

# Analysis of *Escherichia coli* and Total Plate Count of Red Guava Fruit Juice (*Psidium guajava* L.) Sold in Keboansikep Gedangan Subdistrict Sidoarjo

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**Abstract.** Red guava can be processed into a juice drink, but it does not rule out the possibility that fruit juice contains disease-causing microorganisms. This study aims to determine the number of *Escherichia coli* and TPC/Total Plate Count bacteria in red guava fruit juice sold in the Keboansikep, Gedangan, Sidoarjo. The method used is descriptive using a survey technique with a cross-sectional approach. Laboratory experiments are carried out, namely analyzed in the microbiology laboratory-sampling using a saturated sampling technique. Samples were examined to compare the analysis results obtained compared to INS 3719:2014 concerning the Quality of Fruit Juice Drinks. Samples were examined using *Escherichia coli* analysis based on INS 01-2332.1-2006 with the Most Probable Number (MPN) method and Total Plate Count/TPC analysis based on INS 01-2332.3-2006 with the pour plate method. The test results showed that the *Escherichia coli* analysis obtained 100% of the samples showing positive with the lowest MPN index of 9 APM/ml to the highest >1100 APM/ml. The results of the PNA analysis obtained 7% of all samples, indicating that it was safe and met the requirements, namely sample 7A with a TPC value of  $4.9 \times 10^3$  CFU/ml, while 93% of all samples showed that it was unsafe or did not meet the requirements based on INS 3719: 2014. The sample containing the most microbes was sample 5A with a TPC value of  $5,1 \times 10^5$  CFU/ml.

**Keywords:** Red Guava Juice, Most Probable Number, Total Plate Count

## 1 Introduction

Apart from being basic necessities that are useful for maintaining health, food, and drinks can also be a source of disease for humans if they are not handled hygienically. Food and drink can cause disease due to 2 things, namely containing toxic components (heavy metals and toxic chemicals) and being contaminated with pathogenic microorganisms in sufficient quantities to cause disease (*Salmonella thyposa*, *Shigella dysentriae*, hepatitis virus, *Escherichia coli*, and others) [1].

People's mobility and busyness are increasing, especially during the Covid-19 pandemic, increasing the body's immune system must be done as a preventive measure so that it requires optimal health conditions for the body. People in general tend to choose to consume fruit juice which is practical and easy to obtain rather than taking the time to consume real fruit. Ready-to-drink red guava juice effectively gets sufficient vitamins and minerals to meet the body's needs. According to [2], the compounds contained in guava fruit are vitamin A, ascorbic acid, flavonoids, quercetin, and guaijavarin. Ascorbic acid is a compound that can improve the body's immune system. In red guava plants, quercetin is the most active antioxidant, which plays a role in inhibiting the oxidation process, which can cause several disorders, one of which is viral infection, so consuming red guava plants can control these free radicals [3].

The processing process for red guava juice usually sold in the community is crushing and packaging the fruit. It causes microbial contamination such as mold, yeast, *Salmonella sp.*, *Escherichia coli*, and *Staphylococcus aureus*, thereby reducing the quality and shelf life of the juice and can endanger health [4]. The criteria for a fruit juice that is suitable for consumption according to [5] must have a normal taste, smell, and color typical of fruit. Based on Indonesian National Standard 3719:2014 regarding the Quality of Fruit Juice Drinks for TPC (Total Plate Count), it is categorized as suitable for consumption if it does not exceed  $1 \times 10^4$  colonies/g and *Escherichia coli* <3/ml, and is not contaminated by other dangerous microbes.

Fruit juice sold by street vendors contains *Escherichia coli* bacteria in 52% of fruit juice drink samples in the Tembalang area, Semarang [1]. This research also states that this is due to improper washing processes, equipment sanitation, and trash conditions, which causes contamination of *Escherichia coli* bacteria in fruit juice drinks in the Tembalang area.

