turnitin_difference coconut drink using ice cubes and not_sals

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DIFFERENCES IN THE NUMBER AND CHARACTERISTICS OF BACTERIA IN ENDO AGAR MEDIA ON COCONUT DRINK USING ICE CUBES AND NOT IN KLAPANUNGGAL, BOGOR

Abstract

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Introduction: Coconut ice is a traditional processed drink that is widely sold on the roadside and is in great demand by consumers because of its affordable price and easy to find raw materials. Because the price is relatively affordable, many sellers do not pay attention to the cleanliness of their merchandise, there by allowing contamination of *Escherichia coli* bacteria which can cause diarrhea. The water used in making ice cubes must meet the drinking water quality requirements as stipulated in the Regulation of the Minister of Health of the Republic of Indonesia Number 492/MENKES/PER/IV/2010 concerning the requirements for drinking water quality based on microbiological parameters, namely 0 CFU/100 ml of water.

Method: The design of this research is descriptive. Samples were planted using the spread plate method after stratified dilution. The object of the research is coconul drink taken from young coconut ice sellers along Jalan Klapanunggal, Bogor Regency with a population of 10 samples and taken using a non-probability method with purposive sampling technique as many as 5 samples with 2 types of groups. Group 1 did not use ice cubes and group2 used ice cubes. Data analysis using SPSS V.25 software with Wilcoxon statistical test.

Results: The results of the Wilcoxon statistical test obtained a p value of 0.0025 (<0.05), which indicates that there is a significant difference in the amount of growth between samples of coconut drinks that do not use ice cubes and those who use ice cubes

Conclusion: Based on ALT calculations on samples that did not use ice cubes, the results obtained were AKM 1 (0.7 \times 106 CFU/ml), AKM 2 (5.6 \times 103 CFU/ml), AKM 3 (2.4 \times 104 CFU/ml), AKM 4 (2.7 \times 105 CFU/ml), AKM 5 (3.2 \times 105 CFU/ml). As for the samples using ice cubes, the results obtained were AKM 1 (1.0 \times 107 CFU/ml), AKM 2 (1.2 \times 103 CFU/ml), AKM 3 (1.8 \times 108 CFU/ml), AKM 4 (6.8 \times 107 CFU/ml), AKM 5 (2.2 \times 106 CFU/ml). Based on the Wilcoxon test, it was found that 0.0025 (<0.05: Ho rejected) concluded that there was a significant difference in the amount of growth between samples of coconut drink that did not use ice cubes and hose who used ice cubes. The bacteria found from the endo agar medium were Escherichia coli, Klebsiella sp., and non-coliform bacteria. *Key words* : *Brain Heart Infusion Agar (BHIA), Coconut Drink, Coliform, Endo Agar, Escherichia coli, Ice Cubes, Klapanunggal, Total Plate Count Method (TPC)*

INTRODUCTION

Coconut ice is a drink that is very popular with the people of Indonesia. Based on the results of a survey on the amount of daily drink consumption in the city of Bogor, the most selected category of adolescent respondents was coconut ice with a percentage (54%) (Rahayu *et al.*, 2019). The serving of coconut drinks is generally added with ice cubes. Ice cubes in the drink consumed must be hygienic, but currently the level of cleanliness of the water used in making ice cubes shows surprising results. There are ice cubes made from potentially polluted Ciliwung River water which is then given a bleaching agent so that it changes the color of the water from cloudy to clearer. This is known because the main ingredient for making ice cubes comes from water, which indicates the presence of pathogenic bacteria in the water (Elfidasari *et al.*, 2011).

Based on data from the monitoring results of microbiological parameters from the Regional Environmental Management Performance Information Document (DIKPLDH) of DKI Jakarta Province in 2019, high results were obtained, namely exceeding the quality standard value of 5000 jml/160 ml for both total coliforms, and faecal coliform parameters. High microbiological parameters can indicate the presence

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of pollution in the Ciliwung River originating from domestic waste (Anonim, 2019).

