

## Effectiveness of Pineapple Cayenne Waste Extract to Reduce the Number of *Escherichia coli* in the Clean Water Disinfection Process

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### Abstract

Pineapple cayenne waste contains bromelin, a protease enzyme, so it is expected to hydrolyze the protein of *Escherichia coli* bacteria. The purpose of this study was to analyze the effectiveness of pineapple cayenne waste extract to kill *Escherichia coli* bacteria in water disinfection process. The dosage variations in this study were: 1) treatment I: 1000 ml of water intervened with 100 ml of extract, 2) treatment II: 1000 ml of water intervened with 250 ml of extract, 3) treatment III: 1000 ml of water intervened with 500 ml of extract, 4) control: water sample without intervention extract. Further testing of the effectiveness of these four samples to kill *Escherichia coli*. The amount of *Escherichia coli* in treatment I was 104.6 per 100 ml, at treatment II was 47 per 100 ml, in treatment III was 17 per 100 ml and at control was > 2400 per 100 ml. The bacteriological quality of clean water at Intervention II and Intervention III meets the requirements stipulated by Regulation of the Minister of Health of the Republic of Indonesia No. 416 / Menkes / Per / IX / 1990 on Standards of Quality of Clean Water and Drinking Water which states that the content of *Escherichia coli* in water Non piping maximum is 50 per 100 ml. The results of the Kruskal Wallis test and t test showed that at certain concentrations of pineapple cayenne waste effective to kill *Escherichia coli*.

**Keywords:** Pineapple cayenne waste extract, Bromelin, *Escherichia coli*

### I. INTRODUCTION

Pineapple is one type of fruits for export commodities. Pineapple is exported in the form of fresh fruit or in the form of processed foods. This large production has a negative impact, namely the production of solid waste from the remains of pineapple fruit, which is generally not utilized (Kementerian Pertanian RI, 2015). This waste is usually only dumped in the yard of the house causing pollution, such as air pollution (unpleasant odor) and groundwater contamination due to leachate water formed (Salim & Sriharti, 2008).

Pineapple waste that has not been properly managed as described above can actually be recycled into useful materials. Pineapple cayenne waste contains bromelin, a protease enzyme, so it is expected to hydrolyze the protein of *Escherichia coli* bacteria. Thus, the waste can be used as a disinfectant to kill bacteria. During this time the material that is widely used as a disinfectant to kill bacteria in clean water is chlorine.

The purpose of this study was to analyze the effectiveness of pineapple cayenne waste extract to kill *Escherichia coli* bacteria in water disinfection process.

### II. METHODS

Design of this experimental research was One Group Pretest-Posttest. Samples were water taken from clean water storage container in Sumberdodol Village, Penekan Sub-district, Magetan District, East Java Province, Indonesia. The volume of each water take was 5 liters, with 5 repetitions at different times.

The first step in making the extract is to chop the remains of pineapple fruit using a knife, then destroyed using a blender. Next the crushed material is filtered and accommodated in a sterile bottle. The final step is to extract the material by following the Standard of Work Procedure of the Maseration Extraction Method (Atmojo, 2011).

*Escherichia coli* is a bacteria belonging to the coliform group that can dispense lactose, which is characterized by the formation of gas at a temperature of 35<sup>0</sup> Celsius within 24-48 hours. *Escherichia coli* examination was done by Multiple Tube Fermentation method.

The dosage variations in this study were:

1. Treatment I: 1000 ml of water intervened with 100 ml of extract
2. Treatment II: 1000 ml of water intervened with 250 ml of extract
3. Treatment III: 1000 ml of water intervened with 500 ml of extract
4. Control: water sample without intervention extract.

The result of measurement is numerical data so that it refers to Nugroho (2014), the data is presented as the mean value. Kruskal Wallis test and T test was then performed to analyze the effectiveness differences of each sample to kill *Escherichia coli*, because the data distribution was not normal.

### III. RESULTS AND DISCUSSION

The following is the result of laboratory examination from cayenne pineapple waste extract:

1. pH : 3.86
2. Temperature : 28.5 °C
3. Acid : 0.585%
4. Total Acid : 1.71%
5. Protein : 0.42%
6. Bromelin : 0.050 - 0.075 unit / ml.

Table 4. Data from Laboratory Results on Estimation of *Escherichia coli* in Clean Water

Sample sequence number	Variation of extract (ml) + / Mixed with 1000 ml of clean water			
	Control	100 (Treatment I)	250 (Treatment II)	500 (Treatment III)
1.	> 2400	94	46	17
2.	> 2400	130	63	21
3.	> 2400	110	49	23
4.	> 2400	79	43	13
5.	> 2400	110	34	11
Mean	> 2400	104,6	47	17

*Escherichia coli* can grow well at pH 6.8 to 7.5. Laboratory results show that the pH of pineapple cayenne extract was 3.63 to 4, with mean = 3.86. Thus this pH does not support the growth of *Escherichia coli*. The pH shift from 7.0 to lower will inhibit the growth of *Escherichia coli* (Budiyanto, 2010). In acidic conditions, *Escherichia coli* dies because of the difference in osmotic pressure between spaces within the bacterial cell and the environment outside the cell, so that the cell contents will be attracted out (plasmolysis). Enzymes are only able to work on a certain pH, otherwise at a certain pH the enzyme is completely inactive or even damaged. Enzymes are protein molecules whose stability is influenced by the acidity of the environment. At the extreme pH, protein molecules of the enzyme will be damaged (Susanto, 1999).

