

Total Lactic Acid Bacteria and Antibacterial Activity in Yoghurt with Addition of *Ananas comosus* Merr. and *Cinnamomum burmannii*

Lusia Yotista Enggal Parasthi, Diana Nur Afifah*, Choirun Nissa, Binar Panunggal

ABSTRACT

Background: Diarrhea is a gastrointestinal disorder which is the second leading cause of death in children under five years. Food and beverage contamination is the biggest cause of diarrhea in developing countries. Honey cream pineapple (*Ananas comosus* Merr.) and cinnamon (*Cinnamomum burmannii*) have antibacterial properties that can inhibit the growth of *Escherichia coli* and *Salmonella typhi* causing diarrhea.

Objective: This study aims to analyze the total differences of Lactic Acid Bacteria (LAB) and antibacterial activity in yoghurt with the addition of honey cream pineapple and cinnamon extract.

Methods: The study was an experimental study with various treatments in adding honey cream pineapple (0%, 20%, 40%, and 60%) and cinnamon extract (4% and 6%). Total LAB was calculated using the Total Plate Count (TPC) method and antibacterial activity was tested using Kirby Bauer method.

Results: There was no significant difference in yoghurt with the addition of honey cream pineapple and cinnamon extract. Yoghurt with the highest LAB was the one with 40% addition of honey cream pineapple and 6% of cinnamon extract (N40M2) with total LAB $1,43 \times 10^{19}$ CFU/ml. The results of the antibacterial activity showed no significant difference of inhibition zone against *S. typhi*, while there was significant difference of inhibition zone against *E. coli*. The highest activity against *S. typhi* was yoghurt with 60% addition of honey cream pineapple and 4% of cinnamon extract (N60M1) resulting 6,81 mm inhibition zone and the highest activity against *E. coli* was N40M1 resulting 6,77 mm of inhibition zone.

Conclusion: Total LAB yoghurt with the addition of honey cream pineapple and cinnamon extract have met FAO and Indonesian National Standards with LAB minimum 10^7 CFU/ml. Antibacterial activity of all yoghurt treatments categorized as medium inhibition (5-10 mm).

Keywords: antibacterial activity, cinnamon, honey cream pineapple, total Lactic Acid Bacteria (LAB), yoghurt

*Correspondence:

d.nurafifah.dna@fk.undip.ac.id

Diana Nur Afifah

Department of Nutrition Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

INTRODUCTION

Diarrhea is a digestive disease that relates to loose and watery bowel movements more than three times a day.¹ This disease is the second leading cause of child mortality, which kills around 525,000 children under five per year.² Based on The Health Research data in 2018, there were 12.3% episodes of diarrheal disease in children under five.³ Diarrhea leads to dehydration for more than 10% of body mass, the decrease in absorption of nutrients and food intake, and the increase in the catabolism of nutrient reserves and death.⁴ Furthermore, nutritional deficits early in life might cause mental and physical growth disorders.⁵

Escherichia coli and *Salmonella typhi* are the most common bacterias that cause diarrhea on children under five.⁶ Physiologically, *E. coli* is a facultative gram-negative bacterium which accounts for 0.1% of the gastrointestinal microbiota.⁷ Yet this bacterium may be pathogenic if contains a large amount of pathogenic *E. coli* which cause disease especially for hosts with immune deficits. This bacterium causes diarrhea through

various pathways based on the strain by producing enterotoxigenic, intestinal cell invasion and membrane adhesion.⁸

On the other hand, *Salmonella typhi* is a gram-negative bacterium *Salmonella* sp which causes typhoid fever, severe diarrhea, and small intestine perforation.⁹ *E. coli* and *Salmonella* are acid-sensitive enteric pathogens.^{10,11} However, several factors such as polysaccharide capsules in *E. coli* and high gastric pH due to certain food sources can protect them from acidic condition in gastrointestinal tract.^{12,13}