The water used in making ice cubes must meet the drinking water quality requirements as stipulated in the Regulation of the Minister of Health of the Republic of Indonesia Number 492/MENKES/PER/IV/2010 concerning the requirements for drinking water quality which includes microbiological, physical, chemical and radioactive parameters contained in mandatory and additional parameters. Mandatory parameters are parameters that must be checked periodically, while additional parameters will be checked if geohydrological conditions indicate pollution. Microbiological parameters that must be examined include total coliform with s colony forming units in 100 ml of water sample with a maximum limit of total coliform and *E. coli* of 0 m cfu/100 ml (Kementerian kesehatar republik Indonesia, 2010).

cfu//100 ml (Kementerian kesehatan republik Indonesia, 2010). The presence of pathogenic bacteria is caused by hygiene factors and lack of knowledge. The number of traders 46 people and only 1 person who participated in food and beverage hygiene and sanitation training. The application of this low hygiene can affect the presence of pathogenic bacteria in ice cubes (Rifta et al.,

2016). In this study it was found that the main factor for the presence of pathogenic bacteria was poor hygiene, both the tools used and the environment for the coconut ice making process. (Setiawan *et al.*, 2019)

In a study conducted by Sousa *et al* (2021), it was found that from 5 samples of coconut water sold in the Fi City of Fortaleza, Brazil, 1 positive sample contained *E. coli* and 4 other samples were negative for *E. coli* or in accordance with the requirements according to the law. In another study in North Banjarmasin conducted by Hidriya *et al* (2021) from 9 samples, 6 samples (67%) were positive for *E. coli* and 3 samples (34%) were negative for *E. coli*.

Previous studies only identified pathogenic bacteria in ice cubes. Therefore, researchers will conduct research to identify and calculate the number of pathogenic bacterial colonies using the Total Plate Number (ALT) method on coconut ice that does not use ice cubes and uses ice cubes that are sold along the road in Klapanunggal District, Bogor because until now there has been no Research on the content of pathogenic bacteria in ice cubes used in making coconut ice which is sold along the road in Klapanunggal District, Bogor.

METHOD

This research was conducted on February 14, 2022 to March 4, 2022 at the Bacteriology Laboratory of STIKes Mitra Keluarga. The sampling site for this research was conducted along the road in Klapanunggal District, Bogor. The type of research used is descriptive research. The sampling method used is non Article Error (a probability with purposive sampling technique. In this study, bacteria were identified on endo agar media and Article Error (a counted the number of bacteria using the ALT method on young coconut water samples using ice cubes and error (b) sp. (c)

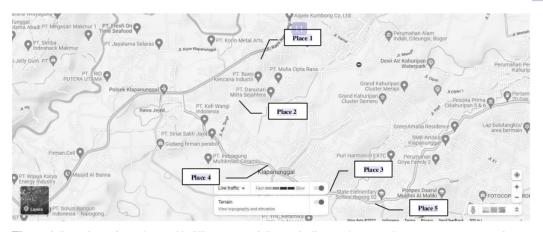


Figure 1. Locations along the road in Klapanunggal, Bogor indicate where to take coconut water samples

Prepare 5 dilution tubes containing 900 l of 0.8% NaCl in each tube. Samples using ice cubes and not using ice cubes were inserted into the first dilution eppendorf tube $(1/10 \text{ or } 10^{-1})$ aseptically. The ratio of the weight of the sample to the volume of 0.8% NaCl is 1:9. After that the sample and 0.8% NaCl were homogenized in the first dilution eppendorf tube. 100 was taken from tube 10^{-1} with a micropipette and then transferred to the second tube (10^{-2}) aseptically. After that, the sample and 0.8% NaCl were homogenized in the second dilution test tube. Transfer is continued to the last dilution tube (10^{-5}) in the same manner. The tips used need to be changed at each level of dilution unless they are used from thesame source (Yusmaniar *et al.*, 2017).or

Take 100 in the last three dilutions and put the sample into sterile BHIA media using the spread plate technique by pouring the sample on the surface of the BHIA media and leveling it using drugalsky. Then cover with plastic wrap. Incubate the media in an incubator upside down at 37°C for 24 hours. After the incubation process is complete, observe and count the growing bacterial colonies using a colony counter (Sholehah, 2019). Count the total plate number on a dish containing 30 – 300 colonies and record the results.

