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IDENTIFICATION OF ESCHERICHIA COLI AND SALMONELLA SP. ON FRIED SNACKS SOLD ON THE UNIMA CAMPUS ENVIRONMENT

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Abstract

This study aims to explain how bacterial contamination and the presence of Escherichia coli and *Salmonella* sp. on fried bananas sold in the UNIMA campus environment. This exploration used clear qualitative subjective observation with the TPC strategy for all total bacterial contamination and involved specific media for Escherichia coli and *Salmonella* sp. From the results of the review, it was found that 1 out of 3 experimental examples of complete bacterial contamination exceeded the threshold set by the Food and Drug Supervisory Agency of the Republic of Indonesia (BPOM RI) in 2016, with the most extreme obstacle being the number of microorganisms in food being 10-4 colonies/ g. Of the 3 samples tried, all samples were identified with Escherichia coli and *Salmonella* sp. Based on the research conducted, it can be concluded that, of all the observed fried samples, all detected bacterial colonies.

Keywords: Fried Food, Total Plate Count (TPC), E. coli, *Salmonella* sp

INTRODUCTION

Sellers awareness of food hygiene needs attention because contaminated food can cause foodborne illnesses. The most common bacteria are *Escherichia coli* and *Salmonella* sp. Food that has been infected with bacteria is caused by food sales that do not pay attention to cleanliness (Arlita, 2014). For example, in the campus environment, especially at Manado State University, selling fried snacks is done freely so you can find many snack food sellers selling in the campus environment.

The campus canteen provides many types of delicious food, especially fried snacks which are most popular with most students (Hikmah et al., 2017). Campus canteens have an important role in meeting students' needs for food while on campus. The research location was chosen on campus because each canteen is in a faculty, although there have been no cases of food poisoning, canteens can pose a risk for transmitting disease through food (Sunarya & Ririh, 2019). Foodborne disease is an infection caused by consuming contaminated food, including diarrhea and food poisoning. Foodborne disease occurs when bacteria can multiply after manufacture (Muna and Khariri, 2020). Foodborne disease is caused by different microorganisms, for example *Escherichia coli* and *Salmonella* sp. (Arlita, 2014). One food that is

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easily contaminated is fried food.

E.coli bacteria is one of the bacteria that has the potential to contaminate fried snacks. *E.coli* is a gram-negative rod bacteria which is normal flora in the digestive tract of humans and animals which easily contaminates water. Pollution by *E.coli* bacteria is influenced by waste water drainage channels, the distance of the business location to the source of pollution, waste storage areas and so on. Contamination of *E.coli* bacteria in fried snacks can have a negative impact on consumers' health, for example causing diarrhea (Julia et al., 2017).

Apart from *E.coli* bacteria, *Salmonella* sp. It is also an indicator bacteria that causes foodborne disease which can cause inflammation of the digestive tract ranging from mild gastroenteritis to bacteremia accompanied by typhoid fever. This disease occurs due to infection with *Salmonella* sp. through fried snacks consumed by the public (Kuala, 2020). Based on the description above, researchers want to identify the bacteria *Escherichia coli* and *Salmonella* sp. in fried snacks sold on the FMIPA UNIMA campus.

RESEARCH METHODS

This research was carried out at the Microbiology Laboratory, Biology Department, Faculty of Mathematics and Science, Manado State University. This research will be carried out in December 2021. The determination of this sample test was fried snacks sold in the Manado State University canteen. The sample to be taken is fried banana.

The tools that will be used in this research are Erlenmeyer tubes, measuring pipettes, suction balls, test tubes, test tube racks, aluminum foil, bunsens, lighters, petri dishes, spatulas, tweezers, L rods, 100µL tips, 1000µL micropipettes, mortal and pestle, analytical balance, hot plate, magnetic stir, cotton, waste paper, autoclave, oven, incubator, laminar air flow, camera, and refrigerator.

