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Fruit Enzyme Activity of Molasses Combination Against Control of S. Aureus and E. Coli Bacteria

Ari Adrianto[1*], Faishal Khalish[1], Dwita Cahaya Pratiwi[1]

[1]Islamic University of Indonesia, Yogyakarta, 55584, INDONESIA

*Corresponding Author

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Abstract: This study aims to determine the effect of the pineapple and orange peel enzymes combined with molasses on the effectiveness of the inhibition zones of gram positive (*S. Aureus*) and gram negative (*E. Coli*) bacteria. The study was conducted using the disc method, where the bacterial samples used were at a concentration of 200 millimicrons. And the addition of enzyme solution with a concentration of 20 millimicrons. In observations using Scan 500, the results obtained in samples of pineapple peel with the highest inhibition zones for *E. Coli* and *S. Aureus* bacteria, respectively, of 11.7 mm and 12.8 mm. Meanwhile, in the test of orange peel samples, the highest inhibition zones for *E. Coli* and *S. Aureus* were 16.6 mm and 13.2 mm, respectively. In addition, the highest pH testing of each sample of orange peel and pineapple was obtained on the third fermentation with values of 4.63 and 4.39.

Keywords: Orange peel, pineapple peel, E. Coli, S. Aureus, bacteria

1. Introduction

Health is an important aspect in the quality of life of every individual. Some cases attack the body's health due to viruses, bacteria, and fungi. Health services need to be improved by making efforts to reduce the prevalence of diseases caused by several factors and one of them is bacteria. Bacteria are organisms that do not have a nuclear membrane and are part of prokaryotes. Bacteria are generally divided into two classes, namely Gram positive and Gram negative. Gram-positive bacteria have a thick cell wall structure ranging from 20-80 nm [1]. Gram-negative bacteria have a thin cell wall structure ranging from <10 nm [1]. In some health cases, the types of bacteria that are often encountered are *Escherichia Coli* (E. Coli) and *Staphylococcus Aureus* (S. Aureus). E. Coli is a gram-negative rod-shaped and classified as a family Enterobacteriaceae. These bacteria are capable of replicating growth within 20 minutes. E. Coli are generally found in bottles, pacifiers, thermometers, eating utensils, intestines, and feces. Staphylococcus aureus is a gram-positive, cocci-shaped bacterium. S. Aureus is classified into the family *Staphylococcaceae*. S. Aureus is commonly found in soil, water, air, nose and human skin. These bacteria are able to grow in media for 20 minutes and produce the carotenoid pigment *staphyloxanthin* [2]. The effects of exposure to E. Coli and S. Aureus bacteria include diarrhea, vomiting, rash, redness, and pain. So that several researchers conducted anti-bacterial research for these two types of bacteria.

One of the bacterial killing treatment methods currently being developed is an enzyme solution derived from organic matter. Where in this study used fruit peel and molasses for the main ingredients. A literature review found that half of the pineapple protein contains the bromelain protease enzyme [3]. In addition, cellulase activity was also investigated from the combination of watermelon rind with citrus fruit of 0.036 U/mL and banana peel mixed with 0.035 U/mL of orange peel [4]. Molasses is used as a substrate to produce alcohol [5]. Molasses is a source of carbohydrates produced from the cane sugar process as a by-product and is widely used in animal food [6]. And molasses contains polymeric sugars which can react during enzymatic hydrolysis. Sugarcane drops usually contain 17-25% water, sugar content (sucrose, glucose, fructose) by 45-50%, and polysaccharides (dextrin, pentosan, polyuronic acid) by 2-5% [7].

*Corresponding author: aadrianto57@gmail.com

In the study of Yagnik et al., 2018 conducted antimicrobial testing of apple cider vinegar against *Escherichia Coli*, *Staphylococcus Aureus*, and *Candida Albicans* to downregulate cytokines [8]. In this study it was found that the percentage of cytokine reduction tends to be high depending on the dose with the results of E. Coli (TNFα, 99.2%; IL-6, 98%), S. Aureus (TNFα, 90%; IL-6, 83%) and *C. albicans* (TNFα, 83.3%; IL-6, 90.1%). This study also states that in apple cider vinegar there are several enzymes that affect bacteria and affect cell integrity. Shin et al., 2020 found that enzymes can destroy bacterial cell walls and kill pathogens.

