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Comparative Analysis Average of *Coliform* and *Escherichia coli* Bacteria in Orange Juice with the Modified Most Possible Number Method

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ABSTRACT
It has been successfully determined the content of *Coliform* and *Escherichia coli* in orange juice using the MPN (Most Probable Number) method. Comparative analyses performed showed that 15 (100%) samples tested were all contaminated with *Coliform* bacteria with the range MPN value of 10 CFU/100 - 240 CFU/100 mL. The test on *Escherichia coli* bacteria showed that 5 of the 15 (33,33%) samples tested were contaminated with *Escherichia coli* bacteria with the average range of MPN value of 2.2 CFU/100 mL - 8.8 CFU/100 mL. Based on these results, it is necessary to take firm action from the local government so that further testing is carried out.
***Keywords**: Coliform; Escherichia coli; Most Probable Number; Orange Juice*

1. INTRODUCTION

Orange juice is a form of natural fruit drink with raw materials for oranges and others which are sold in various places to eat, including in food stalls. Oranges contain vitamin C of 53.2 mg per 100 g. In addition, there are also antioxidants, flavonoids, beta carotene, and hesperidin which function as body antibodies and can increase body immunity. Vitamin C deficiency is thought to make a person more susceptible to disease. People in the condition unhealthy will have an increased need for vitamin C, because of the inflammation process and increased metabolism in body. The nutritional adequacy rate for consumption of vitamin C is 90 mg/day for men and 75 mg/day for women (>15 years) in Indonesia. This adequacy can be met from food, such as vegetables and fruit. For example, oranges, bananas, blah, cabbage, spinach, potatoes [1]

The outbreak of the coronavirus or COVID-19 in the last few months has increased public awareness of the importance of maintaining endurance. So that there is an increase in demand for fruits, especially fruits that contain high vitamin C such as oranges as raw materials for making squeezed orange juice [2][3].Consuming oranges is recommended because it contains many nutrients that are very beneficial for the health of the body. However, it does not rule out the possibility of being contaminated with microorganisms, starting from the process of selecting raw materials to serve. This can happen if the food sanitation hygiene does not meet the requirements. Usually, the juice is better drunk cold, to be added to get a fresher taste. In ice can also be a supporting factor for coliform and Escherichia coli [4][5][6][7].

Coliform bacteria and Escherichia coli are a group of bacteria used as indicators of water pollution. The presence of Coliform bacteria in water indicates the possibility of enter pathogenic and toxigenic microbes that are harmful to health [8][9]. Base on the Regulation of the Minister of Health Indonesia about the requirement of drinking water quality, the value of Coliform and Escherichia coli per 100 mL sample is 0. According to research conducted by Melilisnawaty et al in 2015 regarding the examination of Escherichia coli, Staphylococcus aureus, and Salmonella in orange ice was found in 30% of orange ice contaminated with Escherichia coli bacteria which exceeded the 1991 Ministry of Health standard, while Staphylococcus aureus and Salmonella were not found in orange juice.[10].

According to research conducted by Prajna Paramita, et al in 2016 about knowing the presence of Coliform and Escherichia coli in orange ice packs in the elementary school area of Tembalang sub-district, 84.6% of orange ice samples had Coliform contamination that did not meet MPN <3 requirements [3].

Jalan Gajah Mada Padang is one of the main roads that is often traversed by the public, educational, and office areas. On this road, many people sell squeezed orange. Base on our observations of the area, it is possible that the materials used in making squeezed oranges are contaminated by bacteria. Some of the orange traders pay less attention to cleanliness starting from storage containers, equipment used, hand hygiene of traders, and others who do not carry out the heating process so that there are still bacteria and are not fit for consumption. Based on this, in this study, we studied the contamination of Coliform and Escherichia coli bacteria in squeezed orange juice sold along Jalan Gajah Mada, Padang.

2. MATERIALS AND METHODS

2.1 Material

The number of samples to be studied is 15 samples. The equipment used are Cool Box, Incubator, Waterbath, Test tube rack, test tube, Durham tube, Automatic 10 mL pipette, Automatic pipette 0-1 mL, sterile pipette tip, dropper pipette, Ose, Object Glass, Bunsen, Petri dish, Permanent markers. LB (Lactose Broth), BGLB (Brilliant Green Lactose Bile Broth), Endo Agar, Mac Conkey Agar, SIM Media (Indol Motility Sulfur), TSIA (Triple Sugar IronAgar), and Simmons Citrate Agar (SCA)

2.2 Analysis average of Coliform and Escherichia coli Bacteria in Orange Juice with the Modified Most Possible Number Method

2.2.1 Estimating Test (Presumptive Test)

Purpose: to look for bacteria that ferment lactose and form a gas at 37˚C for 2 x 24 hours. All samples of squeezed orange juice that had been brought to the laboratory were immediately planted into LB (Lactose Broth) media. Prepared 7 tubes, each containing Lactose Broth. Put 10 ml of sample into tube 1 to tube 5, then 1 ml of sample into tube 6, and finally 0.1 ml of sample into tube 7. The tube is shaken slowly so that the water sample is spread evenly throughout the media. The tubes were incubated at 37˚C for 2 x 24 hours. After being incubated for 2x24 hours, each tube was observed for the presence or absence of gas in the Durham tube. If there is gas, it means the estimator test is positive. However, if there is no gas, it means that the estimator test is negative, and if there is gas, it is continued with the acidification test.