Based on research [6][7], fruit juices sold on the streets of Surakarta city have a total plate number content of around  $8 \times 10^7 - 3.7 \times 10^8$  CFU/ml. The level of microbiological contamination of juice sold on the streets of Surakarta is very high and exceeds the limits permitted by BPOM. According to the standards issued, juice sold on the streets of Surakarta City is not suitable for consumption. The research also states that there must be continuity between the community, producers, and related agencies to improve food safety, especially for juice products sold on the roadside.

Another research carried out by [8], namely an examination of the bacteriological quality of red guava juice sold by street vendors in Jalan Margonda Raya, Depok City, showed positive results for total coliform bacteria and *Escherichia coli* in the samples, resulting in results that did not meet the requirements based on BSN (2014) namely the limit for total coliform bacteria in fruit juice drinks is 20 APM/ml while for *Escherichia coli* bacteria it is <3 colonies/ml.

Based on the background of the problem above, researchers are interested in conducting research on "*Analysis of Escherichia coli and Total Plate Count of Red Guava Fruit Juice (Psidium guajava L.) Sold in Keboansikep Gedangan Subdistrict Sidoarjo*", which aims to determine the number of bacteria, especially *Escherichia coli* and TPC bacteria in different red guava fruit juices at different fruit juice traders in Keboansikep Gedangan Sidoarjo Village to determine the quality and suitability for consumption.

## 2 Research method

### 2.1 Material

This research's main ingredient or sample is red guava juice sold in the Keboan Sikep Village area, Gedangan District, Sidoarjo Regency. The materials used for microbiological analysis are distilled water and 70% alcohol.

The growth media used for the analysis of Total Plate Count (TPC) and *Escherichia coli* are Lauryl Sulfate Broth (LSB) media, Brilian Green Lactose Bile Broth (BGLBB) media, *Escherichia coli* Broth media.

### 2.2 Tool

The tools used in the research were Petri dishes, screw test tubes, Durham tubes, pipettes, autoclaves, tube needles, Bunsen, Laminar Air Flow, incubators, analytical balances (Ohaus), test tube racks, micropipette, volume pipettes, hot plates, and water bath.

### 2.3 Research Design

This research used a descriptive survey method with three cross-sectional approaches and continued with laboratory experiments.

The research began with a field survey of Keboansikep Gedangan Sidoarjo Village fruit juice sellers. The number of juice sellers is 15, spread across 8 Neighborhood Units (RW). The survey results obtained 15 fruit juice sellers with different types of sales, namely push carts with display cases, permanent buildings, and semi-permanent buildings. After obtaining data from the survey results, sampling was carried out. The sampling technique used is saturated sampling. Sampling (red guava juice) was done by purchasing red guava juice, labeled. The label contains the name of the place (red guava juice brand), the address of the place, and the time of collection. Sampling is carried out aseptically to avoid external microbial contamination during sampling. Aseptic sampling begins by using sterile gloves that have been sprayed with alcohol. The samples of red guava juice taken were placed in a sterile cool box so that the samples were not exposed to direct sunlight and to prevent microbial growth. After the sample is obtained, proceed with laboratory experiments. The analysis carried out in the microbiology laboratory is the analysis of *Escherichia coli* and TPC.

### 2.4 Research Variable

The variables observed in this research are: Analysis of *Escherichia coli* using Quantitative Methods [9][10] and TPC analysis using Quantitative Methods [11].

## 2.5 Research Time and Location

### 2.5.1 Research Time

Samples were taken from Keboansikep Village, Gedangan District, Sidoarjo Regency fruit juice sellers. Sampling was carried out from 12.00 to 20.00 WIB. The research was conducted from November 2022 to December 2022.

### 2.5.2 Research Location

Quantitative analysis of Total Plate Count (TPC) and *Escherichia coli* was carried out in the microbiology laboratory of PT Tunas Baru Lampung Tbk, Sidoarjo. The research was conducted from November 2022 to December 2022.

## 2.6 Data Analysis Method

The analysis data is discussed descriptively by calculating the number of Total Plate Numbers (PNA) and *Escherichia coli* compared to the Indonesian National Standard 3719:2014 concerning the Quality of Fruit Juice Drinks.

