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# The Sinergy Effect of Essential Oils from Traditional Herbs and Medicines as Antibacterial Materials of Edible Coating on Fresh Fruit

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## Abstract

The selling strategy of peeled honey pineapple is carried out to attract consumers who want fresh, uniform, and ready-to-eat fruit. However, honey pineapple has a short shelf life, primarily when sold as a peel. Hence, additional treatment to prevent damage and maintain the quality of peeled honey pineapple can be done by applying an edible coating. The materials used in this research were jackfruit seed starch, essential oils (ginger, lemongrass, lemon eucalyptus), and citric acid. This research aimed to determine the effect of the edible coating application with the addition of essential oils of ginger, lemongrass, lemon eucalyptus, and citric acid as an antibacterial material on the shelf life of honey pineapple. The treatment variations in this research were the concentration of essential oils (0%; 10%; 30%; 40%), and citric acid added to the edible coating, with pineapple honey storage at room and cold temperature. Antibacterial activity was then analyzed for *Escherichia coli* and *Staphylococcus aureus*. The results showed that the edible coating with 10%, 30%, and 40% ginger essential oils could form an inhibition zone in *S. aureus* bacteria, while the addition of all variations in the concentration of lemongrass essential oil could inhibit the growth of *E. coli* and *S. aureus*. The addition of lemon eucalyptus essential oil revealed an inhibition zone of *E. coli* at a concentration of 40% and *S. aureus* at a concentration of 30% and 40%. It was proven that the addition of citric acid to the edible coating indicated the formation of a greater bacterial inhibition zone than the edible coating without citric acid. The organoleptic test results also uncovered that honey pineapple stored at cold temperatures had the preferred color, texture, aroma for panelists, and longer shelf life than honey pineapple at room temperature storage.

**Keywords:** *edible coating, essential oil, citric acid, antibacterial, shelf life, honey pineapple*

## 1. Introduction

Pineapple (*Ananas Comosus* (L.) Merr.) is an annual fruit plant widely cultivated in Indonesia. According to BPS data in 2018, pineapple production in Indonesia reached 1,805,506 tons and increased to 2,196,456 tons in 2019 [1]. One of Indonesia's leading local pineapple varieties is honey pineapple, which is widely cultivated in Pemalang, Central Java. This honey pineapple is in great demand by consumers because it has a sweeter taste characteristic than other varieties. The sugar content in 100 g of honey pineapple reaches 8.29 g, and the water content reaches 85.3 g [2]. Furthermore, honey pineapple is supplied in the form of fresh or already peeled and ready to eat. Specifically, the sale of peeled pineapple fruit is a strategy to attract consumers who want the fruit to be fresh and attractive when served, with a uniform ripeness level and ready for consumption. However, the selling technique of peeled honey pineapple fruit can accelerate the product's shelf life. Peeled honey pineapple can only last for 2-3 days in plastic or mica packaging. In addition, the high sugar and water content in honey pineapple also accelerate the decay process. The content of sugar and water can also be a medium for the growth of microorganisms that can accelerate the process of fruit decay [3]. In addition to having a short shelf life, serving in the form of cut fruit also causes a faster deterioration in the quality of color, taste, aroma, and texture of the product. This decline in quality is caused by metabolic activity that is still ongoing in the fruit during the shelf life [4]. Metabolic activity that occurs is respiration and transpiration, in which processes can result in loss of substrate and water in the fruit so that fruit weight decreases. This activity will accelerate ripening and can cause fruit rot if not controlled [4]. For this reason, one of the additional treatments that can control the metabolic rate, maintain quality, and extend the shelf life of fruit is with edible coating. The edible coating is a thin layer that serves to reduce water loss in the fruit so that it can maintain the fruit at optimum conditions and as a barrier to the diffusion of O2 and CO2 gases [5]. Edible coatings are also used to reduce moisture loss, improve appearance, as a barrier for gas exchange from products to the environment or vice versa, and antimicrobials [6]. Many studies on coating food products with edible coatings have been carried out and have been shown to extend shelf life and improve product quality [7]. Research on edible coatings is also growing with the addition of antibacterial substances to maximize its function as a natural preservative. Natural antibacterial compounds can be obtained through the isolation method of plant essential oils. In the form of essential oils, the content of antibacterial compounds will be more stable and hygienic if stored for a relatively long time than the raw material. However, the problem with the use of essential oils in food is the change in the organoleptic (aroma and taste) of the product being applied. Therefore, to minimize the level of use of essential oils, edible coatings are utilized as carriers for these natural components. Various types of essential oils from spices and medicinal plants have been reported to be potentially used as food preservatives because they have antibacterial activity. The use of essential oils of spices and medicinal plants is also one of the efforts to utilize Indonesia's abundant biodiversity. Various research results and reviews have described the antibacterial activity of essential oils of traditional medicinal plants, such as ginger, cloves, turmeric, galangal, black ginger, lemongrass, lemon eucalyptus, and other traditional medicinal plants [8-12]. Various types of essential oils from these plants can be used as food preservatives due to their antibacterial activities. Furthermore, essential oils from the rhizome of ginger, lemongrass, and lemon eucalyptus were used in this study. In research conducted by Azkiyah (2020) [13], ginger rhizome essential oil was proven to have antibacterial activity, as evidenced by the formation of an inhibitory zone against the test bacteria E. coli and S. aureus. According to Ali et al.'s (2013) research results [14], the compounds in essential oils that can inhibit the growth of bacteria E. coli and S. aureus are nerol, borneol, β-eudesmol, and zingerone. Meanwhile, the antibacterial activity of lemongrass was reported by Sefriyanti (2020) [11], with an inhibition zone diameter of 3.69 mm for E. coli bacteria and 4.39 mm for S. aureus bacteria. In addition to essential oils, antibacterial activity can be obtained from other ingredients, namely citric acid. Hussain et al.'s (2015) research [15] found that citric acid was shown to be effective and significantly