Protein dialysis is a separation technique that is widely used to remove contaminants by selective and passive diffusion with semipermeable membranes. Many separation studies use this method. This system consists of a tube holding a semipermeable membrane and a PBS solution which is used as a special washing solution for DNA as it helps maintain the pH of the sample [9].

Based on the literature study above, researchers will conduct a study on the effect of fruit peel enzymes on the antibacterial activity of S. Aureus and E. Coli. Several references reported that the content of enzymes such as Amylase, Lipase has effectiveness in intensive pathogen elimination. Variation of fermentation and combination of molasses will be used to see the comparison of each sample. Hopefully, this finding can open the door for the development of enzyme solutions in the future.

2. Materials and Methods

2.1 Materials

The apparatus used in this research include; beaker, buchner funnel, 250 mL funnel, Erlenmeyer 150 mL, vortex, spatula, blender, magnetic stirrer, fermentation vessel, vacuum, incubator, bunsen, wrap, aluminum foil, pH indicator, measuring cup, thermometer, freezer, shaker, UV-Vis spectrophotometer 715 nm and fluorescence reader (Scan 500).

The materials used include; Fruit waste in the form of pineapple peel, oranges, molasses, phosphate buffer (MaxLab), 0.01 M borate buffer, 2% casein, 1% starch, N-Hexane, Oleic acid standard, DNS reagent, Hydrochloric acid (HCl) 6 N and 0.05 N, ammonium sulfate, phosphate saline buffer, and water.

2.2 Fruit Peel Waste Fermentation and Enzyme Production

The orange and pineapple peels were reduced and weighed as much as 160 grams and added 60 ml of molasses and 600 ml of distilled water. Then fermentation was carried out with variations of 1 day, 2 days, and 3 days under anaerobic conditions. Fermentation was carried out in an orbital shaker at a speed of 300 rpm. Fig. 1 shows the result of fermentation of orange peel fermentation variations 24, 48, and 72 hours. It can be seen that there is no change in aqueous terms, but the color for the old fermentation is getting thicker.

Fig. 1 - Enzyme liquid before filtration

2.3 Dialysis Protein - Physical, Chemical and Organoleptic Tests

The solution was separated from aqueous and tar, then added 4 grams of ammonium sulfate to obtain a saturated fraction of the solution. Samples were incubated and covered with aluminum foil for 24 hours.

Organoleptic is a test through a sensory process by seeing using the senses of sight, smell, and touch [10]. In the liquid form test, before filtering the liquid is shaped like a slurry because there is orange peel sediment. After being filtered using 42 Whatman paper, it forms a liquid like water. This test is done by observing the shape, color, smell of the enzyme solution. While, the color of the liquid adjusts to the enzyme shell after the fermentation treatment. And the enzyme solution smells like organic and honey.

Physical tests carried out include testing for viscosity and density. Viscosity testing uses an Ostwald Viscometer tool, by measuring the time for the liquid to pass through two points on the liquid capillary tube. For density testing, a 50 ml pycnometer was used to measure the weight of the sample. And use the calculation formula below:

$$\eta = \eta_o \frac{t \,.\, \rho}{to \,.\, \rho o} \tag{1}$$

$$Sample\ density = \frac{(pycnometer + sample) - (pycnometer\ without\ sample)}{pycnometer\ volume} \tag{2}$$

Descriptions:

Ƞ = viscosity of sample liquid
η_0 = viscosity comparison fluid
t = sample liquid flow time
t_0 = comparison fluid flow time

Chemical tests carried out include pH. Testing the pH using the EcoSense PH100A Meter where by dipping the tip of the stem into the enzyme liquid.

