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DIFFERENCES IN THE NUMBER OF COLIFORM BACTERIA IN 3 TYPES OF ICE CUBES (PLASTIC PACKAGING ICE CUBES, CRYSTAL ICE, AND ICE BLOCKS)

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ABSTRACT
The Ice cubes have a very low temperature, this is an assumption that the ice cubes consumed are relatively safe, even though coliformbacteria at that temperature do not necessarily die. Coliformis a bacterium that is used as an indicator of water contamination. Research Objectives comparing Coliform bacteria in ice cubes (plastic packaging, ice crystals, ice cubes) sold in the Peterongan market. This research is an observational study with descriptive research criteria through a cross sectional approach. All samples of 15 ice cubes consisting of plastic packaged ice cubes, ice crystals, ice blocks sold at the Peterongan Market, Semarang city 6 samples (5, 8, 2, 6, 14, 15) did not meet the requirements set by the Agency National Standardization No. SNI 388:2009 regarding maximum limit of microbial and food contamination is 3/gramMPN index. This is caused by hygiene and sanitation factors such as not providing water for washing hands and washing utensils, poor waste handling, unhygienic serving of ice cubes. In addition, the water factor is the raw material for ice cubes which is not clean and is not cooked first Samples of ice cubes sold by beverage traders at the Peterongan market, Semarang City were all found to contain Coliformbacteria.

INTRODUCTION

Indonesia is a tropical country in Southeast Asia located on the equator. Therefore, some Indonesians prefer to use ice cubes as an additional ingredient in drinks. The product favored by the Indonesian population is ice cubes because the way of presentation is very easy, which is added with drinks and food (Murtiningtyas, 2018). Low temperatures in ice cube products, it is an assumption that ice cubes consumed become relatively safe, even though coliform bacteria at that temperature do not necessarily die. (Nurmalasari, 2019).

Water is one of the important components of living things, almost 98% of living things are composed of water. A vital resource for the life of living beings is water. The main function of water for life is needed in the process of photosynthesis, nutrient distribution, and body temperature control (Suyasa & Santhi, 2018).

Coliform is one of millions of bacteria that can be a benchmark for indicators of poor conditions for water, one example is the bacteria Escherichia coli and Klebsiella

pneumonium. (Saputro & Susilowati, 2019) In food and beverages show bacteria such as coliform, the possibility of microorganisms that are not good for health. Humans may be infected with coliforms caused by factors including consuming contaminated materials, for example through the hands of distributors and sellers, packaging that is less sterile, and the water used is mixed with raw materials that have been contaminated, and many more focal factors. (Aris Widyo Nugroho, Nurahman, & Septiaji, 2016).

Coliform bacteria are a class of intestinal bacteria, which live in the human digestive tract (Feizollah, Anuar, Salleh, & Wahab, 2015). Coliform bacteria are gram-negative, when viewed with staining will be red, while another characteristic property is the ability of these microbes to ferment lactose at temperatures of 35-37 oC (Dimas Hafidh Nugroho, Restu, & Ernawati, 2018). According to (Sunarti, Rahmawati, & Wardani, 2016) there are two types of coliform bacteria, namely: fecal colifom and non-fecal coliform. Bacteria contained in human and animal feces belong to the type of pathogen. There are also bacteria that are often found in water contaminated by human or animal feces including colifoman bacteria, one of which is Escherichia coli bacteria, which are microbes that cause various diseases and include symptoms of abdominal pain, increased body temperature, stomach pain, and vomiting. (Bambang, 2014).

One of the absolute requirements and quality requirements that must be owned in the water content used for making ice cubes according to the Regulation of the Minister of Health of the Republic of Indonesia Number 492 / MENKES / PER / IV / 2010. The most commonly used as indicators of microorganisms of contamination are Escherichia coli and coliform group bacteria. Indonesian National Standard Regulation number 7388 of 2009 concerning the maximum limit of microbial contamination in food explains that the maximum limit for coliform bacteria in ice cubes consumed is 3 / gram. (SNI-7388 of 2009).