reduced the population of E. coli in beef samples. Apart from antibacterial additives from essential oils and citric acid, basic edible coating materials can also be derived from various raw materials, such as starch, pectin, chitosan, and gum exudate. In particular, polymer materials for edible coating raw materials that are potential and easy to obtain are starches. Edible coatings with polysaccharide materials, such as starch, are permeable membranes that are selective for the exchange of CO2 and O2 to extend the shelf life of food products, such as fruit [16]. One of the potential starches is jackfruit seed starch as a tropical fruit waste native to Indonesia. So far, the use of jackfruit is still around the flesh, so it produces waste in the form of jackfruit seeds that are not used optimally. Therefore, the use of jackfruit seed starch as an edible coating material is also carried out to optimize the waste of natural materials and increase its usefulness. Therefore, an edible coating would be developed from jackfruit seed starch incorporating essential oils (ginger, lemongrass, lemon eucalyptus) and citric acid in this study. The edible coating was then applied to honey pineapple to determine the effect of the application of the essential oils from medicinal herbs and citric acid as an antibacterial material on edible coatings to increase the shelf life of honey pineapple fruit.

## 2. Experimental

### *2.1. Materials*

The main raw materials used in this study were jackfruit seed starch, obtained from the extraction of jackfruit seeds, distilled water (Bratachem, Indonesia). As for essential oil, namely, ginger essential oil, lemongrass essential oils, lemon eucalyptus essential oils were purchased from Rumah Atsiri, Indonesia. Other chemicals, such as glycerol, and citric acid were obtained from PT. Merck Chemicals and Life Sciences, Indonesia

### *2.2. Reserch Method*

The research method was carried out in two stages: preliminary research and main research. In the preliminary study, an analysis of jackfruit seed starch's moisture content and ash content was performed. The main study then analyzed the differences in antibacterial activity in each sample. The treatment design for making edible coatings consisted of three factors: different types of essential oils (m), including $m_1$ = ginger, $m_2$ = lemongrass, $m_3$ = lemon eucalyptus. The second factor was the difference in the concentration of essential oils (k), respectively, $k_1$ = 0%, $k_2$ = 10%, $k_3$ = 30%, $k_4$ = 40%. Furthermore, the third factor was the addition of citric acid (s) as an antibacterial material other than essential oils. Meanwhile, the treatment design for the edible coating application process to pineapple was the use of storage temperature in the form of room temperature and cooling temperature. The data was taken once after trial experiments were conducted for three times. Data obtained were analyzed using statistical tools, namely SPSS using ANOVA one way to understand the correlation between the concentration with indicated an inhibition zone of the bacteria growth.