2.4 Bacteria Test

Bacterial effectiveness testing was carried out using the pour plate method. How to do a bacterial culture by diluting the isolate. The dilution was carried out several times, then 1 ml of bacteria was poured into a sterile petri dish and poured with warm sterile media (40-50°C) then tightly closed to be incubated for 1 day at 37°C. The pouring process is carried out aseptically, so the tools used must be in sterile conditions. The media that is poured must be at 37°C (not hot).

3. Results and Discussion

3.1 Physicochemical Properties of Enzyme Solutions

In this study, several tests were carried out such as pH, density, and viscosity. In the pineapple peel density test, the average density was around 1.080. Meanwhile, the density of orange peel obtained an average density ranging from 1.087. The supernatant solution of the orange sample was proven to have a higher density, this was because the suspended solids particles in the solution were more than that of the pineapple. Viscosity data obtained from pineapple samples have a range between 1.176-1.197 and orange peels have a range ranging from 1.183-1.224. The orange viscosity value is higher because it is influenced by the suspension of solids in the solution. When calculating the time value using an Ostwald viscometer, it was found that the travel time of the orange solution was longer. In testing the pH of the pineapple sample, the lowest value in the third fermentation was 4.63. And in the orange sample, the lowest pH was obtained at the 4.39 third fermentation. In table 1, it can be seen that the longer fermentation time treatment proves that the pH value is getting more acidic.

Another study stated that apple cider had a pH value of 4.5 to 3.0 [11]. In addition, the pH of papaya and pineapple has 4.94 and 3.46, respectively. The pH decreases as the enzyme concentration increases [12]. This is because the enzymatic reaction will produce carboxylic acid and galacturonic acid so that the pH decreases [13]. The viscosity of apple cider juice ranged from 0.13-0.09 cP. Enzyme hydrolysis will reduce viscosity [11].

Table 1 - Physicochemical properties

Sample code (Fermentation)	Density	Viscosity	pH
N1	1.081	1.176	5.78
J1	1.089	1.183	5.54
N2	1.080	1.179	5.10
J2	1.086	1.185	5.02
N3	1.080	1.197	4.63
J3	1.087	1.224	4.39

3.2 Effect of pH on the Color of the Enzyme Solution

Organoleptic testing was carried out to determine the smell and color (Fig. 2). Where the orange sample has a mandalay or brownish yellow color while the pineapple sample has a more browns or desert color. In observing the sample, it was found that the longer the fermentation time, the more concentrated the color produced. This is because some of the organic content is broken in the solution. The higher pH relationship also affects the color of the sample, where the higher pH indicates that the sample color is getting brighter. The smell of the two samples is like honey. In addition, the longer the fermentation, the more pungent odor produced when compared to the first fermentation. This is because the concentration of the enzyme produced is higher.