RESEARCH METHODS

This type of research is observational with descriptive research criteria through a cross sectional approach. The population in this study was as many as 15 ice stalls selling in Pasar Peterongan along the road of peterongan quality ice cubes and was in accordance with SNI-7388 regulations of 2009.

RESULTS AND DISCUSSION

Examination of coliform bacteria with MPN method

Table I Organoleptis samples

	Ice Blocks	Plastic Ice	Ice Crystals
Code	E1	E2	E3
Shape	Ice Cube	Ice Cube	Ice Cube
Color	Clear	Clear	Clear

In this study, coliform tests with the 5-1-1 double-series tube method (5 *double lactose broth*, 1-1 *single broth*) as examined in bacteriological as in the instructions. In this test, a variety of

5x10 grams, 1x1 grams, 1x0.1 grams are used, filled with 10 ml media per tube complete with an inverted durham tube. The examination is through 2 ways.

Estimator Test

A series of tubes containing standard LB 5-1-1 media each filled with samples of 10 grams, 1 gram, 0.1 grams using a sterile measuring pipette. Filling is carried out as septically as possible. All tubes are inserted in the incubator at a temperature of 37 $^{\circ}$C and guided 1x24 hours. A positive result can be seen if a gas is formed which can be seen in the form of an empty cavity at the top of the inverted durham tube in the LB media.

Table 2 Ice Block Sample Estimator Test Results

code E1	Sample 10 ml						Sample 1 ml	Sample 0.1 ml
1	+	+	+	+	+	+		_
4	+	+	+	+	+	+		_
5	+	+	+	+	+	+		+
8	+	+	+	+	+	+		+
9	+	+	+	+	+	+		_

According to table 2 of the research conducted, 5 positive samples containing coliform in 1 gram samples and 2 positive samples containing coliform in 0.1 gram samples.

Data on the estimator test of plastic packaging ice cube samples with the 5-1-1 method can be seen in table 3 below:

Table 3 Plastic Packaging Ice Sample Estimator Test Results

Code E2	Sample 10 ml						Sample 1 ml	Sample 0.1 ml
3	+		+	+	+	+	+	_
7	+		+	+	+	+	+	_
10	_		+	+	+	+	+	_
11	+		+	+	+	+	+	_
13	_		+	+	+	+	+	+

According to table 3 of the research conducted, 5 positive samples containing coliform in 1 gram sample and 1 positive sample containing coliform in 0.1 gram sample.

Data presented on the estimator test of crystal ice cube samples with the 5-1-1 method can be seen in table 4 si below.

Table 4 Ice Crystal Sample Estimator Test Results

Code No. E3	Sample 10 ml					Sample 1 ml	Sample 0.1 ml
2	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+
12	+	+	+	+	+	+	_

14	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+

According to table 4 of the research conducted, 5 positive samples containing coliform in 1 gram sample and 4 positive samples containing coliform in 0.1 gram sample.

B. Affirmation Test

Furthermore, after conducting the estimator test, proceed with the affirmation test to ensure the results. Then each positive sample showing gas was grown on GLB media using ose and incubated in an incubator at a temperature of 37 ^{0}C and left for 1x24 hours. If gas forms in several GLB media cylinders, then we equate and we read in the MPN index table 5-1-1.

Data presented on the affirmation test of block ice cube samples with the 5-1-1 method can be seen in table 5si below.

No. kodeE1	Sampel 10 ml					Sampel1 ml	Sampelo,1 ml	Nilai indeks MPN
1	+	+	+	+	+	+	_	256
4	+	+	+	+	+	+	_	256
5	+	+	+	+	+	+	+	<979
8	+	+	+	+	+	+	+	<979
9	+	+	+	+	+	+	_	256

According to table 5 of the research conducted, the results were 979 MPN as many as 3 samples and 256 MPN as many as 2 samples.

Data are presented on the affirmation test of plastic packaging ice cube samples with the 5-1-1 method can be seen in table 6 below:

Table 6 Plastic Packaging Ice Sample Affirmation Test Results

Code No. E2	Sample 10 ml	Sample 1 ml	Sample 0,1 ml	MPN
3	+ + + + +	+	_	256
7	+ + + + +	+	_	256
10	+ + + + +	+	+	22
11	+ + + + +	+	+	256
13	+ + + + +	+	_	22

According to table 6 of the research conducted, the results were 256 MPN as many as 3 samples and 22 MPN as many as 2 samples.