### *2.3 Jackfruit Seed Starch Making Procedure*

The jackfruit seed extraction process was conducted based on the method development used by Nuryati et al. (2019) [17]. Jackfruit seeds were sorted and peeled the outer skin and epidermis. The peeled jackfruit seeds were then washed and thinly sliced. Next, the sliced jackfruit seeds were mashed with a ratio of jackfruit seeds: water = 1: 4. The jackfruit seed pulp that had been refined was filtered, and the filtrate was precipitated for 24 hours. The precipitate obtained was separated from the solution utilizing a Buchner funnel. Then, the resulting starch precipitate was heated in an oven at 60$^{o}$C for three hours. After drying, it was ground with a mortar until smooth and sieved using a 100-mesh sieve.

### 2.4 Making Edible Coating

The edible coating was made with 2.4 g of jackfruit seed starch, dissolved in 60 mL of distilled water (6% m/v). The starch solution was added with 0.5 mL of glycerol and heated at 80°C until gelatinized. In the sample with the addition of essential oil and/or citric acid, the solution was cooled to room temperature.

### 2.5 Antibacterial Testing

Antibacterial tests were also carried out on all variations of the edible coating solution sample using *E. coli* and *S. aureus* bacteria. Antibacterial testing was performed on samples of edible coating solution incorporating essential oils (ginger, lemongrass, lemon eucalyptus) and citric acid employing the disc diffusion method. The edible coating sample solution was dripped onto the PDA agar plate, which had been inoculated with *E. coli* (as an example of gram negative) or *S. aureus* (as an example of gram positive) microbial cultures. Then, it was incubated for 24 hours at 37°C. The effectiveness of the sample solution as an antibacterial compound was seen from the presence or absence of a clear area around the disc, which indicated an inhibition zone for the growth of the test bacteria.

### 2.6 Application of Edible Coating Solution on Honey Pineapple

In the edible coating application process, the honey pineapple was peeled, cut into small pieces, and coated with an edible coating using the spraying method. Samples were stored at different temperatures, namely room and cooling temperatures. Organoleptic tests were also carried out to determine the quality and acceptance of panelists to apply edible coatings with essential oils and citric acid on honey pineapples to know the preferred product. Organoleptic tests carried out included honey pineapple's aroma, color, and texture at room and cooling temperatures. There were 15 respondents involved in organoleptic tests by filling the questioner form. Data from the questioner was selected based on the most preference chosen of each category.

## 3. Results and discussion

### 3.1 Preliminary Research

Preliminary research was carried out with the process of making jackfruit seed starch, analyzing the ash content, and analyzing the moisture content of the obtained starch. The ash content in starch indicates the number of minerals contained in it. According to SNI 3751:2009, the maximum ash content in tapioca flour is 0.70%. In research conducted by Sardiman et al. (2009) [18], the making of jackfruit seed starch was done employing the HTM (Heat Moisture Treatment) method, where the ash content of jackfruit seed starch was 1.23%, exceeding SNI quality requirements. In this study, the extraction method and the deposition process used obtained an ash content was 0.37% so that it met the SNI standard for wheat flour, namely < 0.7%. In addition, the water content analysis in this study was conducted using the gravimetric method (AOAC925.10-1995). In the gravimetric method, the reduction in weight before and after heating is the water content contained in the material. In this study, the water content obtained was 12.03%. Thus, it met the quality requirements of SNI 3751:2009 in wheat flour, less than 14.5%. These results are not much different from Sardiman et al.'s (2009) study [18], which reported that the water content of jackfruit seed starch was 12.91%.

### 3.2 Main Research

The main research was conducted to determine the antibacterial activity of edible coatings and analyze the organoleptic test results.