After counting the colonies with the ALT formula, then take separate colonies and different colonies from BHIA media using a ose needle. Next, do the streak plate technique with the quadrant scratch method and cover the petri dish with plastic wrap. After that, incubation at 37°C for 24 hours. Endo Agar culture was taken using an ose needle, then planted into biochemical media. Cover with a tube cover and cover with plastic wrap. After that, incubation at 37°C for 24 hours.

Then do the gram staining by taking the bacterial colonies separated from the Endo Agar media and placing the colonies on a sterile glass object and fixation. After that, drop the crystal violet and wait for 5 minutes. Then wash with running water. Next, drop Lugol's iodine, then wait 45-60 seconds. Then wash with running water. Then add 95% alcohol drop by drop until the purple color does not flow. Then wash with running water. Then drop fuchsin and wait for 1-2 minutes. Then wash with running water. After that, dry the preparation. The final step is to observe under a microscope with a magnification of 100×(Yusmaniar *et al.*, 2017).

RESULTS

Coconut water samples using ice cubes obtained the growth of bacterial colonies that have macroscopic characteristics such as bacterial colonies on BHIA media in circular shape, moderate in size, yellow in color with entire margins, colonies impermeable to light (opaque) and flat on the entire surface (raised). While the samples that did not use ice cubes obtained characteristics such as bacterial colonies in circular shape, moderate in size, milky white in color with entire margins, opaque colonies and flat on the entire surface (raised).

According to research by Rachmawati *et al* (2016), the results of *Streptococcus mutans* colonies on BHIA media showed that the bacterial colonies were round, whitish yellow in color, the colonies were transparent, had a smooth, slippery texture, sometimes the colonies were piled up and the surface was slightly convex.

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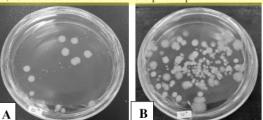


Figure 2. Bacterial colonies on BHIA media (A) bacterial colonies on samples that did not use ice stone (AKM 3 10²) (B) bacterial colonies of bacteria in samples using ice cubes (AKM 2 10²)



Figure 3 shows the number of bacterial colonies obtained from the calculation of the number of colonies on BHIA media from samples that did not use ice cubes and those that used ice cubes, the results were varied, with an average total colony ranging from 5.6×10^3 to $1, 8 \times 10^8$

Based on Figure 3 shows the number of bacterial colonies obtained from the results of calculations using a colony counter, which will then be carried out statistical tests with SPSS, namely the dependent T test. If the results obtained from the normality test are not normally distributed, then use the Wilcoxon test, on the contrary if the results obtained from the normality test are normally distributed, use the Paired T test. The data in this study is less than 50 so using Shapiro-Wilk data. The results of the normality test are listed in table 1.

		Shapiro-Wilk	
	Statistic	Df	Sig.
Coconut <mark>Drinks That Do<mark>n't</mark> Use. Ice S/V @</mark>	.808	10	.018
Coconut Drink Using Ice	.728	10	.002

Table 1 shows the normality test data with Saphiro-Wilk, the p-value for the sample that does not use ice is 0.018, while the p-value of the sample that uses ice is 0.002. it is concluded that the p-value <0.05 so that the data is not normally distributed.