Data presented on the estimator test of crystal ice cube samples with the 5-1-1 method can be seen in table 2 si below

Table 7 Ice Crystal Sample Affirmation Test Results

No. code E3	Sample 10 ml	Sample 1 ml	Sample 0.1 ml	MPN
2	+ + + + + +		_	979
6	++ + + + +		_	979
12	++ + + + +		+	256
14	++ + + + +		+	979
16	++ + + + +		_	979

According to table 7 of the research conducted, the results were 979 MPN as many as 4 samples and 256 as many as 256 MPN as many as 1 sample.

In the estimator test table 2, 3, 4, all ice cube samples were positive, in the 1 gram sample, there were gas grains in the Durham tube and there was a change in color in the LB tube from red to yellow, and there were 8 negative samples in the 0.1 gram sample. In the affirmation test table 5, 6, 7 positive results which mean the presence of gas activity and possible development of *coliforms* on BGLB media. The affirmation test results are entered into the MPN table to find the MPN index value. Next we can see the results of the MPN index of coliform bacteria present in 1gram of ice cube, plastic, and crystal samples. We can see that ice cubes with the lowest to highest MPN values are found in ice cube samples, the lowest are found in Plastic ice cubes with number 10.13 (22/1 gram) then the highest MPN values in Crystal ice cubes with numbers 2, 6, 14, 16 (979/1 gram).

Presented data on hygiene and sanitation observations in my research can be seen in the table below:

Table 8. Hygiene and sanitation observations

No	No lab	Hygiene and sanitation			Total score
		Personal	Equipment	Milieu	
1	1	7	4	2	13
2	4	7	4	2	14
3	5	5	2	1	8
4	8	5	2	1	8
5	9	7	4	2	13
6	3	7	4	2	13
7	7	7	4	2	13
8	10	9	4	2	15
9	11	7	4	2	13
10	13	8	5	2	15
11	2	5	2	1	8
12	6	5	2	1	8
13	12	7	4	2	13
14	14	5	2	1	8

15 15 5 2 1 8

Based on Table 8, samples with numbers 1, 4, 9, 3, 7, 10, 11, 12, 13 are classified as good and samples with numbers 5, 8, 2, 6, 14, 15 are classified as poor in consumption.

Data on MPN and hygiene and sanitation values and SNI standardization are presented in the following table

Table 9 regarding the comparison of MPN and Hygiene and Sanitation values and SNI standardization.

No	No lab	NilaiMPN	Totalskor	SNI-7388 Tahun2009 300/100ml
1	1	256	13	<300
2	4	256	14	<300
3	5	<979	8	>300
4	8	<979	8	>300
5	9	256	13	<300
6	3	256	13	<300
7	7	256	13	<300
8	10	22	14	<300
9	11	256	13	<300
10	13	22	15	<300
11	2	<979	8	>300
12	6	<979	8	>300
13	12	256	13	<300
14	14	<979	8	>300
15	16	<979	8	>300

Based on table 9 regarding the comparison of MPN values and hygiene and sanitation and standardization shows that samples have been contaminated by high amounts of coliform bacteria, so that 6 have MPN values of >3grams and 9 samples have MPN values of <3grams.

Based on the results of research that has been conducted on samples of three types of ice cubes, namely plastic packaging ice, ice crystals, and ice blocks with the MPN (*Most Probable Number*) method with a tube series of 5 1 1. The 5 1 1 tube series is used because ice cubes are materials or samples that have been processed or the number of germs is estimated to be low (Hadi & Radiyatul, 2014). The MPN method examination consists of two test stages, namely the estimator test and the affirmation test. At the estimator test and affirmation test stages, the examination was repeated four times.