Analysis of *Escherichia coli* using the MPN method by observing the Durham tube, which produces gas. The results of the Confirmative Test are in the MPN table.

**Table 1.** Table Most Probable Number (MPN)

Positive Reaction Tube			MPN (Per gr/ml)	Positive Reaction Tube			MPN (Per gr/ml)
1:10	1:100	1:1000		1:10	1:100	1:1000	
0	0	0	<3	3	0	0	23
0	0	1	3	3	0	1	39
0	1	0	3	3	0	2	64
1	0	0	4	3	1	0	43
1	0	1	7	3	1	1	75
1	1	0	7	3	1	2	120
1	1	1	11	3	2	0	93
1	2	0	11	3	2	1	150
2	0	0	9	3	2	2	210
2	0	1	14	3	3	0	240
2	1	0	15	3	3	1	460
2	1	1	20	3	3	2	1100
2	2	0	21	3	3	3	>1100
2	2	1	28				

Analysis of Total Plate Count (TPC) data using the pour plate method by counting all colonies on the petri dish without exception and without paying attention to the characteristics of each colony. The Colony Calculation Formula is as follows:

$$\text{TPC} = \frac{\text{Average Number of Colonies} \times \frac{1}{\text{Dilution Factor}}}{\text{Calculated Amount of Dilution}} \quad (1)$$

## 3 Result and Discussion

### 3.1 Supporting Data

Supporting data observed include location, origin of water used to wash utensils and fruit, water used to make juice when ground, type of ice cubes used, packaging used, and cleanliness of fruit storage windows. More details can be seen in Table 2.

**Table 2.** Supporting Data for Fruit Juice Samples

No	Location	Sample code	Water washing	Water process	Ice type	Packaging type	Etalase condition
1	RW 01	1A	Well Water	Refill water	Block	Plastic bags	+
2	RW 02	2A	PDAM Water	Refill water	Crystal	Plastic cups	++
3		2B	PDAM Water	Refill water	Crystal	Plastic bottles	+
4	RW 03	3A	Well Water	Refill water	Crystal	Plastic bags	+++
5		3B	Well Water	Refill water	Crystal	Plastic bags	++
6	RW 04	4A	PDAM Water	Refill water	Crystal	Plastic bottles	+
7		4B	PDAM Water	Refill water	Crystal	Plastic bags	++
8		4C	PDAM Water	Refill water	Crystal	Plastic bags	++
9	RW 05	5A	PDAM Water	Refill water	Block	Plastic cups	+++
10	RW 06	6A	Well Water	Refill water	Homemade	Plastic bottles	++
11	RW 07	7A	PDAM Water	Mineral water	-	Plastic bottles	+
12		7B	PDAM Water	Refill water	Homemade	Plastic bags	++
13		7C	PDAM Water	Refill water	Crystal	Plastic bags	+
14	RW 08	8A	Well Water	Refill water	Homemade	Plastic cups	++
15		8B	Well Water	Refill water	Homemade	Plastic cups	+

Description: + = dirty, ++ = quite clean, +++ = clean

### 3.1.1 Water washing

Based on data from surveys, PDAM water is used more than well water, with a percentage of 60% compared to 40%. Most traders use PDAM water as a water source for washing equipment and fruit. The use of this water source is adjusted to the availability of the PDAM water installation at the juice-selling location. PDAM installations have yet to reach several locations, so residents take water from the ground to get clean water. Apart from the availability of PDAM water, traders consider PDAM water to be cleaner and of better quality.

The results of research conducted by [12] from 8 PDAM water and 14 dug well water, 50% of the water samples contained *Escherichia coli* more than 0 MPN/ 100 ml. The risk of water pollution from PDAM and dug wells is other pollution such as rubbish, animal waste, and stagnant water, as well as open parts of pipes that allow insects to enter. Equipment in the food processing process must also be kept clean every time it is used and after use. Cleaning equipment properly will produce clean and healthy food processing equipment. It is in the process of washing equipment that determines whether or not bacteria contaminate the equipment. The cleanliness of equipment is very important—because equipment plays a role in the processing of Red Guava Juice; equipment that needs to be cleaned can allow bacterial contamination of drinks [8].