2.2.2 Confirmed Test

Purpose: to determine whether the gas-forming fermentation in the initial test was caused by Coli bacteria. From each tube that is positive for Lactose Broth, 1-2 oses are taken and then put into two tubes containing BGLB (Brilliant Green Lactose Bile Broth). The first BGLB tube was incubated at 37˚C in an incubator for Coliform bacteria examination for 24 hours. The second BGLB tube was incubated at 44˚C in a water bath for the examination of Escherichia coli bacteria for 24 hours. Observe the presence of gas in the Durham tube.

2.2.3 Completed Test

The positive culture formed gas in BGLB which was stored in a water bath at 440C then planted by scratching the colony in a zigzag four sectors into the Endo Agar medium (to see the growth of Escherichia coli bacteria). A positive culture formed gas in BGLB which was stored in an incubator at 370C and then planted by scratching the colony in a zigzag four sectors into McConkey's medium (to see the growth of Coliform bacteria). Incubate in an incubator at 37oC for 2x24 hours. Observe the morphology of bacterial colonies on each medium. Purpose of Gram staining is done to determine the group of gram-positive or gram-negative bacteria. Drop one drop of Physiological NaCl on the object-glass. Take one end of the Ose of the bacterial colony and homogenize it on NaCl drops. Drop Gram A on the preparation and leave it for 1 minute then discard the rest of the stain. Drops of Gram B until the remaining gram A stain dissolves then rinse with running water. Add Gram C for 1 minute and rinse under running water. Add Gram D for 20 seconds and rinse with running water

3. RESULTS AND DISCUSSION

3.1 Coliform and Escherichia coli MPN Value on Orange Juice Estimating Test (Conjecture Test)

Based on table 3.1 above, it was found that all Durham tubes on LB media formed gas (air bubbles). This means that positive results are obtained for all samples in the presumptive test. The positive results obtained in this estimator test are an initial illustration that all samples of squeezed orange juice tested for bacteria are not in accordance with those required by Permenkes no. 492 of 2010. All tubes with positive results were continued to the assay test by planting the sample on LB media into BGLB. Media.

Based on table 3.1, positive results were obtained in all tubes with LB media. This means that all tested samples have been contaminated with Escherichia coli bacteria. To ensure the presence of Escherichia

coli bacteria in the sample, it is followed by an acidification test.

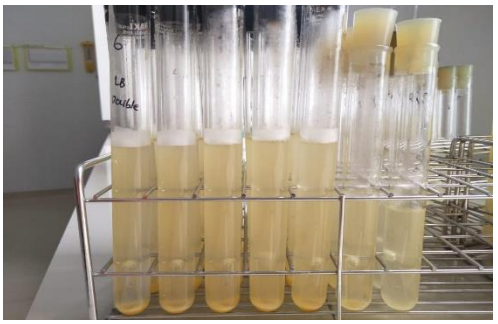

Figure 1. Estimating Test of Testube

Table 1. Presumtive Test *Coliform and Escherichia coli* Bacteria

No. Samples	*Presumtive Test* wit LB Media		
	10cc	1cc	0,1cc
S. 01	+++++	+	+
S. 02	+++++	+	+
S. 03	+++++	+	+
S. 04	+++++	+	+
S. 05	+++++	+	+
S. 06	+++++	+	+
S. 07	+++++	+	+
S. 08	+++++	+	+
S. 09	+++++	+	+
S. 10	+++++	+	+
S. 11	+++++	+	+
S. 12	+++++	+	+
S. 13	+++++	+	+
S. 14	+++++	+	+
S. 15	+++++	+	+

Noted:
+ : There are air bubbles in the Durham tube
- : There are no air bubbles in the Durham tube

From the table 1 above, it is found that all Durham tubes on LB media are gas (air bubbles). This means that positive results are obtained for all samples in the presumptive test.

3.2 Confirmed Test

Based on table 3.2, we can see that positive results were obtained on BGLB media with different values. A positive result in this confirmatory test confirms that coliform bacteria are present in the sample. The total Coliform value was obtained based on matching the number of positive tubes with the MPN index table. The total value of Coliform in each sample varies greatly, namely 10-240 CFU/100 mL

Table 2. *Comfirmed Test of Coliform Bacteria*

No. Sample	BGLB Media (37^0C)			Total *Coliform*
	10mL	1mL	0,1mL	
S. 01	+++++	-	+	96
S. 02	++++	+	+	27
S. 03	+++++	+	+	240
S. 04	+++++	+	+	240
S. 05	++	+	+	10
S. 06	+++++	+	+	240
S. 07	+++++	+	+	240
S. 08	+++++	+	+	240
S. 09	+++++	+	+	240
S. 10	++++	+	+	27
S. 11	+++++	+	+	240
S. 12	+++++	+	+	240
S. 13	+++++	+	+	240
S. 14	+++++	+	+	240
S. 15	+++++	+	+	240

Based on table 2 it was found that 5 samples (33.33%) showed positive results while 10 samples (66.67%) showed negative results. The positive values for the five samples varied from 2.2 to 8.8 CFU/100 mL.