### *3.2.1 Analysis of Edible Coating Antibacterial Activity*

The antibacterial activity of the edible coating, which contained essential oils of ginger, lemongrass, lemon eucalyptus, and/or citric acid, was tested against *E. coli* and *S. aureus* bacteria, which are spoilage bacteria in foodstuffs. This antibacterial test was conducted to determine the effect of adding essential oils of ginger, lemongrass, lemon eucalyptus, and citric acid as antibacterial compounds in edible coatings.

**Table 1:** Inhibitory zone diameter of edible coating without essential oil

| Sample Code | **Diameter of Inhibitory Zone Formed (mm)** *E. coli* | **Diameter of Inhibitory Zone Formed (mm)** *S. aureus* |
|---|---|---|
| B* | 0 | 0 |
| I** | 9.84 | 7.64 |

**edible coating without essential oil and citric acid*

***edible coating with citric acid*

Antibacterial test on edible coating solution without essential oil did not show any inhibition zone for the two test bacteria. Meanwhile, in the edible coating solution with the addition of citric acid, an inhibition zone was formed against *E. coli* and *S. aureus* bacteria. It aligns with Eliuz's (2020) research [19], which showed that citric acid proved effective against *E. coli, S. aureus,* and *C. albicans* bacteria, using broth microdilution and agar-well diffusion assay methods. According to Morales et al. (2003) [20], antibacterial strength is categorized into + or weak (6-10 mm); ++ or active (11-20 mm); +++ or very active (21-30 mm); thus, the antibacterial strength of edible coatings with citric acid is in the + or weak category.

**Table 2:** The diameter of the edible coating inhibition zone with the addition of ginger essential oil and citric acid

| **Essential Oil Concentration (%)** | **Diameter of Inhibitory Zone Formed (mm)** | | | |
|---|---|---|---|---|
| | **Edible Coating + Ginger Essential Oil** | | **Edible Coating + Ginger Essential Oil + Citric Acid** | |
| | ***E. coli*** | ***S. aureus*** | ***E. coli*** | ***S. aureus*** |
| 10 | 0 | 7.87 | 0 | 10.12 |
| 30 | 0 | 10.18 | 0 | 12.84 |
| 40 | 0 | 9.76 | 0 | 15.04 |

Based on the antibacterial activity test results against *E. coli* and *S. aureus*, edible coating with the addition of ginger essential oil at all concentrations could form an inhibition zone against *S. aureus* bacteria. However, the edible coating with ginger essential oil did not show any antibacterial activity against the test bacteria *E. coli*. Ali et al.'s (2013) study [14] reported that the antibacterial activity of ginger formed a greater inhibition zone against *S. aureus* than *E. coli*. This difference can occur because *S. aureus*, a gram-positive bacterium, has a cell wall structure with low lipid content, which is more easily penetrated by antibacterial compounds compared to *E. coli* as a gram-negative bacterium, which has a more complex wall structure. In the edible coating with the addition of ginger essential oil and citric acid, an increase in antibacterial activity was seen compared to the edible coating without citric acid. It

strengthened the synergy between citric acid and ginger essential oil in acting as an antibacterial agent because citric acid has the ability as an antibacterial (Table 1).

**Table 3:** The diameter of the edible coating inhibition zone with the addition of lemongrass essential oil and citric acid

| Essential Oil Concentration (%) | Diameter of Inhibitory Zone Formed (mm) | | | |
|---|---|---|---|---|
| | Edible Coating + Lemongrass Essential Oil | | Edible Coating + Lemongrass Essential Oil + Citric Acid | |
| | ***E. coli*** | ***S. aureus*** | ***E. coli*** | ***S. aureus*** |
| 10 | *Cannot be measured | *Cannot be measured | 9.43 | *Cannot be measured |
| 30 | *Cannot be measured | *Cannot be measured | 13.51 | *Cannot be measured |
| 40 | *Cannot be measured | *Cannot be measured | 10.75 | *Cannot be measured |

Based on Table 3, the edible coating with the addition of lemongrass essential oil showed the formation of the diameter of the inhibition against *E. coli* and *S. aureus* bacteria. The diameter of the inhibition zone could not be measured because the inhibition zone formed was too large, and the bacteria did not appear to be growing. Related to this, lemongrass essential oil has antibacterial activity due to the content of flavonoids, tannins, and saponins, which are capable of damaging the cytoplasmic membrane, disrupting cell permeability, and disrupting the stability of bacterial cell membranes so that the activity of the test bacterial cells is disturbed and even dies [21].