### 3.1.2 Water process

Based on data from a survey using water in the making juice as an additional ingredient uses more refillable water than mineral water, with a percentage of 93% compared to 7%. The survey on mineral water use only used fruit juice sold at Indomaret, because Indomaret has its mineral water products.

The water in the process of making red guava juice should not contain pathogenic germs or any creatures that endanger human health. The water used as a mixture for red guava juice must be of good quality and meet the requirements so that the red guava juice produced is of good quality.

Refill drinking water tends to be cheaper than the price of mineral water. It causes refilled drinking water to be one of the answers for fruit juice sellers to use refilled water from an economic perspective. Refill water undergoes special processing through chlorination, aeration, filtration, and irradiation with ultraviolet light. In

water processing, the increasing level of water pollution, both from household waste and industrial waste, causes refillable drinking water to be susceptible to contamination from various microorganisms [13]. Another study conducted by [14], showed that of the nine refilled drinking water samples tested, all showed that they exceeded the microbial contamination limit.

### **3.1.3 Ice Type**

Based on data from a survey ice cubes are used in making juice as an additional ingredient and more crystal-type ice cubes with a percentage of 53% than block or homemade ice cubes with percentages of 13% and 27%. Ice cubes are added to drinks as a complement and served with drinking water to create a cold and fresh sensation. Therefore, ice cubes are a type of complementary food product. Making fruit juice without adding ice cubes is only sold at Indomaret because it will be placed in the refrigerator after the juice-making process is complete.

Crystal ice is ice cubes in the shape of a tube that has a hole in the middle with a certain diameter and length, which is specifically for drinks because it is cleaner, clearer, has been printed, and does not need to be broken again [15]. Block ice is ice that comes from water and is frozen in the form of blocks, while homemade ice cubes are made by the seller himself because a cooling cabinet at the sales location supports them.

Ice cubes have a low temperature, so bacterial activity, including pathogenic bacteria, can decrease or stop. This is why ice cubes are considered relatively safe, but in several previous studies on ice cubes, there were still pathogenic bacteria in ice cubes on the market [16].

### **3.1.4 Packaging Type**

Based on data from a survey, the packaging used by fruit juice traders as containers mostly uses plastic bag type packaging with a percentage of 46% compared to plastic cup or plastic bottle type packaging with the same percentage, namely 27%. Fruit juice sellers use plastic as packaging for their products because the plastic used is strong, easy to shape and light, non-corrosive, adaptable to the product, practical, long-lasting, has an affordable cost, and is generally used once. Use [17]. On the other hand, consumers also want to buy affordable products, so plastic provides a solution for fruit juice sellers to provide cheap product packaging regardless of the side effects of the chemicals contained in plastic and the negative effects on the environment [18].

Fruit juice sellers widely use plastic bag packaging because it is more practical and the price is more affordable, but the design is less attractive, looks less strong, and the dosage per ml of fruit juice is uncertain or changes. From a design perspective, the packaging for plastic bottles and cups is more attractive with screen printing or brand logos on the bottles and looks strong or does not leak easily. Packaging using plastic cups requires a cup sealer, so this is a consideration for fruit juice sellers from an economic perspective.

### **3.1.5 Etalase Condition**

Based on data from a survey, the cleanliness of the display cases used by fruit juice sellers to store fruit is more clean with a percentage of 13.3%. than the display cases which are quite clean with a percentage of 46.7% and 40% dirty.

The cleanliness condition of the display case is categorized as clean, which can be seen from the cleanness of the display case glass and the display case door which is always tightly closed, the base used in the display case is clean, and the condition of the fresh fruit in the display case. The condition of the display case is categorized as quite clean. It can be seen that it is poorly maintained or rarely cleaned, as indicated by the appearance of the display case glass being opaque and with lots of stains. The condition of the display case is categorized as dirty because the display glass is not maintained or cleaned and there is no cover so many flies land on the fruit. On average, juice sellers who sell fruit products don't pay enough attention to it and mix it with other ingredients for additional food such as cucumbers, gobies, etc.