Non-pathogenic microorganisms are required to prevent the development of enteric pathogens that can withstand from gastric acidity.¹⁴ Probiotics are non-pathogenic microorganism that can maintain the balance of the gut microbiota. Lactic acid bacteria (LAB) is a probiotic that produces bacteriocins and lactic acid which can inhibit the growth of pathogenic bacteria.¹⁵ One of the functional beverage products containing probiotics from the LAB group with a high sales rate (75%) is yoghurt.¹⁶ Yoghurt is a beverage which is easily absorbed that may boost digestive health from intestinal



diseases such as constipation¹⁷, diarrhea¹⁸, colitis¹⁹, and colon cancer.²⁰ Streptococcus thermophilus and Lactobacillus bulgaricus are LAB with mutualism symbiosis and are used as a standard for yoghurt starter.^{21,22}

Yoghurt can be made from cow's milk, goat's milk, skimmed milk, soy milk, and a combination of these milk. Goat milk was used in this study because it contained higher protein, essential vitamins, phenolic compounds, less alpha casein, and was easier to digest than cow's milk.²³ However, the presence of capric fatty acids, caprylic fatty acids, and caproic fatty acids that are easily evaporated can enhance the distinctive smell of goat's milk.²⁴ Nevertheless, goat milk is naturally homogenized because it has smaller fat globules that makes it easier to digest than cow's milk.²⁵ The addition of honey cream pineapple and cinnamon on goat's milk are intended to decrease the smell and increase the nutrient content, LAB, and antibacterial activity of the yoghurt product.²⁶

Glucose on the honey cream pineapple is used by LAB as the main carbon source to produce lactic acid.²⁴ Honey cream pineapple extract had an antibacterial effect to prevent bacteria from sticking on the intestinal mucose due to bromelain as the active compound.²⁷ Other than that, cinnamon had an antibacterial effect as it contains essential oil that can inhibit the growth of bacteria.²⁸ Those compounds have antibacterial effect both on gram-negative and positive bacteria such as LAB which is needed to produce yoghurt.^{29,30} So far, the previous studies just focus on testing the total LAB in soyghurt with honey cream pineapple extract added.³¹ So this research focus on testing the total LAB with honey cream pineapple extract and cinnamon added.

METHODS

This research was a complete randomized experimental study (CRD) with the addition of honey cream pineapple at 0%, 20%, 40%, 60% and cinnamon extract at 4% and 6% in the yoghurt formulas. Each test was carried out 3 times in duplicate. This study focused on the LAB total test and antibacterial activity which was carried out at the Central Laboratory of the Diponegoro National Hospital, Semarang. The ingredients used in this study were fresh goat milk as the main ingredient for making yoghurt, honey cream pineapple, cinnamon extract, and other ingredients such as 0.85% NaCl solution, MRSA (de Man Rogosa Sharpe Agar), MHA

(Mueller Hinton Agar), *Escherichia coli* and *Salmonella typhi* bacteria culture. Fresh goat milk was obtained from *Pedurangan Tengah* while the honey cream pineapple was obtained from Banyumanik, Semarang. Meanwhile, cinnamon was obtained from Banyumanik Semarang traditional market. Based on the results of the determination carried out at the Ecology and Biosystematic Laboratory of Diponegoro University, the honey cream pineapple (*Ananas comosus* Merr.) used has thinner and smoother skin also sweeter taste than ordinary pineapples. In this study we used the pineapple pulp and the log of cinnamon which had an aromatic smell and was brown in color which was originally from Indonesia (*Cinnamomum burmanii*).³² The equipments used in this study were blender, temperature thermometer, pan, stove, filter press cloth, incubator, petri dishes, pipettes, paper discs and Vernier-caliper.