In other words, the five samples also did not comply with the requirements set by standard of Ministry of Health in Indonesia where the results of the examination of Escherichia coli bacteria must be 0 [8][2][4]. To further ensure that the sample contains Escherichia coli bacteria, the positive BGBB tube sample is replanted in the media of Endo agar.

Table 3. *Comfirmed Test Escherichia coli Bacteria*

No. Sample	Media BGLB (37^0C)			Total *E.coli*
	10mL	1mL	0,1mL	
S. 01	-----	-	-	0
S. 02	-----	-	-	0
S. 03	+	-	-	2,2
S. 04	+++	-	-	8,8
S. 05	+	+	-	4,4
S. 06	-----	-	-	0
S. 07	-----	-	-	0
S. 08	-----	-	-	0
S. 09	++	-	+	7,5
S. 10	-----	-	-	0
S. 11	-----	-	-	0
S. 12	-----	-	-	0
S. 13	+	-	-	2,2
S. 14	-----	-	-	0
S. 15	-----	-	-	0

After all the samples were planted into Mc media. Conkey for incubation, the growth of bacterial colonies with a convex round shape of medium size and pink color was obtained. The growing bacterial colonies were then subjected to biochemical and confectionery tests.

Based on table 3, it was found that 5 samples (33.33%) showed positive results while 10 samples (66.67%) showed negative results. The positive values for the five samples varied from 2.2 to 8.8 CFU/100 mL. In other words, the five samples also did not comply with the requirements set by standard of Ministry of Health in Indonesia where the results of the examination of Escherichia coli bacteria must be 0. To further ensure that the sample contains Escherichia coli bacteria, the positive BGBB tube sample is replanted in the media. Endo agar.

3.3 Completed Test (Completed Test)

Before the biochemical and confectionery tests were carried out, a gram staining test was performed on one of the bacterial colonies that grew on each medium, and the results of gram-negative bacteria were obtained in all tested samples. These gram-negative bacteria are rod-shaped and red in color.

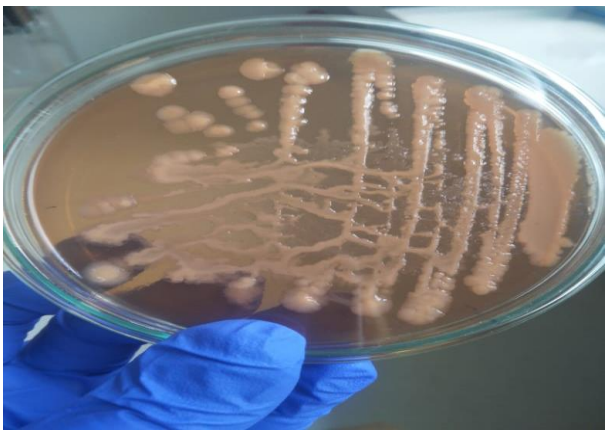

Figure 2. Bacterial colonies of *Coliform* in Mc. Conkey Media

After being planted on an endo agar medium, the growth of colonies in the form of convex rounds, medium to large in size, and metallic in color (metallic luster) was obtained in the five samples. This metallic colored bacterial colony is a specific characteristic of Escherichia coli bacteria

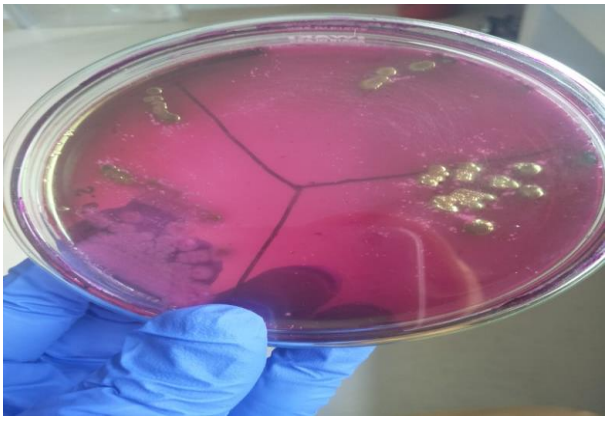

Figure 3. Bacterial colonies of *E.coli* in Endo Media

4. CONCLUSION

Based on the results of research that has been carried out on samples of squeezed orange juice sold along Jalan Gajah Mada Padang, it can be concluded that: All samples of squeezed orange juice that have been tested are contaminated with

Coliform bacteria with different values, with the lowest value being 10 CFU/100 mL and the highest value was 240 CFU/100 mL. A total of 5 samples tested were contaminated with Escherichia coli bacteria with the lowest value of 2.2 CFU/100 mL and the highest value of 8.8 CFU/100 mL.

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