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Table 2. Wilcoxon	test	
	Sig. (2-tailed)	
Coconut Drinks That Don't Use Ice-Coconut Drinks That Use Ice	.005	

Table 2 shows the Wilcoxon test results obtained by sig. of the Wilcoxon test is 0.005. Because the test hypothesis of this study is 1-tailed, so the value of p = 0.005/2 = 0.0025.

Based on the identification of the characteristics of bacterial colonies on BHIA media, several different colonies were obtained in each sample from all the bacterial colonies obtained, different colonies were selected. Then the colonies that have been selected are inoculated on the endo agar medium. The results obtained are listed in figure 4.

Figure 3. Calculation of the number of bacterial colonies using ALT. Method

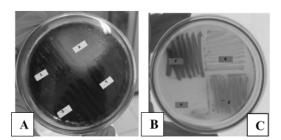


Figure 4. Bacterial colonies on Endo Agar media; A. metallic green color, B. colorless, C. pink color

Sample Code		Color	
1	AKM 1	Metallic green	
2	AKM 3	Pink	
3	AKM 3	Pink	
4	AKM 4	Colorless	

Table 3 . Bacterial colonies on Endo Agar media

Table 3 shows the growth of bacteria on the endo agar medium, in the endo agar medium it can be seen that the bacterial colonies that grew showed metallic green, pink and colorless colors. The results of the study in table 3 sample (1) showed that the colonies that grew were metallic green, samples (2) and (3) showed that the colonies that grew were pink and sample (4) showed that the colonies were colorless.

This study also identified using biochemical tests with SCA media and sugar media, especially lactose and glucose media. After obtaining the difference in 3 color characteristics on the endo agar, then isolation was carried out on SCA media, lactose and glucose media. After incubation for 24 hours at 37°C the results are shown in table 4.

		Biochemical Test Results		
5a	mple Code —	SCA	Glucose	Lactose
1	AKM 1 (1, 3, 4)	-	+	+
2	AKM 3 (5, 8)	+	+	+
3	AKM 3,4,5			
4	(11-20) AKM 1,3,4	+	+	+
	(2, 6, 7, 9, 10)	-	+	-

Table 4. Bacterial colonies on biochemical media

Based on table 4, the results are (1) negative, (2) positive, (3) positive, (4) negative. According to Puspita *et al.*, (2020) the negative SCA test results indicated that there was no color change in the SCA media which initially turned green to blue. On the other hand, in a positive SCA test result, there is a color change, which means that bacteria have the ability to break down/use citrate as the only carbon source. The results of the glucose test on D-glucose media were positive. As for lactose media, the results of (1), (2), (3)were positive, while the results of (4) were negative.

Based on the results of biochemical tests on the SCA test, glucose test and lactose test, it is suspected

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that the type of bacteria E. coli was obtained. Furthermore, the gram staining test used a metallic green colored colony sample from endo agar media. The results of the gram staining show that the gram staining test obtained red coccus-shaped bacteria which are included in the gram negative.

DISCUSSION

Based on macroscopic observations from BHIA media, the characteristics of bacterial colonies that grew were circular in shape, moderate in size, milky white in color with entire margins, colonies were opaque and flat on the entire surface (raised). According to research by Rachmawati *et al* (2016), the results of *Streptococcus mutans* colonies on BHIA media showed that the bacterial colonies were round, whitish yellow in color, the colonies were transparent, had a smooth, slippery texture, sometimes the colonies were piled up and the surface was slightly convex.

The results of the calculation of the number of colonies on BHIA media from samples that did not use ice cubes and those that used ice cubes, obtained varied results, with the average total colonies ranging from 4.6×10^3 to 2.3×10^8 . All samples showed similar results, exceeds the maximum limit of total coliform in water that is allowed by Permenkes No. 492/MenKes/PER/IV/2010 which is 0 CFU/100 ml of water. According to Ariyani & Anwar (2006) in general, snacks and drinks contain relatively high bacteria, with an average of 10^5 CFU/ml. The high content of contamination of the snacks and drinks indicates that there is no preheating and the use of unclean water. From the ALT calculation data, then the data will be analyzed using SPSS which obtained the sig value, of the Wilcoxon test is 0.005. Because the testhypothesis of this research is 1-tailed, so the value of p = 0.005/2 = 0.0025 (<0.05: Ho is rejected) meaning that there is a difference in the amount of growth in the coconut drink samples that do not use ice cubes and those who use ice cubes.