Making Yoghurt Formulations with The Addition of Honey Cream Pineapple and Cinnamon Extract

Preliminary research by making yoghurt formulations with the addition of honey cream pineapple and cinnamon extract was carried out at the Diponegoro University Integrated Laboratory. We used a fresh and perfectly ripe honey cream pineapple to make the pineapple extract. Next steps were peeling off, washing, cutting, and steaming the honey cream pineapple for ± 15 minutes at 90°C. After that, blended the honey cream pineapple without water and filtered it from the pulp to get the extraction. We did the same steps to make the cinnamon extract, (without blending the cinnamon). Meanwhile, to make the yoghurt, we started to pasteurize fresh goat milk at 61°C-63°C then added subcultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* at 5% of the milk. Next, we added honey cream pineapple extract at 0%, 20%, 40%, 60% to the yoghurt formulation as mention on table 1) and cinnamon extract at 4% and 6%. We evenly stirred all the samples, then incubated them in an incubator for ± 5 hours at 37°C. This study used 8 yoghurt samples namely (NOM1, NOM2, N20M1, N20M2, N40M1, N40M2, N60M1 and N60M2) with the addition of honey cream pineapple and cinnamon and also 1 sample of plain yoghurt. The negative control used in the total LAB test and antibacterial activity was sterile distilled water. Positive control for the total LAB test was plain yoghurt. Meanwhile, the positive control for the analysis of antibacterial activity was chloramphenicol.

Table 1. Yoghurt treatment sample with the addition of honey cream pineapple and cinnamon extract (per 1 L of goat milk)

Treatment	% pineapple	% cinnamon extract	% milk+starter
NOM0	0	0	100
NOM1	0	4	96
NOM2	0	6	94
N20M1	20	4	76
N20M2	20	6	74
N40M1	40	4	56
N40M2	40	6	54
N60M1	60	4	36
N60M2	60	6	34



Analysis of Total Lactic Acid Bacteria (LAB) dan Antibacteria Activity

The total LAB test was carried out using the Total Plate Count (TPC) method to determine the amount of LAB contained in yoghurt. One ml of sample was diluted in 9 ml of 0.85% NaCl solution. Pipette was used to put the diluted sample into a sterile petri dish and sterile MRSA (de Man Rogosa Sharpe Agar) was added to each sterile petri dish. After the jelly freezed, the petri dishes were incubated in an incubator at 37°C for 24-48 hours. The total colonies of lactic acid bacteria (LAB) were calculated using the SPC (Standard Plate Count) method in units of CFU/ml. Based on FAO and SNI standards, yoghurt must contain a minimum of LAB 10⁷ CFU/ml.³³ Antibacterial activity tests were carried out using the Kirby Bauer method or disc diffusion.³⁴ This method was used to see the response of bacterial growth inhibition based on the diameter of the clear zone sample against *Escherichia coli* and *Salmonella typhi*. The test step started by pouring MHA (Mueller Hinton Agar) media into a sterile petri dish. Shortly after the media solidified, the pathogenic bacteria suspense was evenly rubbed onto the media surface. Then the disc paper soaked in the yoghurt sample was placed (next placed the disc paper that had soaked in the yoghurt sample) on surface of the MHA media with a little pressure so that it adhered perfectly. Incubation of MHA media was at 37°C for 18-24 hours. Then, the diameter of the clear area/inhibition zone was measured around the disc paper using a caliper. The zone of inhibition was categorized based on the clear zone diameter, which were weak if it was ≤5 mm, moderate if between 5-10 mm, strong if between 10-19 mm, and very strong if it was 20 mm or more.

Data Analysis

The data collected in this study were primary data. Total LAB and antibacterial activity against *E. coli* and *S. typhi* bacteria were analyzed using the SPSS 20 for windows computer program with the Kruskal Wallis statistical test

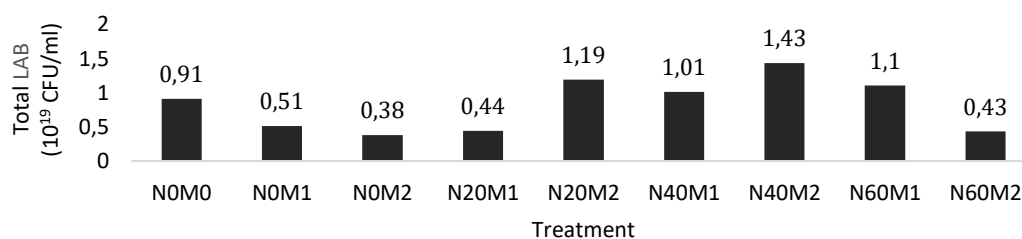
with a 95% level of confidence.