The materials that will be used in the research are fried snacks, sterile distilled water, tissue, masks, handscoon, Nutrient Agar (NA) media, *Salmonella* Shigella Agar (SSA), Eosin Methylen Blue (EMB).

Dilution

The sample was weighed at 5 grams, ground using a mortar, then placed in an Erlenmeyer containing 100 ml of Aquadez and homogenized. After that, take 1 ml of the solution using a 1000µL tip and then put it into a tube containing 9 ml of distilled water so that a dilution level of 10-1 is obtained and homogenize using a vortex. Then it continues like that until the 6th test tube.

Sample preparation

After completing the dilution stage, then take 0.1 ml with a micropipette from a focus level of 10-4 and then put it into a cup containing the media until it reaches the 10-6 test tube. And don't forget to put a name on each petri dish. After that, prepare the L rod and soak it in alcohol. Every time you use the L rod, put it straight back into the alcohol, then pass it over the fire 1-2 times, leave it for a while until it's not hot. Rub the L stick on the agar medium to spread using the spreading method technique (Cruickshank, 1975). The function method is repeated until the focus level is 10-6. Next, let it sit or incubate for 1 x 24 hours at

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370C in an inverted state, after which the amount of precipitation that develops on the media is counted.

Take 0.1 ml of solution from a 10-1 dilution then put it in a petri dish containing EMB media. Absorb the L stem liquid, then pass it over the flame 1-2 times, let it sit for a few moments until the L stem is no longer hot. Rub the L rod over the EMB medium to equalize the solution so that it is the same as the sample. After that, let it sit again for approximately 3 days or 3 x 24 hours. If positive for E. coli, the surface is metallic green, normal in shape with a slightly convex surface.

After the EMB media stage, create another SSA media. Just like EMB media, SSA media also take 1 ml of sample solution from a 10-4 dilution then pour it into a petri dish containing SSA media. Soak the L rod again in the alcohol solution, then pass it over the flame again 1-2 times, let it sit for a few moments until the L rod is no longer hot. Scratch the L bar over the SSA medium to equalize the sample solution. Leave again (incubate) for approximately 3 x 24 hours. If positive for *Salmonella* sp. The colonies are clear (colorless) with a black core.

Data Analysis Techniques

Examination of research data was carried out by describing the morphology of the bacteria and the TPC results on food samples which were presented in tabular form. Checking TPC results uses the Standard Plate Count (SPC) strategy to make it easier to read the results. SPC is a technique for obtaining microbial count results in the range of 30 - 300 CFU (Colony Forming Unit)/ml starting from a dilution of 10-1, up to 10-6.

RESULTS AND DISCUSSION

Fried banana samples were taken from several areas on Universitas Negeri Manado (Unima), at Faculty of Engineering (FATEK), Faculty of mathematics, natural sciences and earth sciences (FMIPA) and Faculty of sports science (FIK), then analyzed at the UNIMA FMIPA Laboratory and carried out based on the same time.

Table 1. Results of testing for *E.coli* and *Salmonella* sp bacteria at FIK UNIMA

Lokasi Pengambilan Sampel	Kode Sampel	Jumlah bakteri			Hasil Deteksi	
		10^{-3}	10^{-4}	10^{-5}	Positif (+)	Negatif (-)
FIK Unima	PG 1A (*E.coli*)	TBUD	73	12	+	
	PG 1B (E. coli)	6	3	2	+	
	PG 1C (E. coli)	TBUD	TBUD	93	+	
	PG 1A (*Salmonella* Sp.)	50	42	10	+	
	PG 1B (*Salmonella* Sp.)	18	2	1	+	
	PG 1C (*Salmonella* Sp.)	25	20	15	+	

Information :
PG = Fried Banana
TBUD = Too Many To Count
A = Bottom Fry
B = Middle Fries
C = Top Fries
TBUD = Too Many To Count

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Table 2. Test Results for *E.coli* and *Salmonella* sp. at FATEK UNIMA