Based on the identification of the characteristics of bacterial colonies on BHIA media, several different colonies were obtained in each sample, from all the bacterial colonies obtained, different colonies were selected. Then the colonies that have been selected are inoculated on the endo agar medium. The results obtained showed the presence of bacterial growth in the endo agar medium, in the endo agar medium it was seen that the bacterial colonies that grew showed metallic green, pink and colorless colors. The results of the study in table 3 (1) showed that the colonies that grew were metallic green which were coliform bacteria that could ferment lactose. Tables 3 (2) and (3) show that the colonies that grow are pink which are still included in the coliform bacteria that can ferment lactose. Table 3 (4) shows that the colorless colonies are included in non-coliform bacteria that cannot ferment lactose.

According to (Kubizniaková *et al.*, 2020) endo agar is usually used to detect coliform bacteria. The selectivity of the endo agar medium was determined by the presence of fuchsin and sulfite as well as the content of carbon substrate in the form of lactose Bacterial colonies that grow can be distinguished based on lactose positive bacteria (pink or red colonies with a greenish metallic luster) and lactose negative bacteria (cream or colorless colonies). According to (Megahati, 2011) endo agar is a selective medium for gram positive and a good growth medium for gram negative bacteria. Colonies that produced a red or shiny metallic color included *E. coli*, whereas *Proteus*, *Salmonella*, *Shigella* produced a faint pink color and *Pseudomonas* did not.

This study also identified using biochemical tests with SCA media and sugar media, especially lactose and glucose media. Based on table 4, the results are (1) negative, (2) positive, (3) positive, (4) negative. According to Puspita *et al.*, (2020) the negative SCA test results indicated that there was no color change in the SCA media which initially turned green to blue. On the other hand, positive SCA test results have a color change, which means that bacteria have the ability to break down/use citrate as the only carbonsource. *E.coli* bacteria are not able to utilize citrate as the only carbon source. From the results of the SCA test, the sugarive results of SCA were in tubes (1) and (4) which were originally on the endo agar medium, which was suspected of being *E.coli* in tubes (1), (2), and (3). including *E.coli* is just a tube (1). In tubes (2) and (3) coliform bacteria are suspected and in tubes (4) non-coliform bacteria are suspected.

Based on table 4, the results of the glucose test on D-glucose media obtained positive results with the discovery of gas in an inverted durham tube. As for lactose media, the results of (1), (2), (3) were positive which was indicated by the presence of gas in the inverted durham tube According to Putri & Kurnia (2018), the presence of carbon gas in the Durham tube chamber is caused by the tightly closed test tube, thelonger the tube is tightly closed it can cause a lot of gas spaces to form and indicate a positive result.

addition, (Kamaliah, 2017) added that the gas formed could also come from lactose fermentation which will form CO_2 gas. According to research (Wandrivel *et al.*, 2012) the presence of gas in the media indicates that there are colonies of coliform basteria in the media. In tubes (2) and (3) showed the presence of turbidity. According to (Kamaliah, 2017) there was turbidity formed because there were bacteria that produce lactic acid from lactose fermentation. Turbidity can be caused by an increase in the amount of lactic acid, so that the lactose will clot. The clumps formed from lactose are what cause turbidity. According to the presence of gas or turbidity.