RESULTS AND DISCUSSION

Total Lactic Acid Bacteria (LAB)

The addition of honey cream pineapple and cinnamon to yoghurt did not show significant differences in the total LAB with p value of 0.947 (p>0.05). Nevertheless, the total value of LAB in all samples still meet the requirements on the number of probiotic bacteria in fermentation products according to the *Indonesian National Standard* and FAO which is at least 10⁷ CFU/ml.³³ It is due to mutualism symbiosis from both LABs that is used. *L. bulgaricus* produced amino acids, valine, glycine, and histidine which stimulate the growth of *S. thermophilus*. Meanwhile, *S. thermophilus* produces formic acid, pyruvic acid, CO₂ and folic acid which supports the growth of *L. bulgaricus*. *S. thermophilus* is a homofermentative LAB which produces lactic acid as the main product. *S. thermophilus* is the only species of bacteria in the genus *Streptococci* that produces the enzyme lactase. The beneficial effect of *S. thermophilus* is by producing a lactase enzyme to digest lactose in milk. This symbiosis has a positive impact on the yoghurt product so that LAB could reproduce well.²²

Based on Figure 1, there was a decrease in the total LAB for the N0M1 and N0M2 treatments. The decrease in total LAB could be caused by essential oils in cinnamon which acts as antibacterial agents as they contains active compounds including cinnamaldehyde, eugenol, saponins, and tannins.²⁸ The antibacterial properties of active compounds in essential oils works to break cell walls, damaged plasma membranes and membrane proteins. It causes cell leakage, and binds enzymes in the cell wall and oxygen which is needed by bacteria so that bacterial growth could be inhibited or died.³⁷ Cinnamaldehyde at 0.2% reduces >3-log CFU/ml of the total LAB.³⁸



Information:

NOM0 = 100% yoghurt with a ratio of honey cream pineapple and cinnamon extract 0%:0%

NOM1 = 96% yoghurt with a ratio of honey cream pineapple and cinnamon extract 0%:4%

NOM2 = 94% yoghurt with a ratio of honey cream pineapple and cinnamon extract 0%:6%

N20M1 = 76% yoghurt with a ratio of honey cream pineapple and cinnamon extract 20%:4%

N20M2 = 74% yoghurt with a ratio of honey cream pineapple and cinnamon extract 20%:6%

N40M1 = 56% yoghurt with a ratio of honey cream pineapple and cinnamon extract 40%:4%

N40M2 = 54% yoghurt with a ratio of honey cream pineapple and cinnamon extract 40%:6%

N60M1 = 36% yoghurt with a ratio of honey cream pineapple and cinnamon extract 60%:4%

N60M2 = 34% yoghurt with a ratio of honey cream pineapple and cinnamon extract 60%:6%

Graph 1. The results of total LAB analysis for yoghurt with the addition of honey cream pineapple and cinnamon



Meanwhile, there was an increase in the total LAB for the N20M1, N20M2, and N40M2 treatments. The increase is due to the addition of honey cream pineapple that contains simple sugar such as glucose 2.32%, fructose 1.42%, and sucrose 7.89%. Sucrose is broken down by LAB into fructose and glucose which is used to obtain energy so that they supported the growth of LAB.²⁴ This is in accordance with the previous research on yoghurt with different sucrose variations which showed that the higher sucrose concentration, the more total LAB is produced.³⁹