## **3.2 Escherichia Coli Analysis**

The results of quantitative analysis of *Escherichia coli* using the MPN (Most Probable Number) method on 15 samples of red guava fruit juice from fruit juice traders in Keboansikep Gedangan Village, Sidoarjo were very varied, starting from the lowest 9 APM/ml to the highest >1100 APM/ml. The MPN method is a cell counting method, especially for calculating *Escherichia coli* bacteria based on the number of closest estimates. The closest estimate is a calculation within a certain range or calculated as a statistically close estimate by referring to the MPN table [19].

The results obtained from the test were said to be positive due to the formation of bubbles/gas, cloudy, and smelly which was suspected to have occurred contamination by *Escherichia coli* bacteria [9]. The positive result of *Escherichia coli* bacteria in the bacteriological quality examination of red guava juice is possibly caused by cross-contamination. If it enters the digestive tract in large quantities, it can be harmful to health and can cause digestive disorders so red guava juice no longer has health benefits. The number of microorganisms tested based on the MPN value in 15 samples can be seen in Table 3.

**Table 3.** *Escherichia Coli* Test Results Data

Sample	Positive tube number			MPN index (APM/ml)
	1: 10	1: 100	1: 1000	
1A	2	1	1	20
2A	3	2	2	210
2B	3	0	1	39
3A	3	3	2	1100
3B	3	3	1	460
4A	1	1	1	11
4B	2	1	0	15
4C	3	2	0	93
5A	3	3	3	>1100
6A	3	3	2	1100
7A	2	0	0	9
7B	3	3	0	240
7C	3	1	0	43
8A	3	3	2	1100
8B	3	1	2	120

Based on the results of research on the number of *Escherichia coli* bacteria in Table 3, it can be seen that all red guava juice samples were positive for containing *Escherichia coli* bacteria. The highest results were in sample 5A (Larasati Juice) with an MPN index value reaching >1100 APM/ml. Positive *Escherichia coli* bacteria are closely related to sales locations located next to highways where they are easily contaminated with bacteria and conditions where traders do not maintain cleanliness, such as using water to wash tools and fruit, water used additionally during the grinding process. Storefront storage conditions must be cleaned and mixed with other food ingredients, causing bacterial contamination. The use of block-type ice cubes can also be an indication of the high level of bacterial contamination in sample 5A (Larasati Juice) because it is very likely that the way the block ice is made is unhygienic or the water as the raw material for the ice cubes is not cooked first/raw. This is supported by the statement [20] that the distribution of ice blocks from producers to consumers is usually carried out using means of transportation, namely trucks or pick-up cars. Sometimes, when distributing ice cubes, producers need to use better packaging, such as only burlap sacks as a cover, and there are even ice cubes that need to be packaged at all or left open.

The lowest result was in sample 7A (Indomaret Juice) with an index value of 9 APM/ml because the condition of the sales place was very clean. After all, it was in a minimarket where the temperature was cold. The water used in making jambi seed juice uses mineral water because Indomaret has its mineral water products. Indomaret Juice (7A) does not use ice cubes as a coolant but uses a refrigerator, thereby reducing bacterial contamination from ice cubes. The condition of the clean and cool display case indicates low contamination by *Escherichia coli* bacteria. Storage at cold temperatures does not kill microorganisms but only inhibits or slows the growth of microorganisms [21].

### 3.3 Total Plate Count analysis (TPC)

Results of quantitative analysis of TPC using the pour plate method on 15 samples of red guava fruit juice from fruit juice traders in Keboansikep Gedangan Village, Sidoarjo. The TPC values tested based on the number of colonies in 15 red guava juice samples can be seen in Table 4.