Table 4 shows that the sample is suspected to be contaminated with *E. coli*, because the lactose and glucose test obtained positive results and the SCA test obtained negative results. Tables 4 (2) and (3) indicate that the suspected sample belongs to *Klebsiella sp*, bacteria, because the SCA test obtained positiveresults and the glucose and lactose tests obtained positive results. As for table 4 (4) shows that the suspected sample SF belongs to non coliform bacteria, because the glucose test obtained positive results and the SCA test obtained negative results. According to Tauran *etal* (2013) the results of the biochemical test of gram negative bacteria using conventional methods on *E. coli* bacteria obtained negative SCA test results and positive lactose glucose tests. *Klebsiella sp*, the results of the SCA test obtained negative and the glucose-lactose test was positive

In the gram staining test, the results obtained are red coccus-shaped bacteria which are classified as gram negative. *E. coli* is a gram negative bacteria. According to Prasetya *et al* (2019), the gram negative bacteria layer has a special characteristic, namely having a thin peptidoglycan layer, while gram positive bacteria have a thick peptidoglycan layer.

CONCLUSION

Based on ALT calculations on samples that did not use ice cubes, the results obtained were AKM 1 (0.7 $\times 10^{6}$ CFU/ml), AKM 2 (5.6 $\times 10^{3}$ CFU/ml), AKM 3 (2.4 $\times 10^{4}$ CFU/ml), AKM 4 (2.7 $\times 10^{5}$ CFU/ml), AKM 5 (3.2 $\times 105$ CFU/ml). As for the samples using ice cubes, the results obtained were AKM 1 (1.0 $\times 10^{7}$ CFU/ml), AKM 2 (14.2 $\times 10^{3}$ CFU/ml), AKM 3 (1.8 $\times 10^{8}$ CFU/ml), AKM 4 (6.8 $\times 10^{7}$ CFU/ml), AKM 5 (2.2 $\times 10^{6}$ CFU/ml). Based on the Wilcoxon test, it was found that 0.0025 (<0.05: Ho rejected) concluded that there was a significant difference in the amount of growth between samples of coconut drink that did not use ice cubes and those who used ice cubes. The bacteria found from the endo agar medium were *Escherichia coli*, *Klebsiella sp.*, and non-coliform bacteria.

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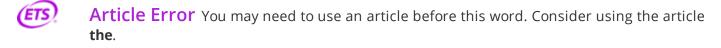
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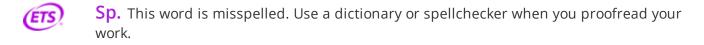
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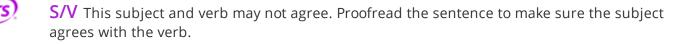
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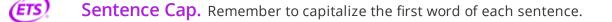


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ETS	Article Error You may need to use an article before this word.
ETS	Run-on This sentence may be a run-on sentence. Proofread it to see if it contains too many independent clauses or contains independent clauses that have been combined without conjunctions or punctuation. Look at the "Writer's Handbook" for advice about correcting run-on sentences.
ETS	Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.
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ETS	Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.
ETS	Sentence Cap. Remember to capitalize the first word of each sentence.
(ETS)	P/V You have used the passive voice in this sentence. Depending upon what you wish to emphasize in the sentence, you may want to revise it using the active voice.



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Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.



Sentence Cap. Remember to capitalize the first word of each sentence.



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Verb This verb may be incorrect. Proofread the sentence to make sure you have used the correct form of the verb.



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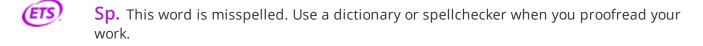


Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.



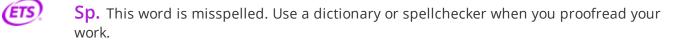
P/V You have used the passive voice in this sentence. Depending upon what you wish to emphasize in the sentence, you may want to revise it using the active voice.

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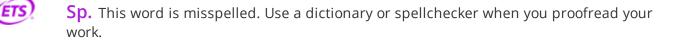


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