The result of the lowest temperature measurement was 27 °C, the highest was 30 °C and the mean was 28.5 °C. *Escherichia coli* belongs to the class of mesophyll bacteria that can live well at temperatures of 5 to 60 °C, with the optimum temperature: 25-40 °C. The optimum temperature for *Escherichia coli* is 37 °C. The rate of growth at sudden high temperatures can be caused by protein denaturation and possibly denaturation of cell structures such as membranes. At the maximum temperature to grow, then the damaging reaction becomes very large. The temperature is usually just how many degrees higher than the temperature for maximum growing speed (optimum temperature). *Escherichia coli* bacteria are more resistant to low temperatures than high temperatures. Temperature plays an important role in regulating the course of metabolic reactions for *Escherichia coli*. High temperatures that exceed tolerance will lead to the denaturation of proteins and other essential cell components so that the cell will die. Low temperatures below tolerance make the cytoplasm more solid so that transport of nutrients will be inhibited and the cell life process will stop (Budiyanto, 2010).

The measurement results show that the lowest humidity was 70%, the highest was 78% and the mean was 74%. *Escherichia coli* requires high relative humidity (RH), which is about 85%. Reduction of water levels of protoplasm will cause cessation of metabolism. *Escherichia coli* will decrease the durability and elasticity of the cell wall as RH environment <84% (Wikipedia, 2012). Free water activity in solution is the ratio between water vapor pressure of the solution and the vapor pressure of pure water or 1/100 of relative humidity. Water activity for *Escherichia coli* in general is 0.90-0.999. As with freezing, the drying process of protoplasm can cause the cessation of *Escherichia coli* metabolism. Drying slowly can cause cell damage due to the influence of osmotic pressure and other effects with increased levels of solutes (Budiyanto, 2010).

The total acid from laboratory test result was 1.71%. Acid is an acidic chemical compound that can inhibit bacterial growth, as described in the preceding section. Other examination results that need to know is the organic acid content. In general, organic acids are weak acids. Acids combined with heat will cause the heat to be more effective against *Escherichia coli*.

The results showed that the protein content was 0.42%. Protein is essential for the survival of *Escherichia coli*. The major atoms of carbon (C), hydrogen (H), oxygen (O) and nitrogen (N) proteins. Proteins are composed of amino acids that make up the polymer so that it is a long compound. Proteins can be damaged by heat, acid and base reaction, proteolytic enzyme activity and other causes (Hingriani, 2010).

Laboratory results showed that the bromelin content was 0.050-0.075 units / ml. Bromelin is a protein-breaking enzyme (protease) that can kill bacteria through the decomposition of proteins, in which protein is one of the important compounds for bacterial life. Protease is an enzyme that serves to hydrolyze the peptide bonds of proteins that break into amino acids (Agustina, 2009). Proteolytic activity results in slight agglomeration. Proteolytics are protein-soluble enzymes produced in cells and then released out (Durham, 1987). With the presence of bromelin, low pH, low humidity, and acid content in pineapple waste extracts, the process of killing *Escherichia coli* may occur more quickly (Susanto, 1999).

Results of laboratory tests on the estimated number of *Escherichia coli* is as follows:

1. Control (before intervention): the mean of the number of *Escherichia coli* was > 2400 per 100 ml.
2. Treatment I (with a dose of 100 ml extract): the mean of the number of *Escherichia coli* was 104.6 per 100 ml. The percentage decrease in the number of *Escherichia coli* compared to the control was: 95.64%.
3. Treatment II (with a dose of 250 ml extract): the mean of the number of *Escherichia coli* was 47 per 100 ml. The percentage decrease in the number of *Escherichia coli* compared to the controls was: 98.04%.
4. Treatment III (with a dose of 500 ml extract): the mean of the number of *Escherichia coli* was 17 per 100 ml. The percentage decrease in the number of *Escherichia coli* compared to the controls was: 99.29%.

Thus, the greater the dose of cayenne pineapple waste, the greater the percentage of *Escherichia coli* killed in clean water. The following is the breakdown of the percentage difference between treatments:

1. Percentage difference between treatment I and treatment II was  $98.04\% - 95.64\% = 2.4\%$
2. Percentage difference between treatment I and treatment III was  $99.29\% - 95.64\% = 3.65\%$
3. Percentage difference between treatment II and treatment III was  $99.29\% - 98.04\% = 1.25\%$

The bacteriological quality of clean water at treatment II and treatment III meets the requirements stipulated by Regulation of the Minister of Health of the Republic of Indonesia No. 416 / Menkes / Per / IX / 1990 on Standards of Quality of Clean Water and Drinking Water which states that the content of *Escherichia coli* in water Non piping maximum is 50 per 100 ml.

Kruskal Wallis test yielded p-value = 0.000 (<0.05) so it was concluded that there were differences in the amount of *Escherichia coli* based on different dosage of cayenne pineapple waste extract in clean water. The results of the t test show that:

1. There is a difference in the effectiveness between treatment I and treatment II to kill *Escherichia coli* in clean water
2. There is a difference in the effectiveness between treatment I and treatment III to kill *Escherichia coli* in clean water
3. There is a difference in the effectiveness between treatment II and treatment III to kill *Escherichia coli* in clean water

#### IV. CONCLUSION

Based on the results of this study it can be concluded that pineapple cayenne waste extract is effective to kill *Escherichia coli* in clean water.

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