(a) (b)

**FIGURE 1**: (a) Antibacterial test with S. aureus test bacteria, and (b) antibacterial test with E. coli test bacteria with disc diffusion method on (B) edible coating sample without essential oil and lemongrass essential oil edible coating sample, with various concentrations (D1) 10%, (D2) 30%, and (D3) 40%

The antibacterial test results of edible coating with the addition of lemon eucalyptus essential oil and citric acid can be seen in Table 4. edible coating with the addition of lemon eucalyptus essential oil showed inhibition zones of *E. coli* at a concentration of 40% and *S. aureus* at a concentration of 30% and 40%. Meanwhile, in the edible coating with the addition of lemon eucalyptus essential oil and citric acid, an inhibition zone was formed on the two test bacteria. In addition, in the addition of lemon eucalyptus essential oil at concentrations of 10% and 30%, the addition of citric acid

could increase the antibacterial activity of the edible coating. In research conducted by Missanjo & Mkwezalamba (2016) [22], the essential oil of lemon eucalyptus (*Corymbia citriodora*) proved to be effective in having a significant effect on antibacterial activity on *E. coli* and *S. aureus* because it contains citronella, citronellol, linalool, or1,8-cineole compounds.

**Table 4:** The diameter of the edible coating inhibition zone with the addition of lemon eucalyptus essential oil and citric acid

| **Essential Oil Concentration (%)** | **Diameter of Inhibitory Zone Formed (mm)** | | | |
|---|---|---|---|---|
| | **Edible Coating + Lemon Eucalyptus Essential Oil** | | **Edible Coating + Lemon Eucalyptus Essential Oil + Citric Acid** | |
| | ***E. coli*** | ***S. aureus*** | ***E. coli*** | ***S. aureus*** |
| 10 | 0 | 0 | 9.91 | 10.68 |
| 30 | 0 | 9.81 | 10.22 | 11.23 |
| 40 | 6.18 | 11.83 | 11.69 | 10.38 |

Further, the statistic test was carried out to understand the effect of addition of antibacterial agents, namely, citric acid and essential oil using ANOVA One Way test. The statistic results showed that adding citric acid into edible coating influenced the growth of bacterial zone inhibition (sig. $0.035 < 0.05$). However, there was no effect on variation of different concentration of essential oil added into the edible coating toward the bacterial zone inhibition (sig. $0.377 > 0.05$). This is because each essential oil has their own antibacterial component which leads to different antibacterial mechanism.

### *3.2.2 Organoleptic Test*

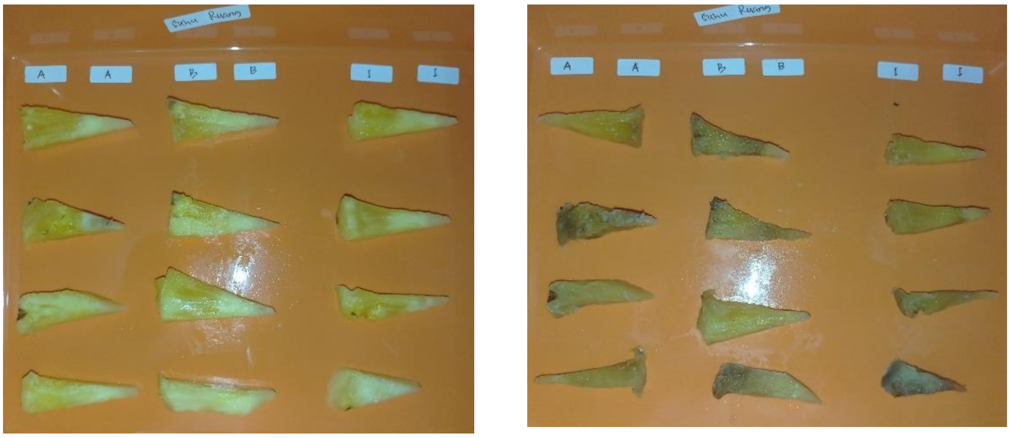
The test was conducted to determine the effect of coating with variations of essential oils (ginger lemongrass, lemon eucalyptus) with various concentrations, namely 0, 10, 30 and 40% and citric acid on the appearance of aroma, color, and texture of honey pineapple, which was stored at room and cold temperatures.