Lokasi Pengambilan Sampel	Kode Sampel	Jumlah bakteri			Hasil Deteksi	
		10^{-3}	10^{-4}	10^{-5}	Positif (+)	Negatif (-)
FATEK Unima	PG 2A (E. coli)	25	15	5	+	
	PG 2B (E. coli)	20	11	7	+	
	PG 2C (E. coli)	TBUD	TBUD	53	+	
	PG 2A (*Salmonella* Sp.)	18	10	3	+	
	PG 2B (*Salmonella* Sp.)	5	4	1	+	
	PG 2C (*Salmonella* Sp.)	20	11	4	+	

Table 3. Test Results for *E.coli* and *Salmonella* sp. at FIMPA UNIMA

Lokasi Pengambilan Sampel	Kode Sampel	Jumlah bakteri			Hasil Deteksi	
		10^{-3}	10^{-4}	10^{-5}	Positif (+)	Negatif (-)
FMIPA Unima	PG 3A (*E.coli*)	5	2	1	+	
	PG 3B (*E.coli*)	2	1	1	+	
	PG 3C (*E.coli*)	8	5	3	+	
	PG 3A (*Salmonella* Sp.)	4	2	1	+	
	PG 3B (*Salmonella* Sp.)	4	2	2	+	
	PG 3C (*Salmonella* Sp.)	12	6	3	+	

Discussion

Based on the location and place of sampling, there are several factors that cause bacteria to grow, including contamination through fried food, such as the water used is not clean and contains fecal matter, the tools used to make fried food, or the spread of microbes through human hands, conditions containers that are open without being closed, and crowded canteen conditions can cause the spread of microbes that are dangerous to human health Sutiknowati (2016).

The time at which samples are taken can also affect the number of bacteria that grow after 6-7 hours of fried food being sold, which will show contamination with *E.coli* and *Salmonella* sp. tall one. *Salmonella* sp and *E.coli* bacteria spread more quickly in warm weather because the bacteria grow well at room temperatures of 37°C - 45°C so fried food can become contaminated quickly.

In the fried banana samples taken from 3 different locations, namely FIK, FATEK, and FMIPA, the one with the most bacteria growing due to ecological conditions was the FIK canteen which was located near the parking lot and near the main road. This causes the danger of contamination of water raw materials with food sources processed to make fried banana dough which can occur through air, soil, touch and the environment. Fried foods that are also placed on the table open without a lid will come into direct contact with the air so that bacteria can also be sent through residue and vehicle vapors carried by the wind.

The low number of bacterial colonies found at FATEK and FMIPA was due to good handling of the

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ingredients for making fried foods which were not contaminated, the possibility of microbes dying during frying and accompanied by very good sanitation in the fried food processing process. Differences in the number of colonies during testing that are not the same due to unhygienic handling of processed products can also be caused by the condition of the canteen. The position of the canteen at Fatek and Fmipa is good and not on the side of the road or close to the parking lot.

Improper processing and tools used during processing can be used as a distribution medium for E. Coli and *Salmonella* sp. Government regulations through BPOM and Law no. 7 of 1996 concerning food and Law no. 8 of 1999 concerning consumer protection have emphasized that any food sold must comply with food safety standards in Indonesia, but there are still many people who do not pay attention to the cleanliness of the food they process. or served (Putri, 2016).

Bacterial Culture Results using the Total Plate Count (TPC) Method

Based on the information from Table 1, it tends to be seen that *E.coli* bacteria were detected in the FMIPA canteen and *E.coli* bacteria were also detected in the FATEK canteen. and in the FIK canteen, E, Coli and *Salmonella* sp bacteria were detected. The presence of *E.coli* and *Salmonella* sp. This could be caused by the way the food is served, or perhaps also by contamination from the raw water used to mix the flour in the fried bananas, or from the equipment used and the methods that are not used. correct in the processing process to serving.