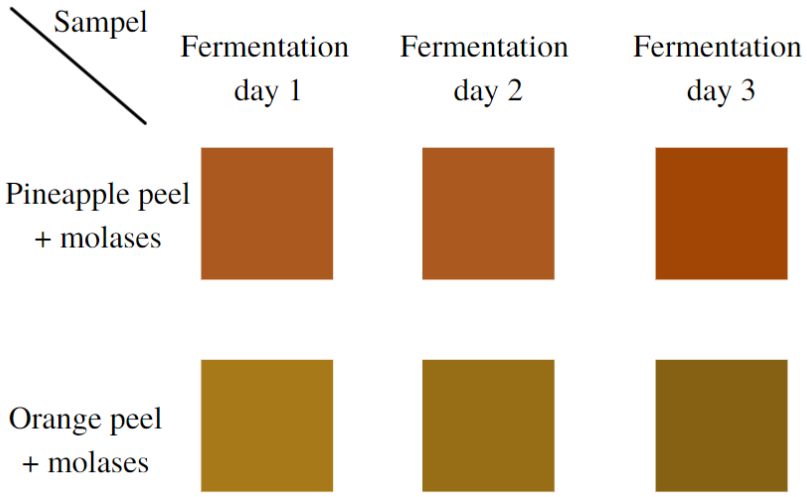

Fig. 2 - Enzyme colour degradation

3.3 Enzyme Effectiveness Against S. Aureus and E. Coli Antibacterial

In this test the effectiveness of the enzyme was carried out to see its effect on the bacterial inhibition zone. In this study, two types of gram-positive bacteria were used, namely *Escherichia Coli* (*E. Coli*) and gram-negative bacteria *Staphylococcus Aureus* (*S. Aureus*). This test was carried out by the disc diffusion method. Based on the results of the study, the results showed that the lowest and highest *E. Coli* inhibition zones in citrus samples were 16.6 mm J3 and 8.2 mm J1, respectively. Meanwhile, in the pineapple sample, the *E. Coli* bacteria tested in the lowest and highest pineapple samples were 0 mm N1 and 11.7 N2.

In the gram-positive test of *S. Aureus*, the highest and lowest inhibition zones of citrus samples were 10.5 fermentation 1 and 13.2 mm fermentation 3. Meanwhile, in the gram-negative test for *E. Coli* the highest and lowest inhibition zones were 11.2 mm and 0 mm. The effect of fermentation affects the inconsistent protein content, it is influenced by several factors such as duration, method, and variations in protein or amino acid profiles. Pranoto, Anggrahini & Efendi [14] reported an increase in protein with the highest fermentation time, 36 hours variation. The results obtained in this study are quite appropriate, the longer the fermentation is carried out the higher the protein content produced which affects the wider bacterial inhibition zone. However, in this study there were some fluctuating results such as pineapple peel on *E. Coli* bacteria. N1 does not have antibacterial activity, so it can be said that the protein content produced is very low. Meanwhile, the fluctuating results in the N2 and N3 fermentation in the *E. Coli* activity test could be caused by factors such as the influence of temperature, the addition of phosphate saline (PBS) buffer, and ammonium sulfate.

Meanwhile, based on observations of the two bacteria. Gram-positive bacteria have a thicker peptidoglycan cell wall than gram-negative bacteria. Gram-positive bacteria have a peptidoglycan thickness of 20-80 nm and gram-negative bacteria have a 2-3 nm-thick wall covered with a bilayer membrane [15]. In the case of this study *E. Coli* acted as gram positive and S. Aureus as gram negative. This theory states that the structure of gram-negative bacteria is weaker than that of gram-positive bacteria. The results showed that the presence of an antibacterial inhibition zone in *E. Coli* was higher than in *S. Aureus*. For example, samples J3 and N2 had a higher *E. Coli* inhibition zone than the S. Aureus treatment. Thus, the fermentation and dialysis methods have an effect on inconsistent protein yields. In the research of Pranoto *et al.* [14] also reported that the decrease in protein after fermentation occurred in the longer treatment time. This change can be influenced by the loss of protein complex due to microorganisms during hydrolysis. From the results of this study, it can be said that the fruit peel enzyme has the potential to inhibit bacteria because it contains protein, although

the inhibition zone is still classified as moderate. However, this can be improved by adding reagents, fruit skin composition, or fermentation time.

Table 2 - Effect of fruit peel solution on inhibition zone on bacteria

Sample	Zone of inhibition on bacteria (mm)	
	E. Coli (Gram Negative)	S. Aureus (Gram Positive)
N1	-	9.5
J1	8.2	10.5
N2	11.7	9.3
J2	1115	1119
N3	11.2	12.8
J3	16.6	13.2

4. Conclusion

Based on the description above, it proves that the enzyme produced from the combination of fruit peel and molasses has a fairly high antibacterial activity. The data of the highest antibacterial activity was orange peel fermented for 3 days which had an antibacterial surface area of 16.6 mm for *E. Coli* and 13.2 mm for *S. Aureus*. This study proves that the effect of fermentation has a significant effect, starting from the efficiency of enzyme disinfection, color, fruit skin type, pH, and organoleptic. Although the antibacterial activity is not so high, it can be increased by several methods of dialysis, composition, addition of compounds, and treatment during fermentation so as to produce maximum enzyme effectiveness.

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