LAB reduction occurred in the N60M1 and N60M2 treatments. This might be caused by the less percentage of milk contained in yoghurt. Lactose content in milk may have a positive effect on the growth of LAB. The addition of lactose in the fermentation process increases the growth rate of LAB. Lactose is the main carbon that could be broken down by LAB into glucose and galactose which are converted into lactic acid.⁴⁰ Goat milk itself has 4.7% lower lactose than cow milk, so the main carbon source that could be used by bacteria is also less.⁴¹

Table 2. Results of Antibacterial Activity Analysis

Treatment	Bacterial Inhibition Zone Diameter (mm)	
	<i>S. typhi</i>	<i>E. coli</i>
NOM0	5.33±0.58	5.17±0.29
NOM1	6.00±0.50	5.33±0.58
NOM2	6.17±0.58	6.10±0.17
N20M1	5.67±0.58	6.27±0.25
N20M2	6.10±0.53	6.43±0.12
N40M1	6.03±0.87	6.77±0.25
N40M2	6.50±0.87	6.63±0.29
N60M1	6.87±0.60	6.33±0.29
N60M2	6.17±0.76	5.50±0.87
<i>P</i>	0.359 ¹	0.019 ²

*Testing with the Kruskal Wallis test; ¹significance >0.05, ²significance <0.05

Information:

NOM0 = 100% yoghurt with a ratio of honey cream pineapple and cinnamon extract 0%:0%

NOM1 = 96% yoghurt with a ratio of honey cream pineapple and cinnamon extract 0%:4%

NOM2 = 94% yoghurt with a ratio of honey cream pineapple and cinnamon extract 0%:6%

N20M1 = 76% yoghurt with a ratio of honey cream pineapple and cinnamon extract 20%:4%

N20M2 = 74% yoghurt with a ratio of honey cream pineapple and cinnamon extract 20%:6%

N40M1 = 56% yoghurt with a ratio of honey cream pineapple and cinnamon extract 40%:4%

N40M2 = 54% yoghurt with a ratio of honey cream pineapple and cinnamon extract 40%:6%

N60M1 = 36% yoghurt with a ratio of honey cream pineapple and cinnamon extract 60%:4%

N60M2 = 34% yoghurt with a ratio of honey cream pineapple and cinnamon extract 60%:6%

The total percentage of cinnamon and honey cream pineapple in the N60M1 and N60M2 treatments was greater than the percentage of milk. Honey cream pineapple and cinnamon have antibacterial agents against gram-negative and gram-positive bacteria, so they could inhibit the growth of LAB.^{29,30} The studies of antibacterial on cinnamon extract against several bacteria showed that gram-positive bacteria were more sensitive than gram-negative bacteria.⁴² Meanwhile, the antibacterial on honey cream pineapple could also inhibit gram-positive bacteria, such as flavonoids and polyphenols because they had the same polar properties as peptidoglycan which made up the cell walls of gram-positive bacteria.²⁹

The higher percentage of honey cream pineapple than milk causes the sugar concentration in yoghurt to be excessive. The sucrose contained in honey cream pineapple has high solubility, the ability to reduce the relative humidity balance and bind water, so that it could be used in food preservation. Concentrated medium concentrations with high levels of sucrose might cause osmosis which water in the bacterial cell comes out through the membrane and flows into the sugar solution. This event causes the bacteria to experience plasmolysis.⁴³ As a result, the water content contained in

yoghurt decreases. The low water content in yoghurt could also inhibit the growth of LAB.³⁹

Water activity (a_w) is closely related to the availability of water and not to foodstuffs. Water content is important for the growth of LAB in the fermentation process. Water is used by LAB as a medium for transporting nutrients into cells, removing metabolites from outside cells, where enzymatic reactions took place, as a medium for cellular complement synthesis, and plays a role in helping biochemical processes such as hydrolyzing polymers into monomers. Based on previous research on yoghurt with the addition of honey cream pineapple and cinnamon, the average water content contained was around 80%. Meanwhile, the water content in yoghurt according to Indonesian National Standard 2981:2009 was 83-84%.