**Aroma**

The addition of essential oils to edible coatings greatly affected the aroma of honey pineapple. The higher the essential oil concentration used, the resulting honey pineapple fruit sample had a more pungent aroma. The aroma of ginger, lemongrass, and lemon eucalyptus essential oils used in the edible coating masked the natural aroma of honey pineapple. Fifteen panelists recommended sample I or pineapple with citric acid edible coating as the product with the most preferred aroma.

**Color**

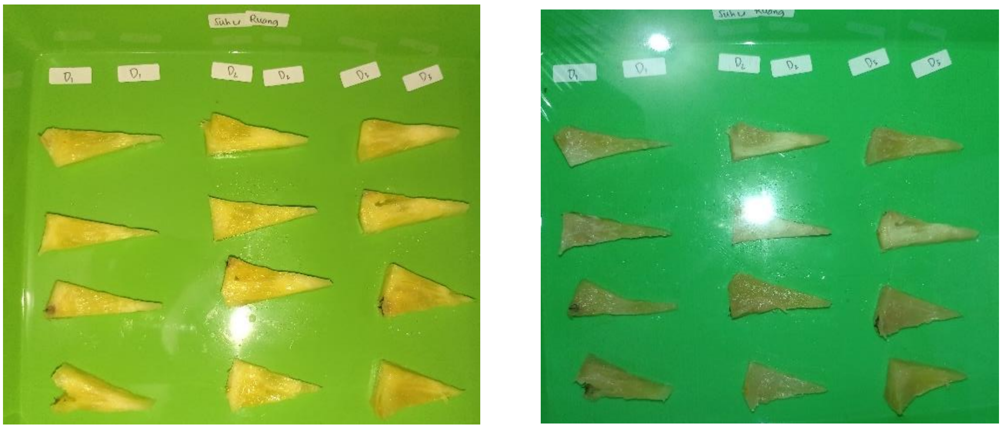
The application of edible coating with the addition of variations of essential oils with/without citric acid did not affect the color of the honey pineapple fruit. The coated pineapple fruit was bright yellow and had no color difference from the control sample. Then, the sample color became browner after storing the sample for five days at room temperature. Meanwhile, there was no color change in the samples stored at cold temperatures until the fifth day of storage.



(a) (b)

**FIGURE 2**: (a) The color of honey pineapple on the first day of storage, and (b) the fifth day of storage at room temperature with variations (A) without edible coating, (B) with edible coating, and (I) with citric acid edible coating

*4*

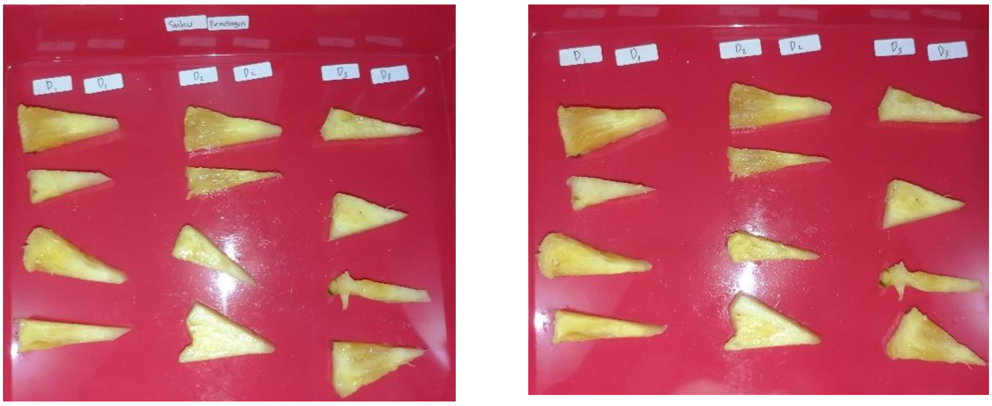


(a) (b)