Based on the data from the TPC testing results of guava fruit juice samples using the Pour plate method in Table 4.3, it can be seen that the sample containing few microbes is sample 7A with a TPC value of  $4.9 \times 10^3$  CFU/ml. The sample containing many microbes is 5A with a TPC value of  $5.1 \times 10^5$  CFU/ml. Pour plate is a method that is usually used to calculate the number of bacteria that grow in liquid samples [22]. The advantage of the pour plate is that it can count several types of bacteria at once (colonies) and can be used to isolate microbes, while the disadvantage is that it cannot differentiate the types of growing microorganisms.

**Table 4.** TPC Test Result Data

Sample	Dilution			PNA Score (Cfu/ml)
	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>	
1A	SPREAD	188	53	3.5 x 10 <sup>5</sup>
2A	196	102	82	3.1 x 10 <sup>5</sup>
2B	74	62	42	1.6 x 10 <sup>5</sup>
3A	243	188	108	4.3 x 10 <sup>5</sup>
3B	174	112	60	2.4 x 10 <sup>5</sup>
4A	119	75	52	2.0 x 10 <sup>5</sup>
4B	134	85	69	2.6 x 10 <sup>5</sup>
4C	164	105	48	2.0 x 10 <sup>5</sup>
5A	236	168	135	5.1 x 10 <sup>5</sup>
6A	149	74	46	1.8 x 10 <sup>5</sup>
7A	49	22	18	4.9 x 10 <sup>3</sup>
7B	92	52	32	1.3 x 10 <sup>5</sup>
7C	149	90	63	2.4 x 10 <sup>5</sup>
8A	227	127	62	2.6 x 10 <sup>5</sup>
8B	79	35	22	2.1 x 10 <sup>4</sup>

The total plate number is a general indicator describing the degree of contamination of food and drink. The total plate count method was used because it can calculate the overall number of bacteria not based on certain types of microbes. It can be a temporary requirement for microbial contamination in red guava fruit juice. Data analysis was carried out by describing the TPC results on red guava juice samples, presented using Standard Plate Counts (SPC) and in table form to make reading easier. SPC is a method for obtaining microbial count results in the range of 25 – 250 CFU (Colony Forming Unit) / ml to minimize the possibility of errors in the analysis process, especially statistical errors.

Dilution is carried out in stages to get colonies that grow separately because samples that are not diluted will be very concentrated and possibly TMTTC (Too Many To Count). Microbial calculations from the plated dilutions were dilutions of 10<sup>-2</sup>, 10<sup>-3</sup>, and 10<sup>-4</sup> CFU/ml because the analysis carried out at the 10<sup>-1</sup> dilution obtained the TMTTC or spread results. According to NSB (2006), dilution of samples is carried out to obtain colonies that grow separately to make it easier to count. It can be especially helpful for samples with very high contamination.

### 3.4 Microbiological Quality of Red Guava Juice

According to [23], the microbiological quality of a food product describes the extent to which it is from microbial contamination and safe for consumption. Counting total microbes plays a role in determining the sanitary status of food or drinks. The microbiological quality of red guava juice can be seen in Table 5, showing that almost all samples exceed the microbial contamination limit. The maximum limit is 104 CFU/ml according to Indonesian National Standard 3719:2014 concerning the Quality of Fruit Juice Drinks. The large total number of microbes can be influenced by the seller's habits of not paying enough attention to factors that can contribute to the high level of contamination in red guava juice, namely the quality of the fruit, water, equipment, and the surrounding environment (air). Of these things, the one that can contribute the most is water.

The main source of contaminants in fruit juice is water, either in the form of added drinking water, ice cubes, or water used for washing ingredients and equipment [6]. Similar results were also presented by [24], one of the critical control points in processing guava into fruit juice is the water quality. The water used in the process should be free of microbiological contamination, and chemical contamination, especially chlorine residue.