LAB growth phase consisted of a lag phase, an exponential phase, a stationary phase, and a death phase. At 4 to 10 hours there was a growth phase where bacterial growth took place very rapidly. The next phase occurred at 11 to 14 hours where there was no increase of the number of bacteria because the number of cells grew was the same as the dead cells. The death phase of LAB occurred from the 15th hour where the number of bacteria began to decline because the nutrients in the



media and energy reserved in the cells began to run low. Research conducted on LAB stored at room temperature showed a decrease in the number of LAB after incubation for 6 hours. Researchers had controlled to inhibit LAB mortality gradually by storing yoghurt in the refrigerator temperature immediately after the incubation process at 5 hours when LAB was still in growth phase. Probiotic products stored at 4° C had shown to maintain stable bacterial counts for 28 days.

However, the limitation of this study was that the refrigerator did not have a temperature thermometer. The laboratory performed temperature control (40C) manually using a thermometer every 3 months, while the research was carried out in the third month after checking the temperature. This allowed the active phase of LAB growth due to the increase in temperature in the refrigerator.

Table 3. Results of the Advanced Antibacterial Activity Test *E. coli*

Perlakuan	NOM0	NOM1	NOM2	N20M1	N20M2	N40M1	N40M2	N60M1	N60M2
NOM0	-	0.796	0.043*	0.046*	0.034*	0.046*	0.043*	0.043*	0.796
NOM1	0.796	-	0.099	0.072	0.034*	0.046*	0.043*	0.068	0.796
NOM2	0.043*	0.099	-	0.346	0.034*	0.046*	0.068	0.239	0.500
N20M1	0.046*	0.072	0.346	-	0.121	0.077	0.178	0.637	0.369
N20M2	0.034*	0.034*	0.034*	0.121	-	0.121	0.480	0.317	0.114
N40M1	0.046*	0.046*	0.046*	0.077	0.121	-	0.487	0.105	0.072
N40M2	0.043*	0.043*	0.068	0.178	0.480	0.487	-	0.261	0.116
N60M1	0.043*	0.068	0.239	0.637	0.317	0.105	0.261	-	0.239
N60M2	0.796	0.796	0.500	0.369	0.114	0.072	0.116	0.239	-

Testing with *Mann Whitney*; *real difference (<0.05)

Information:

- NOM0 = 100% yoghurt with a ratio of honey cream pineapple and cinnamon extract 0%:0%
- NOM1 = 96% yoghurt with a ratio of honey cream pineapple and cinnamon extract 0%:4%
- NOM2 = 94% yoghurt with a ratio of honey cream pineapple and cinnamon extract 0%:6%
- N20M1 = 76% yoghurt with a ratio of honey cream pineapple and cinnamon extract 20%:4%
- N20M2 = 74% yoghurt with a ratio of honey cream pineapple and cinnamon extract 20%:6%
- N40M1 = 56% yoghurt with a ratio of honey cream pineapple and cinnamon extract 40%:4%
- N40M2 = 54% yoghurt with a ratio of honey cream pineapple and cinnamon extract 40%:6%
- N60M1 = 36% yoghurt with a ratio of honey cream pineapple and cinnamon extract 60%:4%
- N60M2 = 34% yoghurt with a ratio of honey cream pineapple and cinnamon extract 60%:6%

Antibacteria Activity

The addition of honey cream pineapple and cinnamon to yoghurt did not show significant differences in antibacterial activity against *S. typhi* ($p > 0.05$). Otherwise, there was a significant difference in antibacterial activity against *E. coli* ($p < 0.05$). However, the inhibition zone formed in *S. typhi* and *E. coli* was still in the moderate category (diameter 5-10 mm). The larger the inhibition zone, the greater the yoghurt ability to inhibit the growth of pathogenic bacteria.³⁵ The low inhibition zone formed could be due to the more complex structure of the gram-negative bacterial cell wall, which has an outer layer in the form of lipoproteins, the thick middle layer called peptidoglycan, and a layer in lipopolysaccharides. This complex cell wall could inhibit antibacterial compounds from LAB, honey cream pineapple, and cinnamon to penetrate bacterial cell membranes.⁴⁶