**FIGURE 3**: (a) The color of honey pineapple on the first day of storage, and (b) the fifth day of storage at room temperature with the addition of lemongrass essential oil with variations in concentration (D1) 10%, (D2) 30%, and (D3) 40%

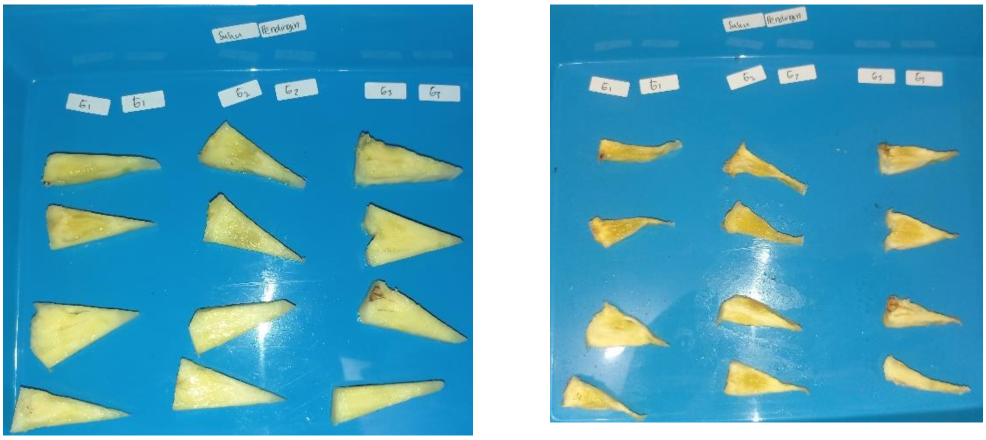
**Texture**

Pineapple fruit coated with the addition of essential oil with/without citric acid had no difference in texture with the control sample. However, the difference occurred after storing the samples at room temperature for five days. In addition, honey pineapple samples stored at room temperature had a softer texture than the previous days. Meanwhile, samples stored at cold temperatures had a drier texture and did not decay. Storage at cold temperatures can slow down the respiration rate of the fruit, thereby slowing down the process of loss of substrate contained in the fruit and prolonging its shelf life [23].



(a) (b)

**FIGURE 4**. (a) The color of honey pineapple on the first day of storage, and (b) the fifth day of storage at a cooling temperature, with the addition of lemongrass essential oil with variations in concentration (D1) 10%, (D2) 30%, and (D3) 40%



(a) (b)

**FIGURE 5**. (a) The color of honey pineapple on the first day of storage, and (b) the fifth day of storage at a cooling temperature, with the addition of lemongrass essential oil and citric acid variations (G1) 10%, (G2) 30%, and (G3) 40%

## 4. Conclusion

The use of essential oils (ginger, lemongrass, and lemon eucalyptus) and citric acid on edible coatings have been shown to have antibacterial activity in several variations. Antibacterial test results also showed that edible coating with ginger essential oils 10%, 30%, and 40% could form an inhibitory zone on *S. aureus* bacteria with weak activity (7.87 – 10.18 mm). The inhibition activity was shifted to active category when both ginger essential oil and citric acid mixed in edible coating with diameter 10.12 – 15.04 mm. In contrast, the addition of all variations in the concentration of lemongrass essential oil could inhibit the growth of *E. coli* and S. *aureus*. In the addition of lemon eucalyptus essential oil, the inhibition zone of *E. coli* was at a concentration of 40%, while *S. aureus* was at a concentration of 30% and 40%, respectively. Most of the samples with the addition of citric acid on the edible coating revealed the formation of a greater bacterial inhibition zone than without the addition of citric acid. These results confirm that there was a synergy effect between essential oils and citric acid to form antibacterial inhibition effect. Further results from organoleptic tests also uncovered that honey pineapple stored at cold temperatures had a color, texture, and aroma that the panelists preferred and a longer shelf life than at room temperature storage.

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