other contaminants. The lid of the tea package is also sealed, which shows that proper sanitation and hygiene measures were taken in the factory.

Several studies on tea water have been carried out, including research conducted by (Ariefiansyah et al., 2015) who examined iced tea in a restaurant by the sea in Purus West Padang that 12 of the 13 test samples were contaminated with *E.coli* and one contained *Klebsella* bacteria. This is influenced by several factors can be from the water used to brew tea, for ice cubes or for washing glasses. Another thing was also found that 7 out of 10 samples of household industry packaged tea drinks circulating in Sungai Dama and Seili Samarinda villages contained *E.coli* (Handayani et al., 2017). Other study conducted by (Khan et al., 2015) revealed that all the studied in some drinks sold in the streets of Dhaka University Campus had a higher microbial load than the specification set for fruit juices in some parts around the world. Regular monitoring of the quality of beverage for human consumption is recommended to avoid any future bacterial pathogen outbreak.

From this study we know that the use of petrifilm is trustworthy for the microorganisms surveyed, facilitating work and producing faster results, which is crucial when it comes to monitoring food-borne outbreaks. The use of the Petrifilm™ system produced more reliable results more easily and quickly, including facilitating the development of work in the laboratory microbiological analyses of food, because there was no need to use large quantities of materials, glassware, petri plates, pipettes, while minimizing material preparation for the analyses (de Souza et al., 2015). It is recommended to count *E. coli* and coliforms in a variety of foods, it is advised that the 3M Petrifilm Rapid *E. coli*/Coliform Count Plate be utilized as the Official First Action status (Bird et al., 2021).

CONCLUSION

According to the findings of the Petrifilm test, none of ten different kinds of packaged tea items exhibited any bubbles or discoloration in their samples (negative). This demonstrates that none of the ten different varieties of packaged tea products contain *E. coli* or Coliform bacteria; hence, they are completely risk-free for human consumption.

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