Ice cube samples using estimator tests grown on Lactose Broth (LB) media with series 5 1 1. Then incubated with a temperature of 37 ^{o}C for 1x24 hours. To the praise of the estimator, all samples showed positive results on all tubes. The estimator test uses *Lactose Broth* media to detect the strength of *Coliform* bacteria. If the LB media is formed acid and gas, this indicates that the sample contains Coliform bacteria. The formation of acid and gas in the media is caused by lactose fermentation carried

out by bacteria (Septyasari, 2014) Coliform. The formation of acid is seen from the turbidity of the LB media and the gas produced is seen in the formation of air bubbles in the durham tube. The formation of gas in Durham's tube does not always indicate the presence of *coliform* bacteria because other bekteri can ferment lactose in LB media by forming gas including lactic acid bacteria. Therefore, it is necessary to conduct an affirmation test. In addition, to ensure the presence or absence of *Coliform bacterial contamination* in the sample, it is necessary to do an affirmation test (Irianto et al., 2013).

The results of the examination on the affirmation test showed that the ice cube that was tested positive was found *Coliform* bacteria. A positive result is shown by coliform bacteria, there are gas grains around the durham tube derived from lactose fermentation. BGLB media contains *brilliant green* which functions to inhibit the growth of gram-positive bacteria (Putri & Kurnia, 2018). Of the three types of ice cubes examined, namely plastic packaging ice, ice crystals, and ice blocks, all found *Coliform* bacteria with a contaminant amount of 22/1 gram, 240/1 gram, and 979/1 gram. This number shows that not all samples meet drinking water requirements according to SNI 7388 of 2009 concerning the Maximum Limit of Microbial Contamination<.

Of the 3 types of ice cubes obtained at Pasar Peterongan Semarang that I studied, crystal ice cubes were ranked first with the highest number of coliform bacteria as many as 4 samples with an MPN index value of 979 and 1 sample of 256 and ranked second ice blocks with the highest number of coliform bacteria as many as 2 samples with an MPN index value of 979 and 3 samples of 256 and ranked number 3 with the lowest number of coliform bacteria were plastic packaging ice cubes as many as 3 samples with MPN index value of 256 and 2 samples 22

From the data above, it is surprising because crystal ice cubes that should be consumed actually contain many coliform bacteria. While ice cubes that should be used to preserve fish actually contain fewer coliform bacteria than ice crystals and plastic packaging ice cubes are the only ice cubes that are suitable for consumption because the number of coliform bacteria is the least. Based on SNI –7388 standardization of 2009 and after adjusting to the results of questionnaires on hygiene and sanitation distributed by sellers, it was found that samples that had an MPN value of >3/gram were classified as inappropriate criteria while samples that had an MPN value of >3/gram were classified as suitable criteria for consumption. There are 9 samples in the category of Suitable for consumption, namely samples (1, 4, 9, 3, 7, 10, 12, 13) and 6 samples in the category of not suitable for consumption (5, 8, 2, 6, 14, 15) because those that are suitable for consumption have a score of >65% and MPN index values of >3 / gram and as many as 13-20 total hygiene and saminya scores, and those that are not suitable for consumption have a score of <65% and MPN index values of >3 / gram and as many as 0-12 total hygiene and sanitation scores. The water contained by microorganisms causes contamination and the water content is no longer sterile. (Khotimah & Masduki, 2016).

For all of us, we must be more careful when buying processed ice cube products so that we can get ice cubes that are good and safe for consumption and farthest from bacteria that are bad for health, it is necessary to pay attention to cleanliness and sanitation during the process of processing and making ice cubes. Because water is the largest water component

in ice cube making products, it is necessary to pay attention and good consideration in choosing the appropriate type of water to obtain a decent ice cube.

CONCLUSION

The conclusion in this study is that ice crystals have the highest MPN index value, rank 2 is block ice cubes and rank 3 is plastic packaging ice cubes with the lowest MPN index value and All ice cube samples consisting of plastic packaging ice cubes, crystal ice, ice blocks sold at the Peterongan Market in Semarang city 6 samples (5, 8, 2, 6, 14,15) have not met the requirements set by the National Standardization Agency No.SNI 7388:2009 concerning the limit of microbial and food contamination of <3/gram of MPN index.

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