The highest inhibition zone against *S. typhi* bacteria was in the N60M1 treatment of 6.87 mm and against *E. coli* in the N40M1 treatment of 6.77 mm. This was due to the antibacterial effect on the added honey cream pineapple. Pineapple contains a protease called bromelain as well as several compounds such as polyphenols, saponins, and flavonoids which are known as antimicrobial agents. The active compounds that work against gram-negative bacteria are bromelain and saponins. Bromelain is a proteolytic enzyme that plays a role in inducing the proteins breakdown of the bacterial membrane that weaken the cell walls and causing

leakage to cell death.²⁹ In addition, saponins play role to change the structure and function of the membrane, disrupt tension of the cell walls surface, and allow antibacterial substances easily entering cell.⁴⁷

Further test results in Table 2, showed that there was a difference in the treatment of NOM0 with NOM2, N20M1, N20M2, N40M1, N40M2, N60M1, and N60M2 on the antibacterial activity against *E. coli*. These differences are due to the addition of honey cream pineapple and cinnamon as antibacterial that inhibited the growth of *E. coli*. However, there was no difference between NOM0 and NOM1 also between M1 and M2 treatments in all groups. The extraction process of cinnamon using water solvent at temperature of 90°C decrease the antibacterial agent compound. Cinnamon contains several main flavonoids known as antibacterial agent, such as cinnamaldehyde, cinnamylacetate, eucalyptol, and eugenol as the main phenol.⁴⁸ Previous studies showed the highest flavonoid levels were found in the maceration method, namely extraction using organic solvents at cold temperature.⁴⁹ Compounds of the flavonoid group were not heat resistant so boiling too long could damage the flavonoid compounds in cinnamon.⁵⁰

There is an increase on antibacterial activity against *E. coli* with the addition of honey cream pineapple and cinnamon. In addition, LAB in yoghurt produces other compounds besides lactic acid, namely bacteriocin and hydrogen peroxide which are also known as antibacterial agent.⁵¹ The main target of



bacteriocins is to damage the permeability of the bacterial cytoplasmic membrane cell by forming pores on the membrane cell and eliminating the proton motive force. LAB could also inhibit the growth of pathogenic bacteria. Lactic acid produced by LAB has a bactericidal effect on pathogenic bacteria so the pH decreased to 3-4.5.⁵² Meanwhile *E. coli* survived in acidic conditions up to pH 4.4 and *S. typhi* at pH 4-9.¹¹ pH checks were carried out by researchers on the incubated yoghurt which showed pH 5. The limitation of this study was the use of pH measuring device using litmus paper, while pH meter could read the measurement results more accurately than litmus paper.⁵³

Based on the results of the total LAB test, all yoghurt treatments with the addition of honey cream pineapple and cinnamon extract were suitable to eat because they fit the Indonesian National Standard and FAO yoghurt standards. All treatments also had antibacterial potential as diarrhea prevention based on antibacterial activity tests. However, the inhibition against *S. typhi* and *E. coli* was still in the moderate category.

CONCLUSION

Total LAB in yoghurt with the addition of honey cream pineapple and cinnamon extract showed no significant difference ($p < 0.05$). The highest total LAB in N40M2 treatment was 1.42×10^{19} CFU/ml. Total LAB for all treatments was 1019 CFU/ml. The results of the yoghurt antibacterial activity test did not show any difference against *S. typhi* ($p > 0.05$) but showed a difference against *E. coli* ($p < 0.05$). The antibacterial activity of all yoghurt treatments was categorized as a moderate inhibition (5-10 mm). The highest antibacterial activity against *S. typhi* was in the N60M1 treatment and against *E. coli* in the N40M1 treatment.

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