Based on the data from the analysis, it was found that the percentage of microbiological contamination that met the requirements was 7%, and microbiological contamination that did not meet the requirements was 93%. These results show that sanitation and hygiene carried out by fruit juice sellers could be better. It is proven that almost all fruit juices need to meet the requirements. The only sample that meets the requirements is 7A, namely Indomaret juice. A clean area and cold storage greatly influence microbiological growth. It is important to pay attention to the air conditions and storage temperature of drinks because they can maintain the microbiological quality of food. Drinks stored at room temperature can increase the number of microbes to twice the original and can be contaminated with pathogenic bacteria. The beverage processing process, especially the temperature used in the process, can influence the quality of beverage microorganisms [25].

**Table 5.** Microbiological Quality of Red Guava Juice

Sample	TPC score (CFU/ml)	Maximum contamination limit (SNI 3719:2014)	Information
1A	3,5 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
2A	3,1 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
2B	1,6 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
3A	4,3 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
3B	2,4 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
4A	2,0 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
4B	2,6 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
4C	2,0 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
5A	5,1 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
6A	1,8 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
7A	4,9 x 10 <sup>3</sup>	10 <sup>4</sup> CFU/ml	Eligible
7B	1,3 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
7C	2,4 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
8A	2,6 x 10 <sup>5</sup>	10 <sup>4</sup> CFU/ml	Not Eligible
8B	2,1 x 10 <sup>4</sup>	10 <sup>4</sup> CFU/ml	Not Eligible

Microbiological contamination is influenced by several aspects, such as the use of water for washing and for the process of making fruit juice. The high level of contamination in snack drink samples indicates the use of unclean water and no prior heating treatment [26]. Sales areas on the side of the road are open to lots of people and vehicles passing by, and storage windows that are small and not tightly closed can cause insects such as flies to enter and become a source of contamination. Microbiological contamination is also influenced by the conditions of the place where fruit juice is sold. Good food and beverage sanitation hygiene needs to be supported by good environmental conditions and sanitation facilities. A hygienic place is one factor to avoid contamination. If this is done, the risk of bacterial contamination in the drinks sold can be reduced [27]. The condition of places selling fruit juice in Keboansikep Gedangan Sidoarjo Village can be seen in Table 6.

**Table 6.** Condition of the Place Selling Fruit Juice

Sample	Conditions of fruit juice sellers
1A	Only Selling Juice
2A	Selling Juice and Food
2B	Only Selling Juice
3A	Selling Juice and Food
3B	Selling Juice and Food
4A	Only Selling Juice
4B	Only Selling Juice
4C	Selling Juice and Food
5A	Selling Juice and Food
6A	Selling Juice and Food
7A	Only Selling Juice
7B	Selling Juice and Food
7C	Selling Juice and Food
8A	Selling Juice and Food
8B	Only Selling Juice

Table 6 shows that only some fruit juice sellers sell fruit juice but also sell food. The condition of the area used for the process of making fruit juice is also used for the process of making food, so bacterial contamination during the making process can be a trigger because of the 15 samples of red guava fruit juice, only 1 sample meets the requirements (Table 5), and this is a trigger that the manufacturing process can be a factor that influences bacterial contamination of red guava fruit juice.

Some fruit juice sellers sell fruit juice and various foods such as pentol, penyetan rice, etc. This condition creates cross-contamination of food ingredients because the tools used for the juice-making process are also used for the food-making process, and the storage of fruit is given less attention and is mixed with food ingredients.



## 4 Conclusion

Based on the results of *Escherichia coli* analysis, show that red guava fruit juice (*Psidium guajava* L) sold in Keboansikep Gedangan Sidoarjo Village does not meet the quality requirements for fruit juice drinks (INS 3719:2014). Meanwhile, the results of the Total Plate Count (TPC) Analysis showed that as many as 7% met the requirements, namely in sample 7A with a TPC value of  $4.9 \times 10^3$  CFU/ml, and 93% did not meet the requirements. The sample containing the most microbes was sample 5A with a TPC value of  $5.1 \times 10^5$  CFU/ml.

Supporting factors that influence the results of *Escherichia coli* analysis and TPC include the origin of the water used to wash equipment and fruit, the water used to make juice during grinding, the type of ice cubes used, the packaging used, and the cleanliness of the fruit storage display case.

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