Food sellers who do not pay attention to cleanliness during the processing of food sources are the main factor influencing *Escherichia coli* contamination in fried food sources. *Salmonella* sp. It can also be caused by food vendors who have been contaminated and the equipment they use is not clean. (Jiastuti, 2018). The neatness and cleanliness of cooking utensils are closely related because cooking utensils are important in the food processing process. If the cooking utensils are not clean, the food being processed will be contaminated. Cleaning equipment used for food using water that has been contaminated with E. coli and *Salmonella* sp. this makes microorganisms stick to food and utensils. Additional water used in the food cleaning cycle must be clean and not contaminated by microbes.

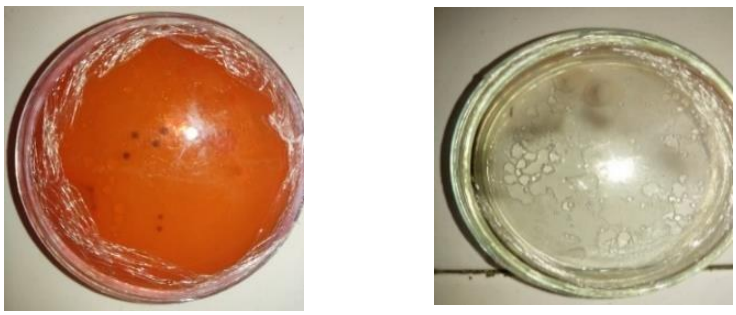

Figure 1. Growth of *Escherichia coli* and *Salmonella* sp bacterial colonies. from samples on EMB and SSA media.

On Endo Agar media, the *Escherichia coli* bacterial colonies that are incubated will form a red color with a metallic sheen. Apart from lactose, Endo Agar also contains sodium sulfite and fuchsin which makes

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this medium special by suppressing the development of gram-positive organisms. Assuming *E.coli* responds with ficshin when fucshin solidifies, this shows a greenish metallic luster on the colony while the red color is caused by lactose Lutpiatina, L. (2015). Meanwhile, *Salmonella* shigella Agar (SSA) media contains lactose which functions as a source of sugar in (SSA). Organisms that ferment lactose produce acid and form pink/red colonies, non-lactose fermenters form colorless colonies. *Salmonella* sp. will not ferment lactose but produces sulfide gas (H2S). Hydrogen sulfide gas will react and form black feces or nuclei. And colonies will appear with black cartilage nuclei or no color ripening (Dewi and Mudatsir, 2013).

Dilution

Based on the results of the research above, the calculation of the number of colonies in this study was a dilution of 10-3 to 10-5 for the bottom, middle and top fried foods. Each dilution produced a different number of microbial colonies. The results of this research observation show that the higher the dilution level, the fewer colonies that can be counted. This is because the number of microbes contained in each volume of inoculant transferred decreases as the dilution process is complete. As shown by (Soesetyaningsih and Jember, 2014), the higher the dilution level, the fewer microbes will be obtained.

TPC calculation results for each sample

In the results table above, it tends to be seen that the 3 samples did not exceed the threshold set by the Food and Drug Supervisory Agency of the Republic of Indonesia (BPOM RI) in 2016, that the most extreme limit for microbial contamination of *Escherichia coli* is 10 /g and *Salmonella* sp. negative/25g. while in other examples the FIK samples exceed the specified limits.

The level of contamination in FIK is caused by food processing processes that are not kept clean, and could also be due to the place where it is served. So fried food is contaminated by microbes. The reduced number of colonies in FATEK and FMIPA is due to good handling, uncontaminated ingredients for making fried foods, the possibility of microbes dying during frying and accompanied by very good sanitation in the fried food processing process.

CONCLUSION

Based on the research conducted, it can be concluded that: Of the 3 samples from fried foods studied, all samples contained E. Coli and *Salmonella* sp; Of all the samples studied, the PG 1C sample had the most *E.coli* bacterial contamination detected; Samples are easily contaminated with bacteria due to various factors, including during food transportation, places where food is sold and processed and how food is served.

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